The Pharma Innovation Journal 2023; 12(9): 1420-1426

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 1420-1426

www.thepharmajournal.com
Received: 08-06-2023
Accepted: 10-07-2023

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Soil nematodes serve as biological indicators for evaluating the soil health: A review

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Abstract
Soil health is critical for sustainable agricultural development, and with increasing social awareness about environmentally friendly agricultural development, soil health demands thorough monitoring. Soil health refers to a soil's ability to perform within ecological boundaries in order to sustain productivity, preserve environmental quality, and promote plant and animal health. Soil health is assessed using physical, chemical, and biological markers; biological indicators include microorganisms, protozoa, and metazoa. Nematodes exhibit a high degree of prevalence among the metazoan kingdom, and their response to contaminants and environmental disruption exhibits considerable variability. Soil nematode populations serve as significant indicators of soil health, as their abundance, diversity, community structure, and metabolic footprint exhibit substantial associations with the soil environment. The dimensions, intricacy, and arrangement of a civilization are indicative of the soil's state. Both free-living and plant-parasitic nematodes serve as valuable ecological indicators, playing significant roles in nutrient cycling and functioning as primary, secondary, and tertiary consumers within food webs. The manipulation of tillage practices, cropping systems, and fertilization strategies can exert a substantial influence on soil nematodes, leading to alterations in the composition and dynamics of soil nematode communities in response to soil perturbations. Given that certain free-living nematodes possess the ability to serve as biological models for investigating soil conditions inside laboratory settings, there is an increasing utilization of soil nematodes as biological indicators for assessing soil health.

Keywords: Biological indicators, community characteristics, soil nematodes, soil health

Introduction

As the global human population continues to grow, there is a corresponding increase in the demand for resources, with a particular emphasis on food. In order to address the growing need for food production, it is imperative to address the detrimental effects of improper soil management practices, such as excessive utilization of chemical fertilizers and pesticides, which pose a significant threat to soil health. Consequently, it is essential to transition towards sustainable agricultural practices in order to ensure long-term viability and productivity. The importance of healthy soils in the context of sustainable agricultural growth has gained significant recognition, leading to a growing trend in the utilization of indicators for the evaluation of soil health. Soil health refers to the ongoing ability of soil to operate as a crucial living entity, operating within the confines of ecosystems and land-use parameters, in order to support biological productivity, preserve the integrity of air and water environments, and enhance the well-being of plants, animals, and humans. The notion of soil health bears a striking resemblance to soil quality, which can be defined as the inherent ability of a particular type of soil to effectively operate within the confines of natural or managed ecosystems. This functionality encompasses the sustenance of plant and animal productivity, the preservation or improvement of water and air quality, and the provision of support for human health and habitation. Please provide more context or information for me to rewrite in an academic manner.

The concept of soil excellence is closely linked to soil function, whereas soil fitness largely pertains to environmental characteristics. Soil fitness can be defined as a vibrant and living system, whose functions are influenced by a diverse range of living species, hence requiring effective management and stewardship [3]. The evaluation of soil excellence involves the consideration of numerous factors. Assessing soil health requires a complete integration of chemical, physical, and biological markers. By examining both trends and typical attributes, the fitness of the soil can be identified [4].

Various techniques are employed for the assessment of soil health, including the Wisconsin Soil Fitness Scorecard [5] and the Cornell Soil Health Evaluation [6]. The primary mediation of soil processes is carried out by living organisms, hence soil fitness can be defined as the capacity of soil to function as an essential living system.

Biological indicators play a crucial role in monitoring soil quality and health. Biological indicators encompass a range of organisms, including bacteria, protozoa, and metazoa. Nematodes, which are the most prevalent form of metazoan [7], inhabit many soil environments. Nematodes exhibit variability in their susceptibility to pollutants and environmental disruptions, and their communities are widely acknowledged as reliable markers of soil quality and health [8]. The presence of soil nematodes, both free-living and plant-parasitic, has been found to be associated with the degree of nitrogen cycling and putrefaction [9]. The presence of free-living nematodes in soil ecosystems has been found to closely resemble the biodiversity and overall health of the soil [10]. Several soil nematode indices are commonly employed to assess the condition of agricultural and natural soils. These indices include the Shannon index, the maturity index (MI) for free-living nematodes, and the plant-parasitic index (PPI) for parasitic nematodes. Indices are utilized to monitor and analyze alterations in land utilization, ecological disruption, and the specific impacts of management strategies.

