Front. Agr. Sci. Eng. 2020, **7**(3): 275–281
https://doi.org/10.15302/J-FASE-2020327

Available online at http://journal.hep.com.cn/fase

REVIEW

A review of soil nematodes as biological indicators for the assessment of soil health

Qiaofang LU*, **Tongtong LIU***, **Nanqi WANG, Zhechao DOU, Kunguang WANG, Yuanmei ZUO** (✉)

College of Resources and Environmental Sciences; National Academy of Agriculture Green Development; Key Laboratory of Plant-Soil Interactions (Ministry of Education), China Agricultural University, Beijing 100193, China

Abstract Healthy soils are essential for sustainable agricultural development and soil health requires careful assessment with increasing societal concern over environmentally friendly agricultural development. Soil health is the capacity of soil to function within ecological boundaries to sustain productivity, maintain environmental quality, and promote plant and animal health. Physical, chemical and biological indicators are used to evaluate soil health; the biological indicators include microbes, protozoa and metazoa. Nematodes are the most abundant metazoa and they vary in their sensitivity to pollutants and environmental disturbance. Soil nematode communities are useful biological indicators of soil health, with community characteristics such as abundance, diversity, community structure and metabolic footprint all closely correlated with the soil environment. The community size, complexity and structure reflect the condition of the soil. Both free-living and plant-parasitic nematodes are effective ecological indicators, contributing to nutrient cycling and having important roles as primary, secondary and tertiary consumers in food webs. Tillage inversion, cropping patterns and nutrient management may have strong effects on soil nematodes, with changes in soil nematode communities reflecting soil disturbance. Some free-living nematodes serve as biological models to test soil condition in the laboratory and because of these advantages soil nematodes are increasingly being used as biological indicators of soil health.

Keywords biological indicators, community characteristics, soil health, soil nematodes

Received February 12, 2020; accepted March 15, 2020

Correspondence: zuoym@cau.edu.cn

*These authors contribute equally to the work

1 Introduction

As the human population increases, so does the demand for resources, especially food. To meet the increasing demand for food, inappropriate soil management such as intensive use of chemical fertilizers and pesticides poses a hazard to soil health, and modern agriculture must be made sustainable. Healthy soils are essential in sustainable agricultural development and the use of indicators to assess soil health is increasingly popular. Soil health is "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health"[1]. The concept of soil health is very similar to soil quality, which is "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation"[2]. Soil quality is related to soil function, while soil health is primarily an ecological characteristic, and is defined as "a living, dynamic system whose functions are mediated by a diversity of living organisms that require management and conservation"[3]. Established soil quality determination considers many parameters, and soil health assessment requires a comprehensive combination of chemical, physical and biological indicators, and both trends and natural attributes can be discerned for soil health[4]. Many methods are used to assess soil health including the Wisconsin Soil Health Scorecard[5] and the Cornell Soil Health Assessment[6]. As most soil processes are mediated by living organisms, soil health is "the capacity of soil to function as a vital living system". Biological indicators are therefore key when monitoring soil quality and health.

Biological indicators include microbes, protozoa and metazoa. Nematodes, the most abundant type of metazoan[7], live in various types of soils. Nematodes vary in terms of their sensitivity to pollutants and environmental disturbances, and the nematode communities are widely accepted as simple indicators of soil quality and soil health[8]. Soil nematode (free-living and plant-parasitic) are correlated with the extent of nitrogen cycling and decomposition[9]. Free-living nematodes reflect the biodiversity of soil ecosystems and soil health[10]. Many soil nematode indices are used to measure the health of agricultural and natural soils such as the Shannon index, the maturity index (MI) for free-living nematodes and the plant-parasitic index (PPI) for parasitic nematodes. The indices are used to monitor changes in land use, environmental disturbance and the effects of management practices.

Soil nematodes are widely used to evaluate forests, grasslands and agricultural systems[11]. Both morphological and molecular methods are currently the most commonly used for identification[12]. In the laboratory several soil nematodes have been used to evaluate soil toxicity, with *Caenorhabditis elegans* the most widely used[13]. This nematode offers many advantages. The genome was completely sequenced in 1998 and many mutant and transgenic strains are available[14]. *C. elegans* is thus ideal for evaluation of soil conditions. Soil nematodes have recently been widely used for soil health assessment, aided by developments in analytical and identification methods.

