

Nematode Biotic Indices: Advancements and Applications in Assessing Soil Health

NETHI SOMASEKHAR* and SATISH NAMDEO CHAVAN

ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad - 500 030, India

*Principal Author E-mail: nssekhar@hotmail.com; ORCID ID: 0000-0002-3666-8171

ABSTRACT: Nematodes are a miracle of evolution with unmatched ecological competence. In soil ecosystems, nematodes are the most abundant and diverse metazoans. Nematodes are present at different trophic levels in soil food webs, ranging from grazers of microorganisms functioning at the interface of living and non-living matter in detritus food webs to parasites of higher plants and animals. Soil nematodes regulate key ecological processes and services. Nematodes regulate primary production, predation, energy transfer, decomposition of organic matter, and nutrient mineralization in agricultural ecosystems directly by feeding on crop plants and indirectly by consuming microflora and fauna. The nematode community in soil, therefore, has a high information content. Nematodes have several characteristics of good bioindicators including ubiquitous presence, a direct relationship between structure and function, diverse trophic groups, a wide range of life history strategies, and the availability of well-tested biotic indices. Nematode biotic indices have progressed over time, evolving from simple community indices based on counts (abundance, frequency, *etc.*) to specialized maturity indices based on colonizer and persister traits (Maturity Index, Plant Parasite Index, *etc.*), and further to the more advanced indices based on functional guilds (Enrichment Index, Basal Index Structure Index, *etc.*) that are valuable in soil food web diagnostics. The most advanced index, the metabolic footprint, estimates nematode contribution to a variety of environmental services and processes. Classical and molecular approaches to nematode community analysis are used for assessing the impact of various kinds of disturbances in the soil environment. Nematode biotic indices have been successfully employed as environmental bioindicators to measure the impact of biological, chemical, and physical factors on soil health and ecosystem services in both natural and managed ecosystems. The available evidence emphasizes the significance of nematodes as bioindicators and their potential for widespread application. The specific characteristics that make nematodes useful bioindicators, the evolution of nematode biotic indices, tools for computing and interpreting these indices, and recent trends in the application of nematode biotic indices in assessing soil health and ecosystem disturbance caused by various agents are discussed in this paper.

Keywords: Bioindicators, ecological services, ecosystem disturbance, nematodes, soil food-web, soil health

INTRODUCTION

Conservation and management of natural resources such as soil, water, and biodiversity, play a crucial role in maintaining and sustaining the productivity of the ecosystem, thereby contributing to the sustainable advancement of humanity. In a pivotal study carried out by the United Nations in 1991, it was estimated that approximately 552 million ha of land, equivalent to 38 per cent of the current cultivated area in the world, had been experiencing varying degrees of degradation due to agricultural mismanagement since World War II (Gardner, 1998). While expanding acreage has been effectively replaced by use of advanced technologies to

boost production, global agroecosystems are growing more susceptible to the adverse impact of these technologies on the environment (Wang and McSorley, 2005). Several advanced technologies influence the ecological environment, contributing to environmental degradation. These modern technologies result in various consequences, including land degradation, soil erosion, deforestation, air pollution, water pollution, and others, subsequently impacting farming and farm produce (Hossain *et al.*, 2020; Abdel Rahman, 2023). These issues have highlighted the importance of maintaining soil health and ecosystem functions on a sustainable basis. Due to the close interaction that biological organisms have with their environment, there has been a lot of

interest in developing bioindicators to assist monitor soil health in recent years (Nielsen and Winding, 2002; Brackin *et al.*, 2017).

Soil contains an extensive array of organisms, with differing functions, requirements, and life cycles, and in which these organisms exist and interact. Out of an estimated five million soil fungi species, only about 100,000 are currently known (Nielsen *et al.*, 2016; Hawksworth and Lücking, 2017). Similarly, despite an estimated one billion species inhabiting soils, only 4,500 species of soil bacteria have been identified (Nielsen *et al.*, 2016). Moreover, out of the millions of estimated nematode species, only around 30,000 nematode species are currently known (Kiontke and Fitch, 2013). In 1914, N.A. Cobb, the father of nematology, said, “In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, valleys, rivers, lakes, and oceans represented by a thin film of nematodes”. In simpler terms, nematodes exhibit remarkable diversity, inhabiting a multitude of environments. They can be found virtually everywhere: from your backyard to forests, from deserts to arctic ice, from hot water springs to the deep sea sediments devoid of oxygen. Evaluating soil health involves examining physical, chemical, and biological parameters. Biological indicators include microorganisms, protozoa, and metazoa. Nematodes, belonging to the metazoan kingdom, are particularly prevalent, and their reactions to pollutants and environmental disturbances demonstrate notable diversity; hence, they serve as one of the important indicators for the assessment of soil health.