Soil nematodes are commonly employed for the assessment of forests, grasslands, and agricultural systems [11]. Currently, both morphological and molecular approaches are widely employed for identification purposes [12]. Various soil nematodes have been employed in laboratory settings to assess soil toxicity, with *Caenorhabditis elegans* being the most often utilized [13]. This particular nematode has numerous benefits. The complete sequencing of the genome occurred in 1998, and a multitude of mutant and transgenic strains currently exist [14]. Therefore, *C. elegans* is an ideal organism for evaluating soil conditions. In recent years, there has been a significant increase in the utilization of soil nematodes for the purpose of assessing soil fitness. This trend has been facilitated by advancements in both analytical techniques and detection methodologies.

Indicator and tools usually used to determine soil health include

The assessment of soil fitness can be accomplished by the integration of several physical, chemical, and biological factors. The physical indicators encompass water storage capacity, bulk density, and texture, with water storage capacity being the most commonly utilized [15]. The chemical markers encompass the quantification of total macrobiotic matter/carbon, the presence of phosphorus and potassium, and the measurement of pH [15]. The concept of soil fitness can be broadly characterized as the ability of a soil to effectively perform its functions within the confines of a natural or controlled ecosystem [16]. In this context, biological indicators have been identified as the most suitable means of assessing soil fitness [17]. The aforementioned entities encompass microbes, protozoa, and metazoa. Utilizing soil organisms as markers of soil fitness presents numerous advantages.

The first point highlights the need of being responsive to changes within an organization. The second point emphasizes the strong correlation between this concept and desirable soil function. The third point underscores the usefulness of this concept in clarifying ecosystem processes. Lastly, the fourth point emphasizes that this concept is both understandable and beneficial for land managers. Microorganisms play a crucial role in maintaining soil fertility. There is a positive correlation between microbiological indicators and levels of soil organic carbon/matter as well as vegetation cover. Conversely, there is a negative correlation between microbial indicators and levels of potentially harmful components in the soil [18]. Soil protozoa play a crucial role in nutrient cycling and energy transfer to higher trophic levels, as they are considered the least primary eaters of bacteria and fungus. The authors assert that they facilitate the connection between soil management practices and the soil microbiota in order to enhance soil health [19]. Earthworms are a significant constituent of the soil faunal biomass and play crucial roles in various ecological processes. These include soil formation, water regulation, nutrient cycling, primary production, climate regulation, pollution mitigation, and agricultural services [20]. Soil nematodes have been identified as potentially effective markers of soil health due to their well-established taxonomy and comprehensive understanding of their feeding behavior [9].

Soil nematodes play a crucial role in nutrient cycling and occupy important positions as consumers within soil food webs at multiple trophic levels. The disturbance of soil has a significant impact on the composition of soil nematode communities, while these soil nematodes in turn influence the soil environment, soil organisms, and plant growth. An instance of this phenomenon is observed in the case of free-living nematodes, which have the ability to indirectly enhance plant growth through the modification of the soil microbial community [21].

Soil nematodes as biological health indicators

Soil nematodes are the most abundant metazoans found on Earth. Plants, microorganisms, micro arthropods, and nematodes are present throughout many trophic levels within the soil food web [22]. The assessment of nematode communities' ecological indices provides insights into the alterations occurring in soil ecosystems, both as a result of natural processes and human activities [23]. Nematodes have been utilized as bio indicators for an extended period. The utilization of soil nematodes as indicators of soil suitability has several benefits, and their implementation in soil assessment is increasing [24] owing to their extensive diversity and abundance, as well as their crucial and varied contributions to ecosystem functioning. Soil nematodes have a crucial role in determining soil fitness, as they contribute to the diversity, community structures, and functions within the soil. Various anthropogenic disturbances in agriculture, including as tillage inversion, cropping patterns, and fertilizer management, have the potential to impact soil nematodes [25]. Assessments pertaining to the organization and operation of nematode communities commonly rely on the quantification and variety of meticulous species, genera, or trophic groupings [10].