2 Common indicators and tools used to assess soil health

As shown in Fig. 1, soil health can be assessed by integrating physical, chemical, and biological data. The physical indicators include water storage capacity, bulk density, and texture, of which water storage capacity is the most frequently used[15]. The chemical indicators include the levels of total organic matter/carbon, available phosphorus and potassium, and pH[15]. Soil health is broadly defined as "the capacity of a living soil to function, within natural or managed ecosystem boundaries"[16] and biological indicators are the most relevant[17]. These include microbes, protozoa and metazoa. Using soil organisms as indicators of soil health has several advantages: (1) sensitivity to changes in management; (2) well correlated with beneficial soil functions; (3) useful for elucidating ecosystem processes; and (4) comprehensible and useful to land managers[4]. Microbes are essential for soil fertility. The microbial indicators are positively related to soil organic carbon/matter levels and vegetation cover, and negatively to soil levels of potentially toxic elements[18]. Soil protozoa are the smallest primary consumers of bacteria and fungi, having essential roles in nutrient cycling and energy transfer to higher trophic levels. They provide the leverage between soil management and the soil microbiota to improve soil health[19]. Earthworms form the largest component of the soil faunal biomass, having important roles in soil formation, water regulation, nutrient cycling, primary production, climate regulation, pollution remediation and agricultural services[20]. Soil nematodes may be the optimum health indicators because their taxonomy and feeding habits are well understood[9]. Soil nematodes contribute to nutrient cycling and have important roles in soil food webs as consumers at various levels. Soil disturbance changes the soil nematode communities and the soil nematodes also exert some effects on the soil environment, soil organisms and the growth of plants. For example, free-living nematodes may promote plant growth indirectly by altering the soil microbial community[21].

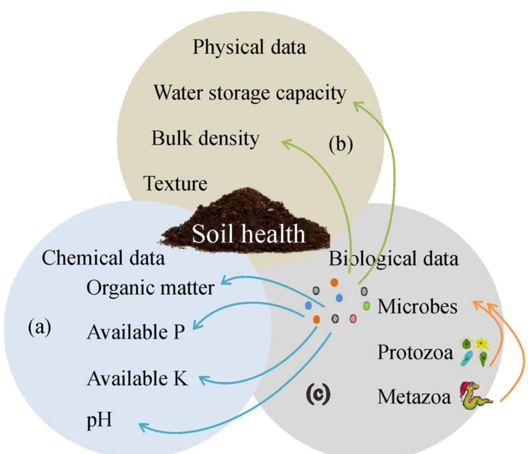

Fig. 1 Common indicators of soil health: (a) microbes are positively related to soil chemical indicators; (b) microbes are essential for soil physical properties; and (c) soil protozoa and several taxa of metazoa feed on microbes.

3 Soil nematodes as biological indicators of soil health

Soil nematodes are the most abundant metazoa on Earth. They are found at all trophic levels of the soil food web in combination with plants, bacteria, microarthropods, and other nematodes[22]. Nematodes have long served as bioindicators and the ecological indices of nematode communities reveal natural and human-induced changes in soil ecosystems[23]. The use of soil nematodes as indicators of health offers many advantages and their use for the assessment of soils is increasing because of their high diversity and abundance and their essential and various roles in ecosystem function[24]. The diversity, community structures and functions of soil nematodes are important indices of soil health; the anthropogenic disturbances in agriculture (tillage inversion, cropping patterns and nutrient management) may influence soil nematodes[25]. Nematode community structure/function evaluation is typically based on the abundance and diversity of certain species, genera or trophic groups[10].

3.1 Ecological and functional characteristics of nematodes determined as indicators

Soil nematodes have been divided by feeding habit into bacterivores, fungivores, herbivores, omnivores and predators[26]. Most soil nematodes are bacterivores and fungivores which feed on bacteria and fungi. They have major roles in the decomposition of organism in soils, and thus changes in bacterivores and fungivores may reflect changes in decomposition pathways[27]. Free-living nematodes (beneficial nematodes), which constitute 60% to 80% of all soil nematodes, include bacterivores, fungivores, omnivores and predators. All engage in nutrient cycling and energy transfer within the soil food web, enhancing that web and soil ecology. Increased microbial activity may enhance the numbers of bacterivores; acidification or metal-induced stress may decrease bacterivore but increase fungivore numbers[7]. Herbivores feed on plants and more than 4100 species of plant-parasitic nematodes have been identified. They cause damage valued at over 80 billion USD annually and the most damaging nematodes are root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.)[28]. Using nematicides to control herbivores is an effective measure but it decreases the populations, diversity, and maturity of fungivores and bacterivores at the same time[29]. Omnivores and predators represent the smaller proportion of soil nematodes, feeding on bacteria, fungi, protist and nematodes. They are sensitive to environmental disturbance and their densities in undisturbed soils are higher than in disturbed soils[30]. Increasing N fertilization may reduce the relative abundance of omnivores and predators in wheat fields[31].