ROLE OF NEMATODES IN SOIL HEALTH

The ability of soil to work within ecosystem boundaries in order to support biological productivity, preserve environmental quality, and advance the health of plants

and animals is known as soil health (Doran and Zeiss, 2000; Somasekhar and Prasad, 2012). Therefore, healthy soil must facilitate essential life processes within its ecosystem. These processes include regulating decomposition and nutrient cycling, providing nutrients and stability for plant growth, sustaining optimal moisture levels, pH balance, and other soil characteristics, fostering soil food web dynamics and energy flow, preserving microbial diversity, mitigating pollutants, and controlling plant pathogens and pests.

Soil nematodes are a diverse group of microscopic roundworms inhabiting soil environments and play a vital role in maintaining soil health. They are recognized as the most abundant and widespread multicellular organisms within soil ecosystems. One square meter of soil may contain over 30 billion nematodes (Sohlenius *et al.*, 1977). Nematode communities within soil comprise a diverse array of trophic and ecological groups, each contributing significantly to essential soil ecosystem functions. Unlike other organisms, nematodes exhibit functionality across multiple trophic levels, serving as primary consumers (plant parasites), secondary consumers (bacterivores and fungivores), and tertiary consumers (animal parasites, omnivores, and predators).

Plant parasitic nematodes (PPNs) are of economic importance in agricultural ecosystems because of their ability to damage plants (Sasser and Freckman, 1987). Nematodes cause an average of 12.3 per cent losses annually in 40 major crops at a global level, which accounts for a monetary loss of about US\$ 80–118 billion per year (Sasser and Freckman, 1987; Nicol *et al.*, 2011). PPNs possess a specialized needle-like structure called a stylet through which they extract plant sap, thereby inflicting damage on the plants. Certain nematode species function as ectoparasites, occasionally feeding on plant roots, while others establish long and intimate relationships with their host plants. In some nematode species, the life cycle is fully synchronized with that of

their plant hosts. Besides directly impacting crop yields, PPNs also contribute significantly to disease complexes involving other pathogens (Back *et al.*, 2002). Entomopathogenic nematodes (EPNs) or insect parasitic nematodes that feed on insects are considered beneficial to the farmers. They play a crucial role in the biological suppression of insect pests that afflict crops, consequently decreasing reliance on harmful pesticides (Somasekhar, 2009; Koppenhöfer *et al.*, 2020). By consuming microbial grazing nematodes, predatory nematodes also regulate nitrogen mineralization in the soil (Wardle and Yeates, 1993).

Although bacteria and fungi are the principal decomposers, microbivorous nematodes play an important role in the decomposition of organic matter, as well as nitrogen (N) and carbon (C) mineralization in soil, both directly and indirectly. Nematodes play a crucial role in the energy route from primary production and detritus to higher trophic groups (Ingham *et al.*, 1985; Freckman, 1988; Ferris *et al.*, 1998). It has been demonstrated that nematodes directly contribute to nitrogen mineralization and the distribution of biomass in plants (Ferris *et al.*, 1998; Trofymow and Coleman, 1982). Plants do better in soils that contain nematode grazers for bacteria, fungi, and other microorganisms than in soils with simpler soil food webs (Ingham *et al.*, 1985). When microbivorous nematodes graze on bacteria and fungi, they release CO₂, NH₄, and other nitrogenous compounds, thereby directly affecting C and N mineralization (Ingham *et al.*, 1985). Nematodes indirectly aid in nitrogen mineralization through several mechanisms: they graze on decomposer microbes, distribute microbial propagules in the soil (Freckman, 1988), consequently, enhance the substrate colonization and nutrient mineralization; they excrete ammonium and other metabolites that potentially stimulate specific bacterial growth, and they immobilize nitrogen in live biomass (Ingham *et al.*, 1985; Beare, 1997; Ferris *et al.*, 1998). Bacterivorous and fungivorous nematodes

contribute to enhancing decomposition rates by favouring the growth of active microbial communities.