The ecological and functional properties of nematodes have been identified and utilized as indicators

Soil nematodes have been categorized into many eating groups based on their dietary preferences, including bacterivores, fungivores, herbivores, omnivores, and predators [26]. Soil nematodes have a bacterivorous and fungivores feeding strategy, as they consume bacteria and fungi. Bacterivores and fungivores perform essential roles in the breakdown of organisms within soil ecosystems. Consequently, any changes observed in these species may indicate corresponding adjustments in the pathways of decomposition [27]. Between 60% and 80% of the total population of soil nematodes consists of free-living nematodes, sometimes referred to as helpful nematodes. This category encompasses several feeding habits, including bacterivores, fungivores, omnivores, and predators. All organisms within the soil food web actively engage in nutrient cycling and energy transport processes, thereby contributing to the interconnectedness and vitality of both the web and the soil ecosystem. The presence of augmented microbial activity has the potential to enhance the population of bacterivores, while acidification or stress generated by metals may have the opposite effect by decreasing the number of bacterivores and promoting the proliferation of fungivores [7]. There is a recognized diversity of over 4,100 species of plant-parasitic nematodes, which are known to be herbivorous organisms that feed on plants. The worms that cause the greatest harm are root-knot nematodes (*Meloidogyne spp.*) and cyst nematodes (*Heterodera and Globodera spp.*) [28].

The utilization of nematicides as a means of herbivore control has proven to be an effective approach. However, it is important to note that this method also has the unintended consequence of diminishing the numbers, diversity, and maturation of fungivores and bacterivores [29]. A small proportion of soil nematodes exhibit omnivorous and predatory behavior, consuming bacteria, fungus, protists, and other nematodes through grazing activities. The species under consideration has a heightened sensitivity to environmental change, with a notable disparity in population densities between undisturbed and disturbed soils, as indicated by a study [30]. The augmentation of nitrogen fertilization possesses the capacity to diminish the proportional prevalence of omnivorous organisms and predatory species within wheat fields [31].

In the context of life strategy, soil nematodes have been classified into two groups: Colonizers, also known as r-strategists, and persisters, often referred to as K-strategists [7]. Colonizers demonstrate abbreviated generation intervals, generate a significant quantity of diminutive eggs, and undergo rapid maturation in environments abundant in nutrients. Persisters display an increased physical magnitude, a diminished reproductive yield, an elongated lifetime, and a restricted reaction to temporary episodes of ample food resources. A numerical scale ranging from 1 to 5 has been utilized to categorize nematodes according to their colonizer persister (c-p) characteristics. The c-p 1 group mostly consists of organisms that engage in bacterial consumption and exhibit significant population growth in soil environments rich in microorganisms. Additionally, they possess the capacity to endure stress induced by pollution. The group known as c-p 5 consists primarily of species that display a range of feeding behaviors, including omnivory, predation, and herbivory. These creatures are recognized to be very vulnerable to various environmental pollutants and perturbations [7].

Soil nematode community and diversity indicators

The soil nematode community may be influenced by soil conditions. The features of soil nematode communities encompass several aspects such as their abundance, diversity, community structure, and metabolic footprints. The characteristics of the soil, including its composition, structure, and metabolic activity, will subsequently influence the size, complexity, structure, and metabolic footprint of the soil nematode community. In order to assess these communities, researchers have employed various metrics including species/community measures, diversity measures, and development indices. The metrics used to assess species or community characteristics encompass many measurements such as the count of distinct groups, the absolute quantities of individuals, and the trophic structure based on relative abundances. The Shannon index, the Simpson index, and trophic diversity are three quantitative measures utilized to assess the level of diversity within a given system [10].

Community indexes have been utilized over an extended period to assess and monitor the condition of soil. The indices employed in this study encompass the MI, enrichment index (EI), channel index (CI), structure index (SI), fungivore/bacterivore (F/B) ratio, nematode channel ratio (NCR = B/(B+F)), ratio of specialized plant parasites to bacterivores and fungivores (Pp/(B+F)), and PPI [10, 32].