Soil nematodes have also been categorized according to life strategy into colonizers (r-strategists) and persisters (K-strategists)[7]. Colonizers exhibit short generation times, produce many small eggs and quickly exploit nutrient-rich habitats. Persisters have larger bodies, lay fewer eggs, live longer and barely respond to transient availability of high-level food. All nematodes have been placed on a colonizer-persister (c-p) scale from 1 to 5. The c-p 1 group includes principally bacterial feeders exhibiting explosive population growth in microbe-rich soils and tolerance to pollution-induced stress. The c-p 5 group is composed principally of omnivores, predators and plant feeders that are more sensitive to pollutants and other disturbances[7].

3.2 Soil nematode community and diversity indicators

The soil nematode community may be influenced by soil conditions, the community characteristics of soil nematodes including abundance, diversity, community structure and metabolic footprints, and then the soil conditions will be reflected in the size, complexity, structure and metabolic footprint of the soil nematode community. Species/community measures, diversity measures and maturity indices have been used to assess these communities. The species/community measures include the number of taxa, absolute abundances and trophic structure based on relative abundances. Diversity measures include the Shannon index, the Simpson index and trophic diversity[10].

Numerous community indices have been used to assess and monitor soil condition. These include MI, enrichment index (EI), channel index (CI), structure index (SI), the fungivore/bacterivore (F/B) ratio, nematode channel ratio [$NCR = B/(B + F)$], ratio of obligate plant parasites to bacterivores and fungivores [$Pp/(B + F)$] and PPI[10,32]. MI is the weighted mean c-p of all individuals in a representative soil sample. MI is inversely related to the extent of soil disturbance, being less than two in nutrient-enriched disturbed systems, but about four in undisturbed environments. EI reflects food availability and soil organic matter enrichment, in turn reflecting the sensitivities of functional nematode guilds[23]. CI is calculated using the numbers of bacterivores and fungivores, revealing the predominant pathway of organic matter decomposition by bacteria and fungi[23]. SI is calculated using an indicator weighting system based on the importance of the functional guilds along hypothesized trajectories of structure and provides location of the food web along the structure trajectory[32]. PPI, which is an MI equivalent for plant-feeding nematodes, is positively correlated with soil disturbance. Changes in the relative abundances of nematodes feeding on bacteria or fungi mirror changes in decomposition routes and energy channel widths. NCR "indicates the contribution of the various primary consumers of detritus in the process of decomposition of soil organic matter"[23]. The value ranges from 0 to 1 (totally fungus mediated to totally bacteria mediated), with higher values in organic systems. $Pp/(B + F)$ describes the differences between detritus and grazing food webs and yields the matter and energy transfer rates from autotrophs to heterotrophs. High values "indicate the consumption of living plant tissue by herbivores and the dominance of the grazing food web", and low values "indicate the predominance of the detritus food web associated with the decomposition of dead tissue by bacteria and fungi"[23].

The soil nematode community has high and useful information content as nematodes respond rapidly to changes in the soil and therefore the structure of the nematode community can be used as an instrument to assess soil conditions.

3.3 Soil nematode community and diversity changes follow soil conditions

Soil nematode community structure and diversity may be influenced by soil management practices and farming patterns (Fig. 2). Soil nematodes closely reflect soil conditions, including those of agricultural soils subjected to continuous cropping, a common situation in China[21].