Predatory nematodes are a group of nematodes that actively hunt and feed on other organisms, primarily other nematodes, but also including small invertebrates like protozoa, and rotifers (Esser, 1987; Khan and Kim 2007; Kanwar *et al.*, 2021). In both traditional and integrated agricultural systems, bacterivorous and predatory nematodes are estimated to contribute around 8 and 19 per cent of nitrogen mineralization in field situations (Beare, 1997). In soil ecosystems, the nitrogen mineralization contribution from bacterivorous nematodes is substantial as compared to the bacteria. This is attributed to the higher carbon-to-nitrogen (C: N) ratio (8:12) and lower nitrogen content of nematodes, along with their smaller growth efficiency (less than 25%) compared to bacteria (with a C: N ratio of 3:4 and growth efficiency exceeding 30%). Consequently, most of the absorbed carbon and nitrogen from bacteria are excreted by nematodes (Wasilewska and Bienkowski, 1985; Hunt *et al.*, 1987). Furthermore, it has been discovered that the abundance of free-living nematodes, especially bacterivorous ones, correlates with the levels of various other soil minerals in fallow fields, indicating the possibility that nematodes may mineralize a variety of soil nutrients (Wang *et al.*, 2004).

In most agroecosystems, decomposers (bacterivores and fungivores) and herbivores constitute the dominant trophic groups, leading to two major pathways of energy flow through the soil nematode community. Herbivorous nematodes act as primary consumers, directly extracting energy from plants. Conversely, the energy flow from plants to nematode decomposers occurs indirectly. This is because bacterial and fungal-feeding nematodes do not directly consume the organic matter but instead feed on the bacteria and fungi responsible for breaking down of organic matter in soil. Understanding the structure and function of nematode communities, as well as their

impact on plant productivity, is crucial for monitoring and managing soil health in agroecosystems. This knowledge is particularly vital for enhancing the profitability of organic farming systems, which rely entirely on biological resources and the regulation of pests and diseases through ecological processes.

BIOINDICATORS OF SOIL HEALTH

Neher *et al.* (1995) suggested criteria for successful bioindicators for soil health. An ideal indicator organism or group of organisms should not only reflect the current state of ecological process structure and function but also exhibit responsiveness to changes in soil conditions resulting from human activities. In the context of large-scale regional and national level monitoring programmes, these indicator organisms should meet several additional criteria. They should be appropriate to all geographical locations, soils, seasons, and vegetation. Additionally, there should be sufficient taxonomic knowledge available to accurately and efficiently identify these indicator organisms. Furthermore, for practical purposes, the cost of collecting and storing samples should be minimal, and sample processing should be relatively easy, rapid, and not require frequent sampling. In line with these criteria, Kennedy and Jacoby (1999) introduced a six-component framework to assess an organism's suitability as a bioindicator, later simplified by Holt and Miller (2010) into four components: 1) Demonstrated indicator capacity: Reflecting responses representative of ecosystem elements. 2) Abundance and prevalence: Prevalent in undisturbed regions and commonly found. 3) Extensively researched: Easily and inexpensively studied. 4) Economic or commercial significance.

The utilization of microbial communities as indicators of soil health encounter inherent logistical challenges and limitations, including transient shifts in populations and diurnal fluctuations in microbial activity. Furthermore, there are difficulties in identifying all bacteria and fungi

