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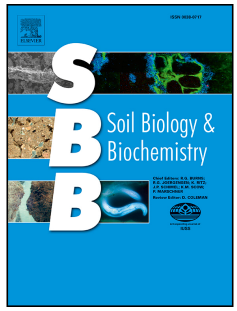

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NEMATODE-BASED INDICES IN SOIL ECOLOGY: APPLICATION, UTILITY, AND FUTURE DIRECTIONS

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ABSTRACT

The health and functioning of soil ecosystems are the foundation of sustainable food production and land management. Of key importance in achieving sustainability, is the frequent measurement of soil health, and indices based on the community structure of nematodes are amongst the most widely used toolsets by soil ecologists. Thirty years after the development of the Maturity Index, we aimed to evaluate the application, utility, and future directions of nematode-based indices (NBIs). This review focused on NBIs that are calculated using the coloniser-persister classification of nematodes. Data from 672 empirical studies in terrestrial environments revealed that the NBIs presented a dissimilar usage trend. The Channel Index and Metabolic Footprints showed the strongest increase in application rates over time, thus indicating a greater interest in studying decomposition pathways and ecosystem functioning, respectively. Furthermore, nematode-based indices were mostly applied in agricultural systems associated with herbaceous crops and in studies investigating, for example, soil nutrient enrichment following manure and/or inorganic fertilizer application. We further provide a framework for selecting a focus-orientated subset of NBIs for testing hypotheses based on the underlying ecological mechanisms. Also, we highlight important considerations, including the unexpected behaviour of some nematode taxa, in the interpretation of NBIs. The improvement of NBIs relies on advancing our understanding of the autecology of nematodes. Finally, we deliver insight into the further development of NBIs considering recent methodological advancements. We highlight that NBIs have been and might become increasingly important in providing valuable information on soil ecosystem health and functioning, especially considering the urgent need for more sustainable land use.

Key words: soil health, ecosystem functioning, food web status, faunal analysis, molecular approaches

1. INTRODUCTION

Soil life is represented by myriad microorganisms that include microbiota (e.g. bacteria, fungi and protists), microfauna (e.g. nematodes), mesofauna (e.g. microarthropods and potworms), and macrofauna (e.g. earthworms) (Kibblewhite et al., 2008; Brussaard, 2012). However, these organismal groups do not exist in isolation, but form part of a complex network in the soil, i.e. the soil food web (Richter et al., 2019). A structured food web that facilitates energy flow is characteristic of a healthy and functioning soil ecosystem, which is pivotal in the delivery of services that include water storage, erosion control, and the production of food and fibre (Kibblewhite et al., 2008). The status of the soil food web is therefore an important consideration in sustainable land management (Bünemann et al., 2018).

Amongst the most widely used bioindicator groups of soil ecosystems are nematodes, which are multicellular, aquatic organisms that inhabit water films surrounding soil particles (Ferris et al., 2001). Nematodes are useful in measuring changes in the function and status of soils, due to their ubiquitous distribution and occupation of a wide range of habitats, as well as being representative of multiple trophic levels in the soil food web. They also reflect changes in terrestrial habitats due to their rapid response to environmental and anthropogenic disturbances (Bongers, 1990; Yeates and Bongers, 1999; Ferris et al., 2001; Zhang et al., 2020). Furthermore, nematodes can be extracted using simple methods (Marais et al., 2017), while the Nematode Indicator Joint Analysis (NINJA) online tool (Sieriebriennikov et al., 2014) eases the calculation of ecological indices. Consequently, nematodes are considered valuable indicators of soil ecosystem health (Ferris and Bongers, 2009; Sánchez-Moreno and Ferris, 2018).

The development of ecological indices based on the life history traits of nematode communities [hereinafter referred to as nematode-based indices (NBIs)] accelerated with the work of Tom Bongers, who originally conceived the idea of the Maturity Index (MI) (Bongers, 1990). Over the years, alterations were made to the MI and additional indices developed (Text Box 1), which resulted in a useful and widely applied framework for soil ecosystem assessments (Ferris and Bongers, 2009; Sánchez-Moreno and Ferris, 2018). This toolset has been extensively used to measure ecosystem health and functioning in a wide range of terrestrial habitats under varying land use systems (Ito et al., 2015; Zhong et al., 2017; Sánchez-Moreno et al., 2018; Jansen van Rensburg, 2020; Tsiafouli et al., 2020) and environmental conditions (Hua et al., 2009; Kitagami and Matsuda, 2020).

Thirty years after the introduction of the MI, we undertook this review as a tribute to Tom Bongers and his contribution to science by evaluating the application, utility, and future directions of NBIs. More specifically, this review aimed at 1) evaluating the application of NBIs to date in different land use and land cover systems, while also considering the relevant study foci, 2) discussing the selection, interpretation, and potential limitations of an appropriate, focus-orientated subset of NBIs, and 3) delivering insight into the potential further development of NBIs considering recent advancements in molecular techniques.

2. METHODS

2.1 Selection of nematode-based indices

This review focused on NBIs that are calculated using the coloniser-persister (cp) classification system (often coupled with trophic grouping) based on the life history traits assigned at nematode family (and sometimes genus) level (Yeates et al., 1993; Ferris et al., 2001). Coloniser-persister values range from 1 to 5, with cp 1 representing r-selected colonisers (short generation times; large population fluctuations; high fecundity; and resistant to adverse environmental conditions) and cp 5 representing K-selected persisters (producing few offspring; generally appearing later in succession; and sensitive to environmental disturbance) (Bongers and Bongers, 1998; Bongers and Ferris, 1999). Nematode trophic groups, in turn, include herbivores, bacterivores, fungivores, omnivores, and predators. Plant-parasitic (herbivorous) nematodes are classified using the same life history trait system, but their classification is denoted as the ‘pp’ range. The studied indices included the Maturity Index (MI), Maturity Index 2-5 (MI2-5), Plant-Parasitic Index (PPI), Sigma Maturity Index (∑MI), Enrichment Index (EI), Structure Index (SI), Channel Index (CI), Basal Index (BI), and Metabolic Footprints (MFs), as well as the coloniser-persister (cp) triangle and faunal analysis (De Goede et al., 1993a; Yeates, 1994; Korthals et al., 1996b; Ferris et al., 2001; Ferris, 2010).