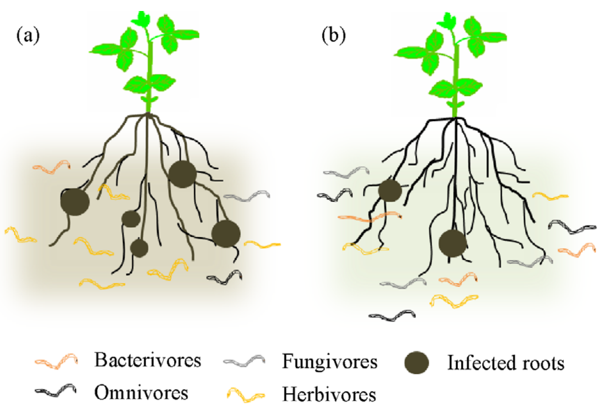

Fig. 2 The soil nematode diversity in (a) continuous cropping soil is lower than in (b) rotation cropping soil.

Continuous strawberry cropping reduces nematode diversity, the predominant decomposition pathway changing from bacterial to fungal as NCR decreases, whereas plant-feeder abundance increases significantly, showing that the soil may be harmful to plant growth under continuous cropping[33]. When banana is rotated with papaya, pineapple or rice, the abundance and functional metabolic footprint of bacterivores, fungivores and omnivore-carnivores were found to increase[34]. Maize-soybean rotation is an agricultural practice in which soybean cyst nematode, *Heterodera glycines*, is effectively controlled[29,35]. Zhong et al. found that reduced-till, no-till and added-residue patterns increased the numbers of bacterivores, omnivores, and carnivores. The latter pattern also increased fungivore numbers but not those of plant parasites[36]. Some studies indicate that soil nematodes may be strongly influenced by tillage management practices[25,37]. Both abundance and diversity were higher in large soil macroaggregates, and soil nematode activity and abundance increased more under no-tillage and ridge-tillage than when standard tillage was employed. In organic soybean production the abundance of all nematode feeding groups was affected by the tillage system[25].

Root exudates act as olfactory compounds and soil chemokines as taste compounds and are nematode attractants across different nematode taxa and feeding guilds[38]. Adding nutrients (chemical/organic) directly influenced the soil nematode community, thus changes in soil nematodes reflect the effects of nutrient applications on soil health[39]. Nitrogen fertilization increased total nematode abundance but decreased nematode generic richness. Increasing N fertilization resulted in minor changes in bacterivores but decreased the abundance of omnivores and predators, and fungivores were suppressed by high N fertilization (300 $kg \cdot ha^{-1} \cdot yr^{-1}$ N)[31]. In tropical secondary forests (P-poor soils), P additions suppresses the density of total nematodes and omnivore-predators and degrade the structure and trophic links of the soil food web[39]. The application of an organic fertilizer such as manure increased the nematode functional metabolic footprints. Plant-parasitic nematodes were controlled by adding organic amendments and then promoted plant growth[40].

Soil nematodes reflect the consequences of anthropogenic interventions (environmental pollution and agricultural activities) and the principal features of natural biocenoses (vegetation type, geographic location and climatic conditions)[23]. The nematode community indices therefore allow useful assessment of soil health.

3.4 Frontier methods of soil nematode community and diversity identification

Nematodes are the most abundant organisms in soils and their communities are very diverse. Over 2270 nematode genera in 256 families containing about 2300 species have been fully described[12]. Morphological and molecular identification are the most common methods (Fig. 3(a)). Morphological identification commenced in the nineteenth century, and nematodes can be identified to the family and genus levels, and some species may be identified based on the morphological features of adult females[41]. However, such work requires extensive training and expertise and is both time consuming and error prone because some nematodes lack distinctive morphological features.

Molecular identification has recently evolved rapidly, identifying specimens that cannot be identified morphologically because they are male or female sub-adults[42]. The PCR-based methods include 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]. qPCR allows accurate real-time measurement of the number of copies synthesized during each PCR cycle. However, these are costly and low-throughput tools compared to high-throughput sequencing (HTS)[44]. Although all these PCR-based methods are widely used to identify nematodes the tests are not standardized. Also, PCR-based techniques identify only known species and are low-throughput, slow techniques[12]. If the species are unknown and the sample numbers high, qPCR-based methods are inadequate. High-throughput identification was commercialized in 2005[45]. Metagenetic approaches can be used to assay many samples simultaneously, with 16S or 18S rRNA gene fragments targeted[46]. The method identifies and quantifies the relative abundances of all or some species in a complex nematode population. Metagenetic approaches are now common, given the developments in Sanger sequencing and second-generation sequencing techniques[45]. HTS is now routine. Many nematode studies have used HTS to define entire nematode communities.