species in samples, particularly the free-living species. A wide variety of organisms can be found in soil ecosystems, such as mesofauna (arthropods and nematodes), microfauna (protozoa, nematodes), and microbes (fungi, bacteria, and algae). Microbial communities play an important role in ecological processes such as nutrient cycling and can respond to soil environmental disturbances such as heavy metal contamination (Nannipieri *et al.*, 1990) and pesticide use (Visser and Parkinson, 1992). Compared to soil microbes, soil fauna possesses advantages as bioindicators. As they occupy higher trophic levels in the food chain, they integrate biological, physical, and chemical properties associated with food sources. Relatively longer generation times of these organisms ranging from days to years, contribute to temporal stability, contrasting with the fluctuating populations of metabolically active microbes, which often respond to ephemeral nutrient flushes in soil (Nannipieri *et al.*, 1990). Among mesofauna, three groups, namely nematodes (Bongers, 1990), collembola (Frampton, 1997), and mites (Ruf, 1998), have been considered as potential biological indicators. Nematodes, in particular, have undergone extensive evaluation for their applicability as indicators in both natural and managed ecosystems due to their significant roles in ecosystem processes and services. Additionally, more comprehensive taxonomic and functional information exists for nematodes compared to other mesofauna groups.

THE IMPORTANCE OF NEMATODES AS BIOINDICATORS

Nematodes have multiple traits that make them useful ecological indicators.:

- 1. Ubiquity and abundance:** Nematodes are ubiquitous and abundant in soil environments, making them readily available for sampling throughout the year in most ecosystems. This characteristic makes them suitable for both local and large-scale regional monitoring efforts (Neher *et al.*, 1995).

2. **Ease of sampling:** The relatively small size and high abundance of nematodes simplify sampling and extraction processes, resulting in lower costs compared to other soil fauna.
3. **Functional diversity:** Soil nematodes encompass a variety of functional or trophic groups, exhibiting different life history characteristics, such as colonizers and persisters (Bongers, 1990).
4. **Central role in soil food-web:** Nematodes are found in many trophic levels and hold a vital place in the detritus food web. They belong to functional guilds of which all members exhibit similar responses to nutrient enrichment and disturbances in the soil environment (Ferris *et al.*, 2001).
5. **Morphological differences:** The structure and function of nematodes are strongly correlated. This allows for easy separation of trophic or functional groups based on morphological structures associated with their various feeding modes (Yeates *et al.*, 1993).
6. **Sensitivity to environmental disturbances:** Nematodes exhibit varying degrees of sensitivity to direct and indirect physical, chemical, and biological disturbances in soil ecosystems. Some species form resistant stages, such as cysts or anhydrobiotic stages, enabling them to survive unfavourable conditions, while others without such stages are more sensitive to environmental disturbances (Fiscus and Neher, 2002).
7. **Biomarker potential:** Nematodes have heat shock proteins, which are activated when exposed to stressors such as heat, metal ions, or organic toxins. These proteins may serve as biomarkers for ecotoxicological assessment of soils (Kammenga *et al.*, 1998).
8. **Taxonomic knowledge and analytical methods:** The availability of taxonomic knowledge and

successful methods for sampling, analysis, computation, and interpretation of indices are more developed for nematodes compared to other soil fauna.

9. **Influence on soil function:** The composition of the nematode community has an impact on soil function that is related to agricultural productivity and sustainability. Nematode maturity indices reflect successional changes in nematode communities in response to anthropogenic disturbances (Wasilewska, 1995).

USE OF NEMATODE BIOTIC INDICES TO ASSESS SOIL HEALTH AND ECOSYSTEM DISTURBANCE

A variety of statistical and graphical methods and indices have been used to describe changes in the environment using nematodes. Ecological indices derived from nematode community analysis serve as valuable indicators for assessing disturbances in the soil ecosystems and the status of the soil food web. Nematodes have been utilized for biomonitoring of aquatic systems since the early 1970s. For instance, the free-living nematode *Panagrellus redivivus* has been employed to assess the toxic effects of approximately 400 individual chemicals (Samoiloff, 1987). Subsequently, the nematode-to-copepod ratio gained popularity for monitoring the condition of aquatic ecosystems. Generally, nematodes exhibit lower sensitivity to environmental pollution compared to copepods. Thus, a high nematode-to-copepod ratio suggests pollution, such as oil spills, sewage, and organic enrichment (Raffaelli and Mason, 1981). Research on utilizing nematodes as bioindicators for monitoring terrestrial ecosystems began in the 1980s. Simple indices based on the abundance, and frequency, of nematodes by trophic groups were proposed initially. Later, more sophisticated indices that can withstand rigorous statistical methods were employed (Table 1).