2.2 Literature survey

The literature survey was undertaken using the Web of Science Core Collection database. We identified three scientific papers, namely Bongers (1990), Ferris et al. (2001), and Ferris (2010), that serve as the foundation and main reference works for scientists and researchers that report on the application of NBIs. Using the Cited Reference Search tool, a search was conducted for works (up until the end of 2020; last search performed 31 March 2021) that cited each of the above-mentioned scientific papers. This returned 1 113, 653, and 122 citations, respectively, for the Bongers (1990), Ferris et al. (2001), and Ferris (2010) papers. The results were combined, duplicates removed, and further refined to include only peer-reviewed scientific articles. Furthermore, all marine and freshwater studies were excluded. Finally, a total of 1 199 papers was included in the analyses.

The next step was to screen each paper to assess whether it met the following basic criteria: 1) empirical study (therefore not a review or meta-analysis), 2) application of at least one NBI, and 3) focused on terrestrial habitat(s). A total of 672 scientific papers met these criteria and, from them, the following information was extracted and recorded: i) which NBIs were applied, ii) location information and spatial scale of the study site(s), iii) study focus, and iv) land use and land cover information [see Table S1 - supplementary material or Du Preez et al., (2021) or https://doi.org/10.5073/20211217-170559].

The study foci were identified based on the knowledge and experience of the authors. The land use and land cover criteria, in turn, were based on SEEA (2012), which defines land use as '*the activities undertaken and the institutional arrangements put in place for a given area for the purposes of economic production, or the maintenance and restoration of environmental* *functions*'. Land use therefore classifies all areas under human management according to its use into five categories namely: 'forestry', 'not in use', 'agriculture', 'built-up and related', and 'maintenance and restoration' (SEEA, 2012). Land cover, in turn, is defined by SEEA (2012) as '*the observed physical and biological cover of the Earth's surface and includes natural vegetation and abiotic (non-living) surfaces*'. Six land cover categories were recognized, namely 'sparse', 'grassland', 'tree-covered', 'shrub-covered', 'herbaceous crops', and 'woody and multi crop' (SEEA, 2012). The 'sparse' category represents both barren terrestrial land and sparsely vegetated land. Further information on each land use and land cover category is provided in the section 3.3.

Although reviews were excluded from this systematic review, some relevant syntheses were previously published. Neher (2001) summarized the characteristics, utility, and ecological meaning of NBIs proposed until then, highlighting the necessity to refine and develop existing and new indices to improve our understanding of soil functioning based on the study of nematode communities. Other relevant reviews include those by Wasilewska (1997), Boag and Yeates (1998), Bongers and Ferris (1999), and Mulder et al. (2005). All of them provided relevant insight into the ecological relevance of using nematodes as environmental indicators.

2.3 Geographic maps

Maps illustrating the location of the study sites, as well as the number of scientific papers published per country, were created in ArcGIS version 10.2 (ESRI, 2013). Only one point was added to the map in areas where the sites were in close local proximity. Proportional land use and land cover categories were also indicated with pie charts on their respective maps. To avoid false impressions through visual inspection of the pie charts on the maps, in the cases where countries were represented by fewer than five scientific papers, the pie charts were outlined in red. In some cases, multiple land use and/or land cover categories were studied in a single paper.

2.4 Statistical analyses

The application rates of NBIs were visualised and studied using regression models, which were created using R Studio version 4.0.2 (RStudioTeam, 2020). Generalized linear models (GLMs) were used to study the development and application of NBIs over time (Crawley, 2007). The model family, i.e. Gaussian or Poisson, was selected based on the lowest Akaike Information Criterion (AIC) following visual evaluation of homoscedasticity and distribution using qq-plots (Motulsky and Christopoulos, 2004). We used F-tests (Gaussian family) and chi-squared tests (Poisson family) to study the significance of the regression lines and curves, respectively. Regression lines and curves were drawn based on the respective model parameters (Motulsky and Christopoulos, 2004). The package "rsq" was used to calculate r-squared values of the regressions (Zhang, 2020).

3. APPLICATION OF NEMATODE-BASED INDICES

3.1 Over a temporal scale

Since the introduction of the MI and PPI (Text Box 1), the annual citation rate of both indices has increased steadily (Figure 1). Noticeable, however, is the relatively lower application rate of the PPI, which is possibly due to its limited explanatory power of actual damage potential. The PPI is based on biological features, such as life cycle characteristics and reproduction rates of plant-parasitic nematodes (Bongers, 1990). Although this index was not originally designed to measure pathogenicity, plant pathologists are typically more interested in damage potential and infection rates for crops.

The ∑MI was not widely applied by nematode ecologists (Figure S1). The reason may be the opposed response of cp and pp nematodes (note that although cp values can be assigned both to non-parasitic and parasitic nematodes, pp specifically refers to cp values assigned to plant-parasitic nematodes). For example, under enriched agricultural conditions, the PPI might increase due to the larger abundances of plant-parasitic species with a long stylet (pp 3 nematodes) that feed on nutrient rich cells in deeper root tissue (e.g. cortex and endodermis) (Bongers and Bongers, 1998). In contrast, the MI decreases due to a higher number of enrichment opportunists (cp 1 nematodes) that profit from bacterial breakdown of nutrient rich crop residues (Bongers et al., 1997; Briar et al., 2012). When considering the MI2-5, a slight exponential increase in citation frequency (Figure S1) was recorded during the past 10 years.

The cp triangle presented a similar citation frequency trend as the ∑MI and has rarely been used by scientists in the past 30 years (Figure S1). This is likely in part due to preferred use of the widely applied faunal analysis. In fact, the faunal analysis, and therefore also the EI and SI, currently show the greatest adoption rate of NBIs with a strong, linear increase (Figure 1) since the publication of Ferris et al. (2001). The ease of drawing (e.g. using Microsoft Excel) and interpretation of the faunal analysis diagrams likely contributed to its popularity. This may further explain why the use of the EI and SI also exceeded that of the MI since the year 2014. In contrast, the direct use of the BI has always been substantially lower (Figure S1). Since the BI is inversely linked with the SI and EI, its use may appear redundant, which potentially explains the BI's low application rate.