HTS shows that bacterivore numbers were reduced, and those of herbivores, fungivores and predators/omnivores increased in burnt compared to unburnt plots. Bacterivores were more abundant in nitrogen-enriched plots[42]. Geisen used morphological and molecular (qPCR and HTS) methods to analyze soil nematode communities and the results show that HTS afforded the highest taxonomic resolution and sample throughput[47], although the various methods yielded similar outcomes.

Combined use of morphological and molecular methods has afforded new insights. When sample numbers are large, HTS is optimal. HTS does not require expertise in morphological identification, and is therefore more feasible for scientists with little knowledge of morphological identification[47]. Both the morphological and molecular methods can effectively identify and analyze nematode community structure, and this has a critical role in evaluating soil health using the soil nematode community structure.

3.5 *Caenorhabditis elegans* as an example to evaluate soil health

Soil nematode data (community diversity and the ecological indices) can be used to assess soil condition (Fig. 3(b)). For example, many laboratories use free-living nematodes (principally *C. elegans*) for preliminary assessment of soil condition[48]. The advantages of *C. elegans* are (1) a short lifespan, (2) simple and inexpensive cultivation (300–350 eggs per nematode), (3) availability of the complete genome sequence and many genetically modified strains, and (4) a rapid response to environmental change[49]. *C. elegans* can be used to rapidly assess chemical toxicity, with soil pollutants inhibiting *C. elegans* growth and reproduction[50]. Kim et al. used an offspring counting assay as a bioassay for soil ecotoxicity evaluation to evaluate the chemical toxicity of several metals[51]. The toxicity trend for each metal was classified according to the number of offspring. They also found that cation exchange capacity, water holding capacity, and clay and silt fractions reduced the numbers of offspring, whereas the electrical conductivity and sand fractions increased the numbers[52]. Physical, chemical and biological parameters affect the growth, reproduction and movement of free-living nematodes such as *C. elegans*, and these organisms can be used for the preliminary evaluation of soil condition.

4 Future perspectives

Nematode community structure reflects soil condition. Evaluating soil quality based on changing diversity and structure of the soil nematode community is becoming more popular. Unhealthy soils are associated with the excessive use of chemical fertilizers and pesticides, especially in long-term monocropping systems. Soils are increasingly subject to degradation, pollution, deterioration in physicochemical properties, reduced biodiversity and low productivity. Soil nematodes are involved in nutrient cycling, energy transfer and global soil carbon cycling. Increasing bacterivore and fungivore abundance may enhance the decomposition of soil organisms and reduce soilborne pathogen impacts because of their trophic structure. Lowering herbivore abundance can promote plant growth. Also, predators which are sensitive to environmental change can be used to evaluate fertilizer impacts (Fig. 4). Classifying soil nematodes using molecular approaches will increase the accuracy rate. *C. elegans* is a free-living nematode that can be used to evaluate soil health and some environmental impacts under laboratory conditions. In summary, the use of soil nematodes for soil health assessment may be more widely adopted and particularly molecular approaches can be used more widely. Application of the *C. elegans* model to address agricultural and ecological questions is just the beginning. Importantly, a set of comprehensive integrated and optimized approaches to increase crop productivity and nutritional quality while decreasing nematode disease in harvested products will become increasingly widely used.

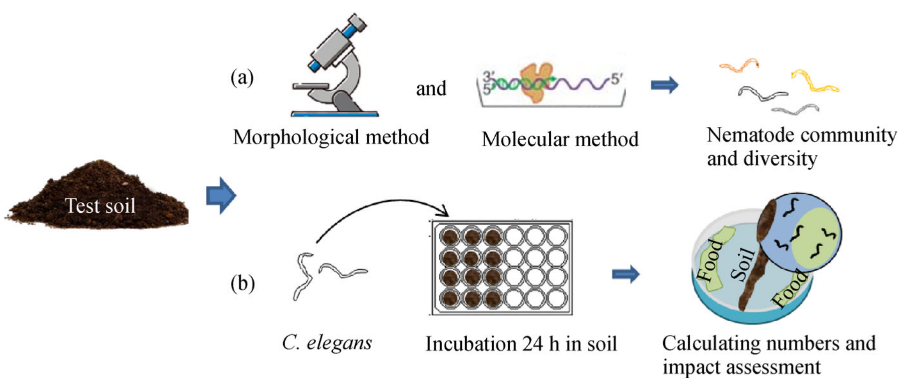

Fig. 3 Methods for soil health assessment: (a) determination of soil nematode community and diversity; and (b) evaluation of soil health with the nematode *Caenorhabditis elegans*.