Table 1. Nematode biotic indices used for monitoring soil health and ecosystem disturbance

Index	Interpretation	Reference
<i>Species/Community measurers</i>		
Abundance	Indicates population density of a species/taxa.	Heip <i>et al.</i> , 1988
Species richness	Number of species/taxa.	Heip <i>et al.</i> , 1988
Trophic structure	Describes the abundance of different trophic groups in the nematode community.	Heip <i>et al.</i> , 1988
<i>Diversity indices</i>		
Shannon diversity index (H')	This diversity index gives more weight to rare species. The higher the index value, the greater the diversity.	Shannon and Weaver, 1949
Simpson's Dominance Index (λ)	This diversity index gives greater weight to common species. A higher value denotes lower diversity.	Simpson, 1949
Trophic Diversity (T) Index	Describes the diversity of trophic groups within the nematode community.	Freckman and Ettema, 1993
Similarity index	Describes the similarities between nematode communities in two different ecosystems.	Topham <i>et al.</i> , 1991
<i>Maturity indices</i>		
Maturity index (MI)	The nematode Maturity index is computed using only free-living nematodes. Indicates environmental disturbance resulting from Perturbations. A lower index indicates disturbance (Range 1–5).	Bongers, 1990
Plant parasite index (PPI)	The Nematode Maturity index is computed using only plant parasitic nematodes. A low value indicates the dominance of small and medium-sized ectoparasites and a higher index value indicates the dominance of medium/large-sized semi/endo parasitic/virus-transmitting nematodes (Range: 2–5).	Bongers, 1990
Σ MI	Combined maturity index for free-living and plant parasitic nematodes in a sample. Low index value (<2.0) indicates the presence of high amounts of nutrients and low plant-parasitic nematode pressure (Range: 1–5).	Yeates, 1994
PPI/MI	The PPI/MI ratio is lower in nutrient-deficient situations than in nutrient-rich conditions. It is a measure of enrichment in agroecosystems.	Bongers <i>et al.</i> , 1995
<i>Enrichment and Structure indices</i>		
Enrichment index (EI)	Indicates the food availability and nutrient enrichment. Low (0–30), middle (30–60), and high (60–100) values represent similar levels of food availability and nutritional enrichment (Range: 0–100).	Ferris <i>et al.</i> , 2001
Structure index (SI)	Indicates the soil food web structure and complexity, as well as disturbance due to environmental or anthropogenic causalities. The structure index characterizes a soil ecosystem as structured (high SI) or disturbed (low SI) (Range: 0–100).	Ferris <i>et al.</i> , 2001

Index	Interpretation	Reference
Basal index (BI)	Reflects the complexity and organization of the food web structure. High (60–100) and low (0–30) values denote corresponding degrees of soil disturbance. Increased values (>50) reflect a degraded and disturbed soil food web (Range: 0–100).	Ferris <i>et al.</i> , 2001
Channel index (CI)	It describes the dominant decomposition channels in a food web. Increased dominance of bacteria in decomposition is indicated by lower values (<50), whereas an increase in the dominance of fungus in decomposition is shown by higher values (>50) (Range: 0–100).	Ferris <i>et al.</i> , 2001
Metabolic footprint (MF)	Estimates nematode contribution to a range of ecosystem services and functions. The higher MF values signify enhanced carbon channeling, leading to an augmented role in supporting soil ecosystem functions and services (Range: 0–infinite).	Ferris, 2010

A significant advancement in nematode community analyses occurred with the introduction of the colonizer-persister (c-p) continuum, ranging from 1 to 5, which classifies nematode families based on their life history traits. This classification system was accompanied by the development of the Maturity Index (MI) by Bongers in 1990. Nematodes with a c-p value of 1 typically exhibit small body size, a short lifespan, high fecundity, and resilience to environmental disturbances. They thrive in enriched environments and form dauer larvae as microbial blooms subside. Conversely, nematodes with c-p values of 5 tend to have a larger body size, longer lifespan, lower fecundity and are highly sensitive to disturbances. They do not form dauer stages and are predominantly omnivores or predators (Bongers, 1990). Taxa within the same c-p classes respond similarly to disturbances. The MI, determined as the weighted mean frequency of the c-p scaling throughout the whole nematode community, presents a representation of the nematode community and also the state of the soil environment (Bongers, 1990; Yeates, 1994; Neher and Campbell, 1996). Nematode MIs have been widely utilized to monitor changes in natural as well as managed ecosystems caused by various factors (Bongers *et al.*, 1991; Ettema and Bongers, 1993; Freckman and Ettema 1993; Ferris *et al.*,

1996; Neher and Campbell, 1996; Somasekhar *et al.*, 2002). Neher (2001) provided a comprehensive review of different types of MIs and their responses to various disturbances.