Both the CI and MFs showed exponential increases since the publication of Ferris et al. (2001) and (Ferris, 2010), respectively. The CI is popular among soil ecologists due to its value in studying bacterial and fungal decomposition pathways without the need for expensive and highly specific laboratory equipment (e.g. measurement of phospholipid fatty acids using chromatography) (Briar et al., 2011). Metabolic Footprints, in turn, are often well correlated with soil organic carbon (Luo et al., 2021), while in agricultural systems, the number of bacterivores, and therefore also their footprint, present positive links with microbial biomass and microbial respiration (Schmidt et al., 2020). The CI and MFs indices provide indirect measures of ecosystem functionality, such as degradation processes, nutrient turn-over rates, water storage, or soil suppressiveness towards pests and diseases and hence, rapidly earned a reputable place in the toolbox of modern soil ecologists. Ultimately, the adoption and application of NBIs substantially increased following the development of especially the food web diagnostic indices (i.e., EI, SI, BI and CI), as well as the MFs.

3.2 Over a spatial scale

Scientific studies that utilized at least one NBI were recorded from every continent on Earth, but a clear agglomeration was evident in the northern hemisphere, particularly in China, Europe, and the United States of America (Figure 2). Especially China presented a substantial increase in the usage of NBIs since the late 2000s (Figure S2), which was mainly driven by a predominant focus on environmental assessments, as well as nutrient and crop management (see section 3.4). However, limited access to information, the lack of trained personnel and funding, as well as a primary research focus on food production and thus plant-parasitic nematodes (Sikora et al., 2018), may be major reasons for the low application of NBIs in Africa, the Middle East, South America, and South-East Asia. A study by Cortada et al. (2019) investigated nematology as a training and research discipline in Africa and found that this region especially suffers from insufficient local expertise resulting from a lack of funding and capacity in both the private and public sectors.

When considering the spatial scale of the studies included in this review, approximately 66% focussed on local areas, such as a specific field, forest, or grassland. About 25% of the studies presented a regional focus that included two or more sites (forests, fields, grassland, etc.) within a larger region (valley, federal state, commune, etc.). Studies applied on national and global scales were, however, scarce and represented only 5% of the total number of studies. Reasons for the low publication rate of national and global scale studies are likely the financial costs and the excessive need for contributing partners that provide facilities (e.g. field experiments or sampling sites) for investigation. Furthermore, such results are often published in meta-analyses, which are not considered in this review.

Nonetheless, NBIs can be especially useful when applied in studies undertaken over large spatial scales. It has been well established that nematode communities are affected by several environmental factors, such as soil texture, moisture, and temperature, as well as organic carbon and nutrient content (Bongers, 1990; Du Preez et al., 2018; Girgan et al., 2020). Therefore, nematode species that are common in one site may be missing in another site, which would impede a direct comparison. But here lies the advantage of the NBI toolset, which is not based on species abundance, but rather on the life history classification of the nematode community. If a cp 2 bacterivore genus in Field A (e.g. *Acrobeles*) is missing in Field B, but replaced by another cp 2 bacterivore genus (e.g. *Cephalobus*), most NBIs remain unaffected. This allows more direct comparisons across multiple landscapes and across larger spatial scales.

3.3 Land use and land cover systems

Studying the application of NBIs in different land use (Figure 2) and land cover (Figure 3) systems provided a better perspective on the versatility and usability of the NBI toolset in different landscapes. Overall, agriculture was the dominant land use system with 57% of the total published research papers (Figure 2). This was followed by land not in use (e.g. deserts, glaciers, etc., 15%), maintenance and restoration (e.g. nature protection areas, 14%), and forestry (12%). The application rate of NBIs on built-up and related areas (e.g. city parks, mines, industrial areas, etc.) were only represented by 2% of the studies. This even though soils associated with mines and industrial areas are often contaminated with chemical and biological waste, which creates an opportunity to test the applicability of, for example, the MI2-5 (Korthals et al., 1996b). Contaminated soils may also be used to validate the sensitivity of nematodes in different cp groups, also sentinel taxa, to environmental disturbance (Fiscus and Neher, 2002; Ekschmitt and Korthals, 2006).

Interestingly, there was no clear trend on the application of NBIs in specific land use systems across continents, climate zones, nor countries. One exception was western Europe (Figure

2B), where the focus was predominantly on agriculture. The frequency of studies that focused on forestry, land not in use, and maintenance and restoration, was higher in eastern Europe. This is possibly the result of many eastern and northern European countries (also the western European countries Switzerland, Sweden, and Finland) having more than 50% of terrestrial land covered by forests and semi-natural areas (ETC-ULS, 2020). This is either the result of topography and bedrock in high altitudes (e.g. Alpine region in Switzerland; Carpathian Mountains in Romania and Slovakia), or of climate, as especially Sweden and Finland are located in boreal environmental zones (Jongman et al., 2006), where agricultural land use is limited.

The land cover statistics (Figure 3) presented similar trends to that of land use. The highest prevalence was reported for herbaceous crops (i.e. cultivated graminoids and forbs) with 36% of the studies applying NBIs, grassland (i.e. grasslands, prairies, and savannahs) with 21% of the studies, and woody crops (i.e. tree and shrub crops) with 7% of the studies. These are typical agricultural systems. Tree-covered areas (16% of the studies), which include natural and planted trees, were largely represented by natural forests. Terrestrial barren land (i.e. areas with less than 2% natural vegetation) and sparsely vegetated land (i.e. areas with between 2% and 10% natural vegetation) were concatenated under ‘Sparse’ as illustrated in Figure 3 (10% of the studies). Shrub-covered land (i.e. areas covered with 10% or more natural shrubs) were represented by 6% of the studies.