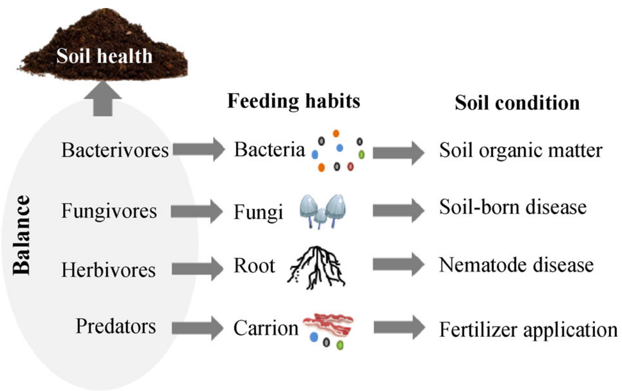

Fig. 4 Soil nematodes are valuable bioindicators of soil health due to their regulated balance between plants and microorganisms in the rhizosphere ecosystem.

Acknowledgements This study was supported by the National Key Research and Development Program of China (2017YFD0202102, 2016YFD0200405, and 2016YFE0101100).

Compliance with ethics guidelines Qiaofang Lu, Tongtong Liu, Nanqi Wang, Zhechao Dou, Kunguang Wang, and Yuanmei Zuo declare that they have no conflicts of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Doran J W, Sarrantonio M, Liebig M A. Soil health and sustainability. *Advances in Agronomy*, 1996, **56**: 1–54
2. Karlen D L, Mausbach M J, Doran J W, Cline R G, Harris R F, Schuman G E. Soil quality: a concept, definition, and framework for evaluation. *Soil Science Society of America Journal*, 1997, **61**(1): 4–10
3. Doran J W, Zeiss M R. Soil health and sustainability: managing the bioticcomponent of soil quality. *Applied Soil Ecology*, 2000, **15**(1): 3–11
4. Mann C, Lynch D, Fillmore S, Mills A. Relationships between field management, soil health, and microbial community composition. *Applied Soil Ecology*, 2019, **144**: 12–21
5. Romig D E, Garlynd M J, Harris R F, Mcsweeney K. How farmers assess soil health and quality. *Journal of Soil and Water Conservation*, 1995, **50**(3): 229–236
6. Moebius-Clune B N. Comprehensive assessment of soil health: the Cornell framework manual. New York, USA: *Cornell University*, 2016
7. Bongers T, Bongers M. Functional diversity of nematodes. *Applied Soil Ecology*, 1998, **10**(3): 239–251
8. Griffiths B S, Römbke J, Schmelz R M, Scheffczyk A, Faber J H, Bloem J, Pérès G, Cluzeau D, Chabbi A, Suhadolc M, Sousa J P, Martins da Silva P, Carvalho F, Mendes S, Morais P, Francisco R, Pereira C, Bonkowski M, Geisen S, Bardgett R D, de Vries F T, Bolger T, Dirilgen T, Schmidt O, Winding A, Hendriksen N B, Johansen A, Philippot L, Plassart P, Bru D, Thomson B, Griffiths R I, Bailey M J, Keith A, Rutgers M, Mulder C, Hannula S E, Creamer R, Stone D. Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function. *Ecological Indicators*, 2016, **69**: 213–223
9. Neher D A. Role of nematodes in soil health and their use as indicators. *Journal of Nematology*, 2001, **33**(4): 161–168
10. Urzelai A, Hernández A J, Pastor J. Biotic indices based on soil nematode communities for assessing soil quality in terrestrial ecosystems. *Science of the Total Environment*, 2000, **247**(2–3): 253–261
11. Liu J S, Chen Y, Du C, Liu X X, Ma Q H, Zhang X, Wang D L. Interactive effects of nitrogen addition and litter on soil nematodes in grassland. *European Journal of Soil Science*, 2019, **70**(3): 697–706
12. Seesao Y, Gay M, Merlin S, Viscogliosi E, Aliouat-Denis C M, Audebert C. A review of methods for nematode identification. *Journal of Microbiological Methods*, 2017, **138**: 37–49
13. Nigon V M, Félix M A. History of research on *C. elegans* and other free-living nematodes as model organisms. *WormBook*, 2017, **2017**: 1–84
14. Hunt P R. The *C. elegans* model in toxicity testing. *Journal of Applied Toxicology*, 2017, **37**(1): 50–59
15. Bünemann E K, Bongiorno G, Bai Z G, Creamer R E, De Deyn G, de Goede R, Fleskens L, Geissen V, Kuyper T W, Mäder P, Pulleman M, Sukkel W, van Groenigen J W, Brussaard L. Soil quality—a critical review. *Soil Biology & Biochemistry*, 2018, **120**: 105–125
16. Doran J W. Soil health and global sustainability: translating science into practice. *Agriculture, Ecosystems & Environment*, 2002, **88**(2): 119–127
17. Stone D, Costa D, Daniell T J, Mitchell S M, Topp C F E, Griffiths B S. Using nematode communities to test a European scale soil biological monitoring programme for policy development. *Applied Soil Ecology*, 2016, **97**: 78–85
18. Niemeyer J C, Lolata G B, Carvalho G, Da Silva E M, Sousa J P, Nogueira M A. Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil. *Applied Soil Ecology*, 2012, **59**: 96–105
19. Xiong W, Jousset A, Guo S, Karlsson I, Zhao Q, Wu H, Kowalchuk G A, Shen Q, Li R, Geisen S. Soil protist communities form a dynamic hub in the soil microbiome. *ISME Journal*, 2018, **12**(2): 634–638
20. Blouin M, Hodson M E, Delgado E A, Baker G, Brussaard L, Butt K R, Dai J, Dendooven L, Peres G, Tondoh J E, Cluzeau D, Brun J J. A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 2013, **64**(2): 161–182
21. Neher D A. Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology*, 2010, **48**(1): 371–394
22. Ferris H. Contribution of nematodes to the structure and function of the soil food web. *Journal of Nematology*, 2010, **42**(1): 63–67
23. Matveeva E M, Sushchuk A A. Features of soil nematode communities in various types of natural biocenoses: effectiveness of assessment parameters. *Biological Bulletin*, 2016, **43**(5): 474–482
24. Mulder C, Schouten A J, Hund-Rinke K, Breure A M. The use of nematodes in ecological soil classification and assessment concepts.