The metric of microbial biomass, denoted as MI, is calculated as the summation of the product of the c-p values and their corresponding weights for all individuals present in a representative soil sample. There exists an inverse relationship between MI and the degree of soil disturbance. In ecosystems that have seen disturbances and exhibit high nutrient levels, the Mutual Information (MI) metric is observed to be below two. Conversely, in areas where the soil remains undisturbed, the MI value is approximately four. The EI (Ecological Index) provides an assessment of the quantity of food resources and the amount of organic matter present in the soil. These factors have a direct impact on the sensitivity of functional nematode groups [23]. The quantification of bacterivores and fungivores is employed to determine the Compositional Index (CI), which represents the predominant mechanism by which bacteria and fungi decompose organic substances [23]. The calculation of the structural integrity (SI) metric involves the use of a weighting approach to indicators, which takes into consideration the relative significance of functional guilds along postulated paths of structure. This indicates the position of the food web inside the trajectory of the structure [32]. The Plant Parasitic Index (PPI), which serves as the counterpart to the Microbial Index (MI) for plant-consuming nematodes, exhibits a positive correlation with soil disturbance. The alterations in the feeding behavior of nematodes, namely their consumption of bacteria or fungus, are indicative of shifts in the processes of decomposition and the extent of energy flow within ecological networks.

The study conducted by NCR demonstrates the manner in which many primary consumers of waste actively participate in the decomposition process of organic matter within the soil [23]. The numerical scale ranges from 0 to 1, representing a continuum from predominantly fungus-mediated to predominantly bacteria-mediated processes. Organic systems tend to exhibit greater values on this scale. The ratio of primary production to the sum of biomass and fecundity (Pp/(B+F)) serves as an indicator of the contrasting characteristics between debris food webs and grazing food webs. Additionally, this ratio provides insights into the rate at which matter and energy are transferred from autotrophs to heterotrophs. High values suggest that herbivores consume living plant tissue, highlighting the significance of the grazing food web. Conversely, low values show the prominence of the detritus food web, which comprises bacteria and fungus responsible for decomposing deceased organic matter. The user provided a numerical reference [23]. Nematodes exhibit rapid responsiveness to soil fluctuations, hence rendering the composition of the nematode community a viable indicator for assessing soil conditions.

Soil conditions affect the community and diversity of soil nematodes

The composition and diversity of soil nematode communities can be influenced by the management practices employed for soil and crop cultivation. Soil nematodes serve as valuable indicators of soil conditions, particularly in regions characterized by continuous cultivation of crops, as is commonly observed in China [21]. The practice of continuous strawberry cropping has been observed to have a negative impact on the diversity of nematode species. As the nematode community richness (NCR) decreases, there is a noticeable shift in the dominant decomposer organisms from bacteria to fungi. Simultaneously, there is a notable increase in the population of plant-consuming nematodes, indicating a potential degradation of soil quality for sustained crop cultivation [33]. When the banana is replaced with alternative fruits such as papaya, pineapple, or rice, there is an observed rise in the population of bacterivores, fungivores, omnivores, and predators, along with an increase in their functional metabolic footprint (Source: 34). Crop rotation has been found to effectively manage the soybean cyst nematode, scientifically known as Heterodera glycines, through the alternating cultivation of maize and soybean crops [29, 35]. In their study, Zhong *et al.* observed an increase in the abundance of bacterivores, omnivores, and carnivores in agricultural systems characterized by reduced-till, no-till, and the addition of waste materials. The population of fungivores exhibited an increase, while the population of plant pests did not see a corresponding rise [36]. Several studies have indicated that the management approaches employed for tillage can significantly impact soil nematodes (25, 37). The abundance and diversity of nematodes were found to be greater in large soil macroaggregates. The population and abundance of nematodes in the soil exhibited a greater rise in response to no-till and ridge-till practices compared to standard-till practices. The manipulation of soil management practices resulted in alterations in the abundance of nematode-feeding groups within the context of organic soybean cultivation [25].