From a continental perspective, NBIs were predominantly applied to land under herbaceous crops in Africa, while in Australia and New Zealand, studies on grasslands were more frequent. China (with 147 studies) and the Unites States of America (with 135 studies) showed a similar pattern with a major focus on herbaceous crops and grasslands, and a relatively even distribution among the remaining land cover types. When considering Europe (Figure 3B), there was no clear dominance of a particular land cover type. However, herbaceous crops and grassland were the main land cover types and similarly frequent in many countries. In some countries, for example, Czech Republic, Slovakia, Spain, and Sweden, however, 25-40% of the studies were conducted in tree-covered areas. Most of these countries are located in mountainous regions or boreal climate zones (Jongman et al., 2006), where natural vegetation and grassland dominates with agriculture often being uneconomic. This is well reflected by the permanent grassland area (on average 35% of total agricultural area) in Europe (Smit et al., 2008). Ultimately, the application of NBIs were generally more frequent in grassland than herbaceous crops when the proportion of grassland was indeed above this European average. For example, the proportion of permanent grassland of the total agricultural area in Austria, Ireland, Switzerland, and the United Kingdom, is more than 46% and thus reflects the use of NBIs in this land use system (Figure 3B). Lastly, it is worth noting that natural grassland and forest ecosystems can serve as baseline systems when investigating the effect of intensive agricultural practices on soil ecosystem health and functioning using NBIs (Girgan et al., 2020).

3.4 Distribution of study foci

The largest proportion of studies (34%) focused on environmental assessments (Figure 4), with assessments of environmental gradients (e.g. soil moisture, slopes, soil types, and soil texture) as typical examples of this category. These were generally conducted in built-up and related areas, in areas under maintenance and restoration, and in land not in use, and therefore mirror the publication frequency of these land use systems (i.e., 31%, Figure 2). A considerable number of studies (20%) also focussed on fertilizer and nutrient application. The application rate of NBIs was similar among the other seven study focus categories ranging from 3% (cover crops) to 9% (organic agriculture, Figure 2). Six of the categories (namely rotation, tillage, organic, cover crops, fertilizer/nutrients, and pest control) are closely related to agricultural land use systems that represented 57% of all studies applying NBIs.

The prevalence of the study focus fertilizer and nutrients (i.e. application of soil amendments for the purpose of enrichment) can be best explained by the rapid response of cp 1 nematodes to the addition of nutrients to soil (De Goede et al., 1993a; Ferris et al., 2001). For this, selected

NBIs (see also Figure 5) are useful for measuring the subsequent effect on the soil ecosystem. This explains why the use of NBIs are popular in fertilizer and organic enrichment experiments. For the same reason, several research papers focused on organic farming, which is predominantly based on diverse crop rotations and organic fertilizer applications. The status of soil ecosystems in organic practices can be typically and clearly differentiated from conventional systems with the use of NBIs (Neher, 1999; Landi et al., 2018). However, Ilieva-Makulec et al. (2016) recorded only minimal differences in NBIs between these systems as affected by seasonal variation. This highlights the importance of environmental conditions (i.e. abiotic and biotic effects) also being accounted for when more in-depth studies (e.g. comparing treatments under field conditions) are conducted.

4. UTILIZATION OF NEMATODE-BASED INDICES

4.1 Selecting an appropriate subset of nematode-based indices

Nematode-based indices are extensively used to assess the condition of the soil ecosystem and the effects of natural and anthropogenic impacts on soil (Blakely et al., 2002; Zhong et al., 2017; Sánchez-Moreno et al., 2018; Jansen van Rensburg, 2020). Different NBIs may be appropriate in theoretical and empirical studies depending on the hypotheses being tested or the observations interpreted. There are several NBIs available (Ferris and Bongers, 2009; Sánchez-Moreno and Ferris, 2018), and the temptation to apply them all to analysis of a dataset often leads to confusion rather than enhanced understanding. Generally, it is more useful to carefully select and apply NBIs that are appropriate for understanding, or testing hypotheses on, the underlying ecological mechanisms. Always, a solid hypothesis on the mechanisms which drive the observed patterns is necessary to allow correct interpretation of the information derived from the NBIs. Therefore, the following text provides information on the relevance of nematode groups given specific conditions, which is followed by a framework (Figure 5; Table 1) for the selection of a focus-orientated subset of NBIs.

Considering the most sensitive trophic guild to the studied perturbation may help in the choice of a useful NBI (Figure 5). For example, the soil microbiota (i.e. bacteria and fungi) typically blooms after the application of organic amendments to the soil (Ren et al., 2019). Depending on the quantity and quality of the amendment, the soil microbiota may respond to changes in composition, biomass, or both (Böhme et al., 2005; Ji et al., 2020; Urra et al., 2020). Organic amendments with low C:N ratios favour bacteria over fungi and, in response to such amendments, the bacterivores may increase (DuPont et al., 2009). In contrast, organic amendments with high C:N ratios, or in advanced states of decomposition with only recalcitrant organic structures remaining, favour fungi and consequently fungivores (Ferris and Matute, 2003; Ferris and Bongers, 2006). In addition, information on the occurrence of dauer larvae, i.e. non-feeding, resting L3-stages of cp 1 nematode taxa, can provide insight into soil nutrient dynamics in the period preceding assessment of the nematode assemblage. A high dominance of dauer larvae indicates low current microbial activity, but high activity in the recent past (Vazquez et al., 2019). Depending on the specific aims of a research project, dauer larvae could be excluded from the index calculations (when the aim is to estimate only the current food web activity) or included (when information is needed spanning a larger period).

Therefore, when considering the selection of NBIs, *structural* changes in the microbiota components of the soil food web are reflected in the EI and CI, while the magnitudes of *functional* changes are reflected in the Enrichment Footprint (EF), Bacterivore Footprint (BF), and Fungivore Footprint (FF) of the MFs (Ferris et al., 2012b; Kou et al., 2020; Song et al., 2020; Zhang et al., 2021). Changes in the quality and quantity of organic materials in the soil result in changes in the composition and biomass of the microbial community. That alters the resources available for microbivore nematodes and, consequently, in the magnitudes of MFs. In general, while high values of the EI, the EF, and the BF might be considered indicators of soil enrichment and fertility, high values of the CI and the FF indicate progression to recalcitrant organic matter exploited by soil fungi (Ferris et al., 2001; Ferris, 2010). Examples of agricultural practices that affect the soil microbiota and that might result in soil enrichment include cover crop incorporation or diversification of organic inputs through exogenous amendments and crop rotation (Figure 5) (Nivelle et al., 2016; Urra et al., 2020).