Ecotoxicology and Environmental Safety, 2005, **62**(2): 278–289
25. Ito T, Araki M, Komatsuzaki M, Kaneko N, Ohta H. Soil nematode community structure affected by tillage systems and cover crop managements in organic soybean production. *Applied Soil Ecology*, 2015, **86**: 137–147
26. Yeates G W, Bongers T, De Goede R G M, Freckman D W, Georgieva S S. Feeding habits in soil nematode families and genera-an outline for soil ecologists. *Journal of Nematology*, 1993, **25**(3): 315–331
27. Schratzberger M, Holterman M, van Oevelen D, Helder J. A worm's world: ecological flexibility pays off for free-living nematodes in sediments and soils. *Bioscience*, 2019, **69**(11): 867–876
28. Jones J T, Haegeman A, Danchin E G J, Gaur H S, Helder J, Jones M G K, Kikuchi T, Manzanilla-López R, Palomares-Rius J E, Wesemael W M L, Perry R N. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 2013, **14**(9): 946–961
29. Grabau Z J, Chen S Y. Influence of long-term corn–soybean crop sequences on soil ecology as indicated by the nematode community. *Applied Soil Ecology*, 2016, **100**: 172–185
30. Ferris H, Bongers T. Nematode indicators of organic enrichment. *Journal of Nematology*, 2006, **38**(1): 3–12
31. Song M, Jing S S, Zhou Y Q, Hui Y, Zhu L L, Wang F, Hui D F, Jiang L, Wan S Q. Dynamics of soil nematode communities in wheat fields under different nitrogen management in Northern China Plain. *European Journal of Soil Biology*, 2015, **71**: 13–20
32. Tsiafouli M A, Bhusal D R, Sgardelis S P. Nematode community indices for microhabitat type and large scale landscape properties. *Ecological Indicators*, 2017, **73**: 472–479
33. Li X, Lewis E E, Liu Q, Li H, Bai C, Wang Y. Effects of long-term continuous cropping on soil nematode community and soil condition associated with replant problem in strawberry habitat. *Scientific Reports*, 2016, **6**(1): 30466
34. Zhong S, Zeng H C, Jin Z Q. Response of soil nematode community composition and diversity to different crop rotations and tillage in the tropics. *Applied Soil Ecology*, 2016, **107**: 134–143
35. Hu W, Strom N, Haarith D, Chen S, Bushley K E. Mycobiome of cysts of the soybean cyst nematode under long term crop rotation. *Frontiers in Microbiology*, 2018, **9**: 386
36. Zhong S, Zeng H C, Jin Z Q. Influences of different tillage and residue management systems on soil nematode community composition and diversity in the tropics. *Soil Biology & Biochemistry*, 2017, **107**: 234–243
37. Zhang S X, Li Q, Lü Y, Zhang X P, Liang W J. Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. *Soil Biology & Biochemistry*, 2013, **62**: 147–156
38. Dong L, Li X, Huang L, Gao Y, Zhong L, Zheng Y, Zuo Y. Lauric acid in crown daisy root exudate potently regulates root-knot nematode chemotaxis and disrupts Mi-flp-18 expression to block infection. *Journal of Experimental Botany*, 2014, **65**(1): 131–141