Root exudates serve as chemical molecules that worms are capable of perceiving through olfaction, whereas soil chemokines function as chemical compounds that nematodes can detect through gustation. Both categories of chemicals have the ability to attract nematodes belonging to various types and feeding groups (Reference 38). The introduction of chemical or organic nutrients exhibited a discernible impact on the soil worm population. The alteration in soil nematode populations serves as an indicator of the impact of nitrogen supplementation on soil health [39]. The application of nitrogen fertilization resulted in a general rise in nematode abundance, while concurrently leading to a reduction in nematode species diversity. An increase in nitrogen fertilization resulted in minimal alterations in the population of bacterivores, but a decline in the abundance of omnivores was observed. The application of high levels of nitrogen fertilizer at a rate of 300 kg ha–1 yr–1 N resulted in a decrease in the abundance of hunters and fungivores [31].

The addition of phosphorus (P) in tropical secondary forests characterized by nutrient-poor soils has been observed to result in a reduction in the overall abundance of nematodes and generalist consumers, hence negatively impacting the structural integrity and trophic interactions within the soil food web [39]. The application of organic fertilizers, such as manure, resulted in an increase in the beneficial metabolic footprints of nematodes. The inhibition of plant-parasitic nematodes and subsequent promotion of plant growth were achieved with the use of organic amendments [40]. Soil nematodes exhibit the combined influence of anthropogenic activities, such as pollution and agricultural practices, as well as the fundamental attributes of natural biocenoses, including vegetation type, spatial distribution, and climatic conditions [23]. Consequently, the utilization of nematode community measures serves as a reliable means to assess the soil's overall health.

Frontier methods of soil nematode community and diversity identification

Nematodes, which encompass a diverse array of taxa, are the predominant creatures found in soil. A comprehensive description has been provided for about 2,270 genera of worms, distributed over 256 families, with approximately 2,300 distinct species [12]. The utilization of morphology and DNA analysis is commonly employed by individuals to determine the taxonomic classification of an organism. The commencement of morphological identification of nematodes can be traced back to the 19th century. Nematodes possess the capability to be classified at the family and genus taxonomic levels, while certain species can be distinguished based on the morphological characteristics exhibited by adult females [41]. However, undertaking such tasks necessitates extensive training and proficiency, entails a significant time investment, and is susceptible to errors due to the absence of discernible characteristics in certain worms.

In recent years, there has been a rapid advancement in the field of molecular recognition, enabling the determination of the gender of a specimen even when it cannot be discerned based on its physical characteristics [42]. Several PCR-based techniques have been developed for various purposes, including PCR-restriction fragment length polymorphism identification, random amplification of polymorphic DNA, PCR single strand conformational polymorphism identification, amplified fragment length polymorphism identification, and PCR-denaturing gradient gel electrophoresis [43]. Quantitative polymerase chain reaction (qPCR) enables precise and real-time measurement of the number of copies generated in each cycle of the PCR process. However, it should be noted that these instruments are somewhat costly and exhibit worse performance compared to high-throughput sequencing (HTS) methods [44].

Despite the widespread use of many PCR-based methods for worm identification, it is important to note that these tests exhibit variations in their characteristics and outcomes. Additionally, it should be noted that PCR-based techniques are limited to the identification of species that have already been characterized. Furthermore, these methods exhibit a sluggish pace and possess a reduced capacity for high-throughput analysis [12]. Quantitative Polymerase Chain Reaction (qPCR)-based methodologies may not be deemed sufficiently reliable in scenarios when the species under investigation remains unidentified and a substantial number of samples are involved. In the year 2005, the commercialization of high-throughput recognition technology became feasible [45].

Metagenomic techniques that specifically focus on the amplification and sequencing of 16S or 18S rRNA gene fragments have the capacity to simultaneously analyze many samples [46]. This method enables the determination and quantification of the relative proportions of all or a subset of species within a diverse population of worms. Metagenetic approaches have gained popularity due to advancements in Sanger sequencing and second-generation sequencing techniques [45]. High-throughput sequencing (HTS) has become widely utilized, and numerous studies have employed HTS techniques to characterize entire assemblages of nematodes. The high-throughput sequencing (HTS) analysis reveals that there was an increase in the population of herbivores, fungivores, and predators/omnivores in the burned plots, whereas the number of bacterivores exhibited a decline. The prevalence of bacterivores was higher in plots characterized by elevated nitrogen levels [42]. Geisen (year) conducted an investigation on soil nematode communities, employing both morphological and molecular techniques, specifically quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS).