The impact of agricultural soil management on plant roots, plant-soil interactions, and therefore underground plant-parasitic nematodes can also be considered (Sánchez-Moreno et al., 2006; Talavera et al., 2019; Ferreira et al., 2020). Changes in the proportion of plant-parasitic nematodes in the community are reflected in the ∑MI, the PPI and, when the biomass of plant-parasitic nematodes changes, in the Herbivore Footprint (HF) (Zhong et al., 2016; Bongiorno et al., 2019; Hodson et al., 2019). Such indicators may be related to C-fixation rates, plant biomass, and crop yields (Ferris et al., 2001; DuPont et al., 2009).

Finally, soil chemical and physical disturbances predominantly affect sensitive omnivores and predators (Korthals et al., 1996a; Korthals et al., 1996c; Korthals et al., 1998). When nematodes at high trophic levels in the soil food web disappear due to disturbance, both their relative abundance and their biomass is reduced, and both structural (MI2-5, SI, BI) and functional [Omnivore Footprint (OF), Predator Footprint (PF), Structure Footprint (SF)] indicators reflect such changes (Zhang et al., 2015; Bongiorno et al., 2019; Jackson et al., 2019). When basal resources are available and no other perturbation occurs, predators and omnivores may increase due to bottom-up transfer of resources to higher trophic levels (Ferris et al., 2012a; Song et al., 2020).

4.2 Ranges and expected results of nematode-based indices

Although maturity indices have bounded ranges (0-5) (Table 1), extreme values are uncommon and intermediate values (in the range of 2 to 3 for the MI) are typically reported. In desert soils with <250 mm rain/year, MI values around 2-2.5 are reported in bulk soil and plant rhizospheres (Pen-Mouratov, 2008; Ma et al., 2018), but values are as high as 3.5 in soils under mature biological crusts (Darby et al., 2007). In tropical forests with 2000-5000 mm rain/year, mean MI values are reported in the range of 2.8-3.3 (Zhong et al., 2017; Varela

Benavides, 2018; McQueen and Treonis, 2020), while in highly organic peat, MI values range from 2.1 to 3.6 (Sohlenius, 1999; Wasilewska, 2002; Hoschitz and Kaufmann, 2004). Clearly, a system with an MI close to 5 must be unstable (De Goede et al., 1993b), being dominated by predators and/or omnivores with an absence of prey (except in rare cases in which the dominant cp-5 nematodes are generalist predators with a broad spectrum of prey). Alternatively, agroecosystems may present lower MI values due to the levels of anthropogenic disturbances and resource inputs.

Soil food web diagnostic indices (EI, SI, CI, BI) have a theoretical range of 0-100 [see, e.g. (Berkelmans et al., 2003; Ferris et al., 2001; Renčo and Baležentiené, 2015; Zhong et al., 2017; Sánchez-Moreno et al., 2018; Zhang et al., 2020) for examples in different habitats and/or crops], but extreme values are less common [e.g. as in Djigal et al. (2012)] and, as with high MI values, would probably represent unstable soil food web conditions. The original presentation and calibration of the faunal analysis chart for interpretation of the indices (Ferris et al., 2001) was based on datasets of nematode assemblages from different environments and ecosystems: annual crop agriculture with frequent disturbance and exogenous inputs (SI<50 and EI>50, Quadrat A); perennial crop or more sustainable agriculture with exogenous input but minimal physical disturbance (SI>50, EI>50, Quadrat B); natural forests and grasslands, undisturbed with recycling of endogenous resources (SI>50, EI<50, Quadrat C); and resource deprived systems in stressed environments (SI<50, EI<50, Quadrat D). However, for any specific location, the assemblages of nematode fauna commonly fall into a singular quadrat based on the taxa that are present [see, e.g., Minoshima (2007); Cheng et al. (2008); Sánchez-Moreno et al. (2008); Song et al. (2020)]. Since the SI is derived from the proportional abundance of organisms with long life cycle and relatively low fecundity (Ferris et al., 2001), management to relieve a stressor or to promote recovery from perturbation will, in the short term, move the SI only by small increments within the parent quadrat for that location.

If they are not already present, soil management is unlikely to introduce sensitive cp 4 and cp 5 nematodes into the system and, even when present, their increase may take many years.

Consequently, increases in the SI may be very gradual (DuPont et al., 2009). Most of the faunal analysis "action" in response to management at a single location will probably be in the form of increases or decreases in the EI, which moves significantly following either an intended enrichment event or an enrichment facilitated by access to new resources. Conversely, changes in the ecosystem in response to major perturbations, for example, soil fumigation with broad spectrum pesticides, or experimental climate manipulation, may reduce the SI substantially, and the assemblage may move from one quadrat to another in a relatively short time (Berkelmans et al., 2003; Biederman et al., 2008; Sánchez-Moreno et al., 2010; Cesarz et al., 2015). When interpreting the effects of management on nematode bioindicators at a single location, the faunal analysis diagram using the four-quadrat system may not be necessary and smaller scale analyses and depictions, for example, within the quadrat that accommodates the relevant taxa present at that location, might be more informative. The four-quadrat faunal analysis diagram is most useful for explaining differences between locations, cropping systems, or ecosystems.

The indices built on the MI framework indicate the structure of the nematode assemblages in the ecosystems to which they are applied. However, they do not measure the magnitude of the functions or services performed by the components of the assemblage. Those assessments require consideration of the abundance of individuals in a taxon or functional guild and their rate of carbon utilization. Ferris (2010) provided a basis for estimating the magnitude of ecosystem functions and services by MFs for all the nematodes in a functional guild or trophic group. The calculations estimated life-time carbon utilization based on the mass of the adult nematode body and the rate at which individuals use and excrete carbon through respiration. In contrast to the various indices, MFs have no upper limit since the abundances of taxa in the system and their carbon utilization can be extremely large. In a further development in the evolution of NBIs, Ferris and Tuomisto (2015) recognized that the nematodes within a functional guild may differ from each other in terms of behaviour, activity, size, and foraging capabilities. They pointed out that species diversity is another probable component of the magnitude of an ecosystem function. They suggested that diversity-weighted biomass or diversity-weighted MF of the species within a functional guild would increase the precision, at least in concept, of the magnitude of an ecosystem function or service.