39. Zhao J, Wang F M, Li J, Zou B, Wang X L, Li Z A, Fu S L. Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest. *Soil Biology & Biochemistry*, 2014, **75**: 1–10
40. Ferji Z, Mayad E H, Alfalah M. Management of root knot nematode affecting banana crop by using organic amendment and biological products. *Biology Agriculture and Healthcare*, 2013, **3**(17): 82–85
41. Gasser R B, Bott N J, Chilton N B, Hunt P, Beveridge I. Toward practical, DNA-based diagnostic methods for parasitic nematodes of livestock—bionomic and biotechnological implications. *Biotechnology Advances*, 2008, **26**(4): 325–334
42. Darby B J, Todd T C, Herman M A. High-throughput amplicon sequencing of rRNA genes requires a copy number correction to accurately reflect the effects of management practices on soil nematode community structure. *Molecular Ecology*, 2013, **22**(21): 5456–5471
43. Pontes T, D'Amelio S, Costa G, Paggi L. Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. *Journal of Parasitology*, 2005, **91**(6): 1430–1434
44. Li Y, Lawrence G W, Lu S, Balbalian C, Klink V P. Quantitative field testing Heterodera glycines from metagenomic DNA samples isolated directly from soil under agronomic production. *PLoS One*, 2014, **9**(2): e89887
45. Schadt E E, Turner S, Kasarskis A. A window into third-generation sequencing. *Human Molecular Genetics*, 2010, **19**(R2): R227–R240
46. Esposito A, Kirschberg M. How many 16S-based studies should be included in a metagenomic conference? It may be a matter of etymology. *FEMS Microbiology Letters*, 2014, **351**(2): 145–146
47. Geisen S, Snoek L B, ten Hooven F C, Duyts H, Kostenko O, Bloem J, Martens H, Quist C W, Helder J A, van der Putten W H, Kembel S. Integrating quantitative morphological and qualitative molecular methods to analyse soil nematode community responses to plant range expansion. *Methods in Ecology and Evolution*, 2018, **9**(6): 1366–1378
48. Tejeda-Benitez L, Olivero-Verbel J. Caenorhabditis elegans, a biological model for research in toxicology. *Reviews of Environmental Contamination and Toxicology*, 2016, **237**: 1–35
49. Bouyanfif A, Jayarathne S, Koboziev I, Moustaid-Moussa N. The nematode *Caenorhabditis elegans* as a model organism to study metabolic effects of ω-3 polyunsaturated fatty acids in obesity. *Advances in Nutrition*, 2019, **10**(1): 165–178
50. Höss S, Jänsch S, Moser T, Junker T, Römbke J. Assessing the toxicity of contaminated soils using the nematode *Caenorhabditis elegans* as test organism. *Ecotoxicology and Environmental Safety*, 2009, **72**(7): 1811–1818
51. Kim S W, Moon J, An Y J. A highly efficient nonchemical method for isolating live nematodes (*Caenorhabditis elegans*) from soil during toxicity assays. *Environmental Toxicology and Chemistry*, 2015, **34**(1): 208–213
52. Kim S W, Moon J, Jeong S W, An Y J. Development of a nematode offspring counting assay for rapid and simple soil toxicity assessment. *Environmental Pollution*, 2018, **236**: 91–99