A notable advancement in utilizing nematodes as bioindicators is the development of the concept of nematode functional guilds, which combines nematode feeding groups with the c-p scaling into functional guilds (Ferris *et al.*, 2001). This concept has enhanced nematode community analysis, transforming it into a more potent tool for assessing soil health and food web conditions. Ferris *et al.* (2001) utilized functional guilds to calculate three key indices: the enrichment index (EI), structure index (SI), and channel index (CI). The enrichment index (EI) is derived from the predicted responsiveness of opportunistic guilds (such as bacterivores with c-p = 1 or Ba1) to food enrichment. Therefore, EI indicates whether a soil ecosystem is nutrient-enriched or depleted. On the other hand, the structure index (SI) aggregates functional guilds with c-p values ranging from 3 to 5. SI indicates whether a soil ecosystem is structured or disturbed. The channel index (CI), measured as the percentage of fungivores among all fungivores and opportunistic bacterivores, indicates the primary decomposition

channels in a food web. These indices offer more comprehensive insights than analyses focused solely on biomass, diversity, or plant parasitic species. They combine both qualitative and quantitative information about the soil ecosystems (Bongers, 1990; Ferris and Motute, 2003).

It is established that nematodes belonging to specific functional guilds occupy degraded soils. These guilds constitute a basal community (Ferris *et al.*, 2001; Ferris and Matute, 2003). The nematode community reacts to the physical and chemical characteristics of the soil above this basal community. These indices have been widely utilized to evaluate soil health in managed and natural ecosystems across diverse geographical regions (Bongers, 1990; Ferris *et al.*, 2001; Berkelmans *et al.*, 2003; Hohberg, 2003; Stirling *et al.*, 2004). Various types of biotic indices based on nematode analyses used in evaluating soil health and their interpretations are summarized in Table 1.

METABOLIC FOOTPRINTS OF NEMATODES WITHIN THE SOIL FOOD WEB

The metabolic footprint estimates the contribution of nematodes to various ecosystem services and activities (Ferris *et al.*, 2010). The main resources that regulate the size and activity of the food web are carbon and energy. Nematodes not only use carbon to produce their bodies and eggs, but they also have size-dependent metabolic costs (Klekowski *et al.*, 1974; Ferris *et al.*, 1996). Metabolic footprint assessment is used to calculate the biomass and metabolic activity linked to each attribute of the food web. It comprises both production and respiration components (Ferris *et al.*, 2010). The production component takes into account the cumulative carbon allocated to growth and egg production during the lifespan, while the respiration component considers the utilization

of carbon in metabolic activities. Metabolic footprints of nematode assemblages offer metrics for assessing the ecosystem services carried out by individual functional guilds.

INTEGRATING NEMATODE COMMUNITIES WITH OTHER SOIL BIOLOGICAL HEALTH INDICATORS

The ability of the physical, chemical, and biological components of the soil to support plant productivity, preserve animal health, and enhance the quality of the air and water is known as soil health (Doran and Zeiss, 2000). A healthy soil ecosystem thrives on beneficial soil components that support biological productivity and provide essential ecosystem services. Bio-indicators of soil health are quantifiable characteristics that delineate the living components within the soil. They have the potential to serve as metrics for assessing soil functionality across diverse ecological conditions (Bhaduri *et al.*, 2022). Among the crucial bio-indicators for soil health are microbial biomass, respiration, enzymatic activity, molecular gene markers, microbial metabolic chemicals, and microbial community (Bhaduri *et al.*, 2022). Indicators and soil health outcomes often do not significantly correlate in the field of soil biological health (Sprunger *et al.*, 2024). Exploratory factor analysis (EFA) is an empirical approach to quantifying the underlying soil health characteristics (Zhang *et al.*, 2018). Quantitative analyses based on EFA can then be connected to soil health outcomes and ecosystem processes (Wade *et al.*, 2020). Utilizing EFA, Martin *et al.* (2022) showed that some of the soil bio-indicators, such as soil mineralizable carbon (C), permanganate oxidizable carbon (POXC), soil protein, and enzyme activity can be integrated with the nematode feeding groups. They demonstrated how the nematode feeding groups may be easily integrated into the soil health framework for future soil health assessments.