4.3 Important considerations in the application of nematode-based indices

During the three decades of applying NBIs, several limitations in their use and interpretation have become apparent. The limitations result, in most cases, to our fragmentary knowledge of the autecology of many nematode taxa. Specific nematode taxa may behave or respond differently than expected, or be unexpectedly abundant, when considering their trophic or cp group (Li et al., 2005). As an example, the omnivorous genus *Mesodorylaimus* is classified as cp 4 and is thus considered a persister that is sensitive to perturbation. However, it may also behave as an opportunistic coloniser (Austin et al., 2009) by responding positively to organic enrichment (Zhao and Neher, 2013) and presenting high abundances in extreme environments such as Antarctic soils and lakes in active volcanoes (Nedelchev and Peneva, 2000; Muschiol and Traunspurger, 2009). Adaptability to extreme environments is also exhibited by other omnivore genera, including *Eudorylaimus* and *Aporcelaimellus* (De Goede et al., 1993b; McSorley, 2012). Other nematode taxa, in turn, might be exceptionally abundant in certain ecosystems. For example, *Discocriconemella*, a root-feeding, slow-moving nematode, is highly abundant in tropical forests and sensitive to land use change (De Cardoso et al., 2015; Franco-Navarro and Godinez-Vidal, 2017). Similarly, rhabditid enrichment opportunists, commonly associated with pulses of new resources (Bongers, 1990; Bongers and Ferris, 1999), may be as common in mature tropical forest soils as in adjacent agricultural crops (De Cardoso et al., 2015).

In some instances, additional information generated on the autecology of nematode taxa resulted in adjustments to their life history classification. Leptolaimidae were moved from cp 3 to cp 2, because of their occurrence in polluted conditions (Bongers et al., 1991).

Monhysteridae were changed from cp 1 to cp 2 as they occur in resource-limited conditions and do not form dauer larvae, and because of opposing reasons, Myolaimidae were downgraded to cp 1 (Bongers et al., 1995). Wasilewska (1995, 1997) suggested to also recognise an opportunistic class of plant-parasitic nematodes and proposed to classify *Paratylenchus* as pp 1 (Bongers et al., 1995). However, we are not aware of any published studies adopting this idea of recognising an opportunistic class of plant-parasitic nematodes. Species of the polyphyletic genus *Filenchus* (Qing and Bert, 2017), previously classified as an epidermal cell and root hair feeder, can be cultured on fungi growing on agar plates (Okada et al., 2002). As *Filenchus* is known to be very dominant in certain ecosystems (De Goede and Bongers, 1994), its inclusion in the calculation of the NBIs can have a great impact on the results. Another example of an important change in the life history classification of a nematode taxon was when the predatory genus *Seinura* was separated from the fungal feeding Aphelenchoidae taxa. These examples indicate that refining the cp classification of nematode taxa to, e.g., genus level, requires fundamental decisions that cannot always be unequivocally judged. In the case of *Filenchus*, maybe we must accept that in agreement with Sohlenius et al. (1977), the feeding group 'epidermal cell and root hair feeders' not only comprises species that feed in the rhizosphere on root hairs and epidermal cells, but also on (mycorrhizal) fungi (Okada et al., 2005). As our knowledge of nematodes advances, it may become apparent that interpretation of the effects of soil disturbances and ecosystem enrichment differ with taxa assemblages that are associated with specific ecosystems, geographic locations, and local climate (Neher et al., 1998; Neher et al., 2005; Van den Hoogen et al., 2019). The identification of genera known to respond to perturbations in specific habitats, possibly also the identification of sentinel taxa, will therefore improve the assessment of soil health and interpretability of NBIs.

Another important consideration is the calculation of MFs as an assessment of the magnitude of ecosystem functioning. This requires the estimation of the biomass of nematode functional guilds in the system being studied. Although measuring a significant number of nematodes in each sample has sometimes been done (Mulder and Vonk, 2011; Zhang et al., 2015), it is usually considered unmanageable in terms of available resources. The more frequently used approach to estimating nematode biomass and calculating MFs is through the Nematode Indicator Joint Analysis (NINJA) online tool (Sieriebriennikov et al., 2014) (https://shiny.wur.nl/ninja/), which utilizes a large repository of nematode ecophysiological data. However, a certain bias is assumed since MFs calculated through NINJA are based on the size and biomass of adult females as an estimate of lifetime biomass potential for each taxon (species, genus, or family). These metabolic footprints thus represent the potential lifetime carbon utilization, not the carbon utilization or estimate of ecosystem function at one point in time.

Lastly, the NBI toolset is currently not directly linked to soil functions. With an increased interest in soil health, more emphasis should be placed on validating the functional significance of the NBI toolset. Zhang et al. (2017), for example, evaluated the relationships between N-mineralization and abundance of various bacterivores. Also, the effects of omnivores and predators in regulating populations of opportunists have been inferred and tested in microcosm experiments (Sanchez-Moreno and Ferris, 2007; Steel and Ferris, 2016). The true potential of the NBIs will increase greatly when soil ecosystem functions can be directly measured using this toolset. However, the current inferences on soil function provide an important basis in hypotheses that can be tested experimentally.

5. FUTURE DIRECTIONS

The current toolset of NBIs make use of microscopy to identify nematodes based on their morphology. This approach is certainly not trivial as extensive training is needed to acquire the necessary identification skills. In addition, the time required to identify nematodes to a sufficient taxonomic level is considerable, which limits the number of samples that can be processed (Geisen et al., 2018). However, even with the further advancement of molecular approaches, training in nematode taxonomy remains essential. Available short courses on the identification of nematodes include the summer courses in Wageningen (Netherlands) on the identification of plant-parasitic, terrestrial and freshwater nematodes (https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Nematology/Education-at-the-Laboratory-of-Nematology/Training-courses-for-professionals.htm), the nematology short course in Potchefstroom (South Africa) (http://sanematodes.com/symposia/short-courses/), the short course on the identification of plant-parasitic nematodes at Clemson University (USA), and the plant nematology course at the Universities of Göttingen and Kassel in Witzenhausen (Germany) (https://www.uni-kassel.de/fb11agrar/en/sections-/-facilities/honorary-professor-for-science-management-in-international-organic-agriculture/nematology).