The findings indicate that high-throughput sequencing (HTS) demonstrated superior taxonomic clarity and sample throughput [47], despite the comparable outcomes observed across the various methodologies employed.The integration of morphological and genomic methodologies has yielded novel insights. High-throughput sequencing (HTS) demonstrates optimal performance when a substantial number of samples are available. Researchers lacking expertise in morphological identification might utilize High-Throughput Sequencing (HTS) as it eliminates the requirement for extensive knowledge in this field [47]. Nematode community structure can be investigated and evaluated through the utilization of both morphological and molecular techniques. The utilization of nematode community structure holds significant importance in the assessment of soil health.

Caenorhabditis elegans as an example to evaluate soil health

Community diversity and ecological indices are commonly employed to assess the soil's overall health status. For instance, numerous laboratories employ free-living nematodes, predominantly *Caenorhabditis elegans*, as a preliminary approach to assess the condition of the soil [48]. *Caenorhabditis elegans*, commonly referred to as *C. elegans*, possesses several advantageous characteristics that make it a valuable model organism in scientific research. Firstly, its relatively short life span is conducive to studying various biological processes within a manageable timeframe. Additionally, *C. elegans* is known for its ease and cost-effectiveness in cultivation, with each nematode producing a substantial number of eggs (ranging from 300 to 350). Moreover, the availability of its complete genome sequence and the existence of numerous genetically modified strains further enhance its utility as a research tool. Lastly, *C. elegans* exhibits a remarkable responsiveness to environmental changes, enabling investigations into the effects of such fluctuations on its physiology and behavior [49]. The nematode *Caenorhabditis elegans* serves as a valuable model organism for assessing chemical toxicity due to its sensitivity to soil pollution, which hinders its growth and reproductive capabilities [50]. In their study, Kim *et al.* (year) employed a scientific methodology known as the "offspring counting assay" to evaluate the potential chemical toxicity of various metals present in soil [51]. The classification of metals into four groups was based on their respective levels of toxicity, taking into consideration the number of children affected. Additionally, it was discovered that the presence of electrical conductivity and sand fractions positively correlated with the number of offspring, but cation exchange capacity, water holding capacity, and clay and silt fractions exhibited a negative correlation with the number of offspring [52]. The growth, reproduction, and mobility of free-living nematodes, such as *C. elegans*, are influenced by several physical, chemical, and biological variables. These animals can serve as an initial approach to assessing the soil's health.

Future perspectives

The spatial arrangement of nematode colonies provides insights into the characteristics and properties of the soil. An increasing number of individuals are evaluating the soil's quality based on the alterations in the variety and structure of the soil nematode population. Excessive utilization of chemical fertilizers and pesticides, particularly within extended monocropping frameworks, might result in soil degradation. The degradation of soils is increasingly attributed to various factors, including pollution, the decline of physicochemical attributes, the loss of biodiversity, and reduced productivity. Nematodes present in the soil play a crucial role in facilitating the redistribution of nutrients, energy transfer, and global carbon cycling. The acceleration of the decomposition process and mitigation of soil-borne diseases can potentially be achieved by augmenting the population of bacterivores and fungivores, owing to their role in the intricate dynamics of food chains. The removal of herbivorous organisms can facilitate the growth of plants. Furthermore, the utilization of predators with a heightened sensitivity to alterations in their immediate habitat can serve as a means to ascertain the impact of fertilizer on the surrounding ecosystem. The utilization of molecular techniques for the classification of soil worms is expected to enhance the precision of the classification process. *Caenorhabditis elegans*, often known as *C. elegans*, is a nematode worm that exhibits a free-living lifestyle. It is frequently employed as a model organism in laboratory settings to assess soil health and investigate certain environmental impacts.

In summary, it is recommended that soil nematodes be employed with greater frequency as a means of assessing soil health, whereas molecular techniques should be employed more extensively. The utilization of the *C. elegans* model is an initial step towards addressing inquiries pertaining to agriculture and the environment. Significantly, an increasing number of individuals will adopt a comprehensive, cohesive, and refined set of methodologies to enhance agricultural yield and improve nutritional value, while concurrently mitigating the prevalence of nematode diseases in harvested crops.

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