MOLECULAR TOOLS FOR BETTER EFFICIENCY

Traditional studies of soil nematode communities require the identification of 100–200 nematodes based on morphological features and extrapolating them to the total number of live nematodes recovered from a 100 g representative soil sample. Due to constraints in morphological identification and the larger objectives of functional ecology analyses, traditional assessments typically focus on identifying nematodes only up to the family or genus level. Molecular and high-throughput tools are facilitating swift identification and quantification, extending the analysis of nematode communities up to the species level. This advancement enhances our comprehension of intricate plant-soil interactions (Mathesius and Costa, 2021; Sikder *et al.*, 2021). Several researchers have recently successfully utilized DNA-metabarcoding to characterize nematode communities and to assess soil health (Waeyenberge *et al.*, 2019; Schenk *et al.*, 2020a; Schenk *et al.*, 2020b; Bell *et al.*, 2021).

COMPUTATION OF NEMATODE BIOTIC INDICES

Individual researchers have developed numerous quantitative analyses for nematode communities that have been devised and employed in biological monitoring initiatives. However, the computation of these metrics is intricate. Manual calculations using spreadsheet software are time-consuming and typically demand a substantial learning process. Sieriebriennikov *et al.* (2014) developed ‘NINJA’, an automated system for calculating nematode-biotic indices. ‘NINJA’ code is written in R language and is designed to perform these complex calculations efficiently. This code is compiled into HTML format and made available online. Importantly, it is accessible to all users at no cost and features a user-friendly interface. Utilizing ‘NINJA’ merely requires a table comprising

taxonomic inventory data, and it generates output within a few seconds. Notably, this tool offers the calculation of 40 metrics commonly utilized in nematode-based biological monitoring.

CONCLUSION

Nematodes, being the most ubiquitous and abundant multicellular organisms in soil, play a pivotal role in soil food webs, influencing important ecosystem functions such as nitrogen cycling and plant growth. Consequently, the structure of soil nematode communities harbour significant informational value. Due to their numerous attributes, nematodes serve as valuable ecological indicators for assessing soil health and monitoring ecosystem disturbances. Biotic indices based on nematodes have proven effective in evaluating soil health in both managed and natural ecosystems across diverse geographic regions. However, a primary challenge in utilizing nematodes as bioindicators is the difficulty in species-level identification and the scarcity of taxonomic experts in many places. However, this challenge could be addressed by employing trophic group categorizations and functional guilds, which necessitate only basic taxonomic knowledge. The molecular and high-throughput metabarcoding tools can also aid in identifying nematodes at the species level, particularly in situations where taxonomic experts are unavailable.

In the past, various nematode species and indices were utilized to assess soil health. However, there is a pressing need to develop a single, robust nematode-based index using one or a few cosmopolitan nematode species. This advancement would significantly enhance the utilization of nematodes in biomonitoring of soil health. There is significant potential for developing or integrating nematode indices with pressing contemporary issues like climate change, plastic waste, and electronic waste. Nematodes have heat shock proteins, which are activated when exposed to stressors such as heat, metal

ions, or organic toxins. These proteins could be used as bioindicators to detect toxicants in soil. Achieving these goals requires improved and more robust international collaboration among renowned institutions and laboratories working in this field.

Utilizing nematodes as bioindicators integrates both biotic and abiotic factors, offering insights into the function and structure of soil ecosystems. In India, the predominant focus of nematology research lies in managing plant parasitic nematodes, which contribute to crop losses. However, investigations regarding the application of nematode biotic indices for evaluating soil health are scarce. Hence, there is a pressing need to enhance research efforts in this crucial domain. Nematode community analysis serves as a potent tool that, when combined with traditional physical and chemical assessments of soil, enables a comprehensive understanding of how management practices impact soil health across diverse agro-climatic zones.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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