Nonetheless, efforts to provide alternative, robust, faster, and cost-effective nematode identification methods have been initiated by various scientists, which range from biochemical (enzymatic-based) to molecular (DNA- and RNA-based). The former, however, do not provide sufficient taxonomic resolution to be used for NBIs (Block and Powers, 2009; Xu et al., 2010) as normally required by ecological studies. Several modern molecular methods, on the other hand, exhibit versatility to various experimental requirements in that discrimination can be made between nematode taxa. While methods like quantitative PCR (qPCR) (Cavallero et al., 2014; Li et al., 2014) are increasingly used in agricultural nematology, such as for the specific detection of plant-parasitic species, only high-throughput sequencing methods provide information on entire nematode communities that can be used for NBIs. In fact, DNA- and RNA-based techniques have almost entirely replaced conventional methods to study microbes at community level and are now increasingly being used to investigate soil nematodes (Porazinska et al., 2009; Geisen et al., 2018). However, information on nematode community structure obtained by these nucleic acid-based metabarcoding techniques differs from that obtained via morphologically identified nematodes (Geisen et al., 2018; Griffiths et al., 2018). As such, and despite the fact that reproducible ecological information can be generated using both approaches (Geisen et al., 2018; Griffiths et al., 2018), information obtained from sequencing approaches cannot currently be used to accurately calculate NBIs (Griffiths et al., 2018). In a case where the molecular profiling of nematode communities was successfully used to observe the effects of soil management on soil quality (Bongiorno et al., 2019), the indices derived from molecular data were not comparable to those obtained by traditional methods. Calibration of molecular to morphological information is therefore urgently needed. Fortunately, there is some hope: While abundances cannot be obtained using molecular approaches, a combination of molecular characterization of nematode community structure coupled with simple quantification of all the nematodes in a given sample can be performed (Wilschut et al., 2019). Especially in combination with whole-community biomass estimations, sequencing techniques can provide reliable information on biomass distributions of individual nematode taxa (Schenk et al., 2019). Also, the application of Artificial Neural Networks (i.e., visual imagery classification and analysis) has shown promising potential for nematode identification (Uhlemann et al., 2020) and this technology could be further developed for nematode biomass estimations.

There are also some additional considerations when investigating the calculation of NBIs using molecular approaches. Conventional nematode extraction methods that utilize, for example, the Oostenbrink elutriator, Seinhorst elutriator, or Baermann funnel, rely on the active movement of nematodes. This generates reliable information on the abundance and composition of motile nematodes in soils. Microbial ecologists, in turn, study active, inactive, and even dead microbes directly in soils by nucleic acid extraction. These extractions are most often done in small soil samples of 0.25g, while a reliable estimate of nematode communities is suggested to require >100g of soil (Wiesel et al., 2015). Unfortunately, this amount of soil surpasses the range of most DNA extraction methods. A possible solution to this problem is thoroughly homogenising soils by freeze drying and subsequent mixing. This approach has shown that microarthropods that are far larger than nematodes can reliably be studied in 0.25 g of homogenised soil (Oliverio et al., 2018). As such, nematode communities might be studied directly from soils without the need for conventional extraction methods. However, this approach would also first need to be calibrated to the established morphology-based NBIs (Griffiths et al., 2018), or new (molecular-based) NBIs need to be developed.

Finally, NBIs are calculated using family or genus level information. However, differences in traits, including size and growth rates, can be profound between individual congeneric nematode species (Mulder and Vonk, 2011). This level of differentiation is not achievable for ecologists and is even impossible with microscopy techniques only, nor with current molecular tools. New sequencing tools such as PacBio, Oxford Nanopore, or LoopSeq might allow sequencing long reads and thereby allow taxonomic resolution to species level (Krehenwinkel et al., 2019; Tedersoo and Anslan, 2019; Callahan et al., 2021).

6. FINAL REMARKS

Thirty years after the foundational work on nematode ecology by Tom Bongers, nematode-based indices are widely used to generate a powerful suite of information on soil ecosystem health and functioning in multiple land use and land cover systems. The development of especially the original MI, food web diagnostic indices, and MFs, represent critical achievements that enhanced the versatility and applicability of NBIs. However, for multiple reasons, but mainly due to the lack of funding and trained personnel, this valuable toolset is not readily applied by ecologists in, for example, Africa. Fortunately, efforts by various stakeholders and research institutions (e.g. M.Sc. Nematology program from Ghent University) are making a positive impact on creating awareness and facilitating the training of Nematology graduates (https://studiekiezer.ugent.be/international-master-of-science-in-agro-and-environmental-nematology-en/2021). The current 'Nematology Education in Sub-Sahara Africa (NEMEDUSSA)' initiative is another example of a multinational and -institutional project particularly aiming at increasing awareness and education (https://nemedussa.ugent.be/). As part of this initiative, students are trained in the identification of major nematode groups, which is a critical skill in the application of NBIs.

We also provided a holistic overview and guide to the selection and interpretation of NBIs, which will increase the power and usability of NBIs. Furthermore, current limitations and important challenges in the application and further development of NBIs were highlighted. Of key importance is our fragmented knowledge on the autecology of many nematode taxa and increased efforts should be made to study the life history traits of nematodes at genus or even species level. This will allow the refinement of cp and trophic group classification and possibly the identification of sentinel taxa, which will increase the accuracy of NBIs.

The establishment of an international committee on the use of nematodes as environmental bioindicators should also be considered. Such a committee can oversee the standardisation of nematode sampling, extraction, and identification protocols, as well as consider and recommend changes in the life history classification of nematode taxa.

Finally, methodological advances such as the application of Artificial Neural Networks can help identify nematodes and potentially quantify biomass. Also, novel molecular approaches promise to determine species compositions and increase the applicability and information obtained using NBIs. Ultimately, the continued use of NBIs, as well as enhancing the toolset's versatility and value for soil ecologists, are reliant on efforts to expand our knowledge on the autecology of nematodes, while also thinking of new and better ways of measuring nematode community structures.

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Table 1: Information on the purpose, ranges, and ecological relevance of nematode-based indices that will assist in the choice and interpretation of these indices. Adapted from Sánchez-Moreno and Ferris, 2018

	Indicates	Range	Ecological relevance
Maturity Index (MI)	Environmental disturbance resulting from perturbations	1 – 5	Low values (<2) indicate an early (primary or secondary) successional stage or a temporary level of increased nutrient availability. Values close to 2 indicate a high level of disturbance with low soil food web structure, while intermediate values (2.5 – 3) indicate some soil food web maturity. High values (>3) indicate a well-structured and complex soil food web likely with connectivity and energy flow between trophic levels.
Maturity Index 2-5 (MI2-5)	Environmental disturbance resulting from perturbations unrelated to nutrient enrichment in agricultural fields	2 – 5	Low values (close to 2) indicate substantial disturbance resulting from perturbations unrelated to nutrient enrichment. High values (>3) indicate greater maturity with minimal or no effect resulting from perturbations.
Plant-Parasitic Index (PPI)	Assemblage composition of plant-parasitic nematodes	2 – 5	Low values (close to 2) indicate plant-parasitic nematode assemblages dominated by small and medium-sized ectoparasites that feed on single plant cells. Higher values indicate assemblages dominated by medium and large (semi-) endoparasitic (e.g., Meloidogyne and Heterodera spp.) or ectoparasitic virus transmitting nematodes (e.g., Xiphinema and Longidorus spp.).
Sigma Maturity Index (∑MI)	Environmental disturbance resulting from perturbations in non-agricultural soils	1 – 5	Low values (<2) indicate a high level of nutrient availability and minimal plant-parasitic pressure, while values close to 2 indicate a high level of disturbance with low soil food web structure. Intermediate values (2.5 – 3) indicate some soil food web maturity. High values (>3), in turn, indicate a well-structured and complex soil food web likely with connectivity and energy flow between trophic levels, which might include larger plant-parasitic nematodes. This index is less sensitive to enrichment in agricultural soils.
Enrichment Index (EI)	Food availability and nutrient enrichment	0 – 100	Low (0 – 30), intermediate (30 – 60), and high (60 – 100) values indicate equivalent levels of food availability (e.g., labile organic carbon) and nutrient enrichment.
Structure Index (SI)	Soil food web structure and complexity, as well as disturbance due to environmental (e.g., salinity and drought) or anthropogenic (e.g. tillage, mining, and chemical pollution) causalities	0 – 100	Low (0 – 30), intermediate (30 – 60), and high (60 – 100) values indicate equivalent levels of soil food web complexity. Lower values are indicative of perturbed soil food webs, while higher values indicate a structured soil food web.
Channel Index (CI)	Predominant decomposition pathway of organic matter	0 – 100	Lower values (<50) indicate increasing decomposition dominance by bacteria, while higher values (>50) indicate increasing decomposition dominance by fungi. Bacterial dominance indicates the presence of rapidly decomposed organic matter, while fungal dominated decomposition indicates the slow breakdown of more complex organic matter. The focus on opportunistic bacterial and fungal feeders makes this a highly

			responsive index, which can be used to detect alternating decomposition pathways over time.
Basal Index (BI)	Food web structure and complexity	0 – 100	Low (0 – 30), intermediate (30 – 60), and high (60 – 100) values indicate equivalent levels of soil perturbation. Therefore, higher values (>50) are indicative of a depleted and damaged soil food web.
Metabolic Footprints (MFs)	Magnitude of ecosystem functions and services fulfilled by nematode community	0 – infinite (no upper limit)	Higher metabolic footprint values are indicative of greater carbon channelling and therefore an increased contribution to the fulfilment of soil ecosystem functions and services. This can be considered per trophic group (e.g. bacterivore footprint), or per component of the nematode community that indicate enrichment (enrichment footprint) and structure (structure footprint).

FIGURES

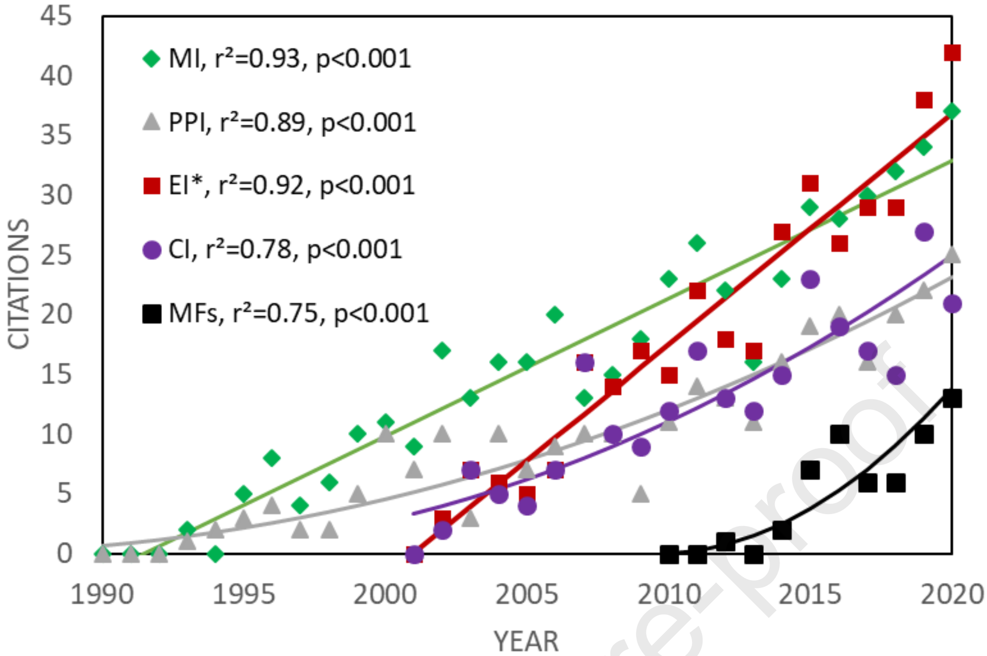

*Figure 1: Citation frequency per year of the five most widely used nematode-based indices introduced by Bongers (1990): Maturity Index (MI), Plant-Parasitic Index (PPI); Ferris et al. (2001): Enrichment Index (EI), Structure Index (SI), Channel Index (CI); and Ferris (2010): Metabolic Footprints (MFs). Regression lines and curves were created using generalized linear models (GLM) and F-/chi²-tests; *EI (y= 1.84x-19.6) and SI (y=1.81x-18.8) are highly correlated (Pearson's r: 0.99, p<0.001) and therefore only EI is illustrated*

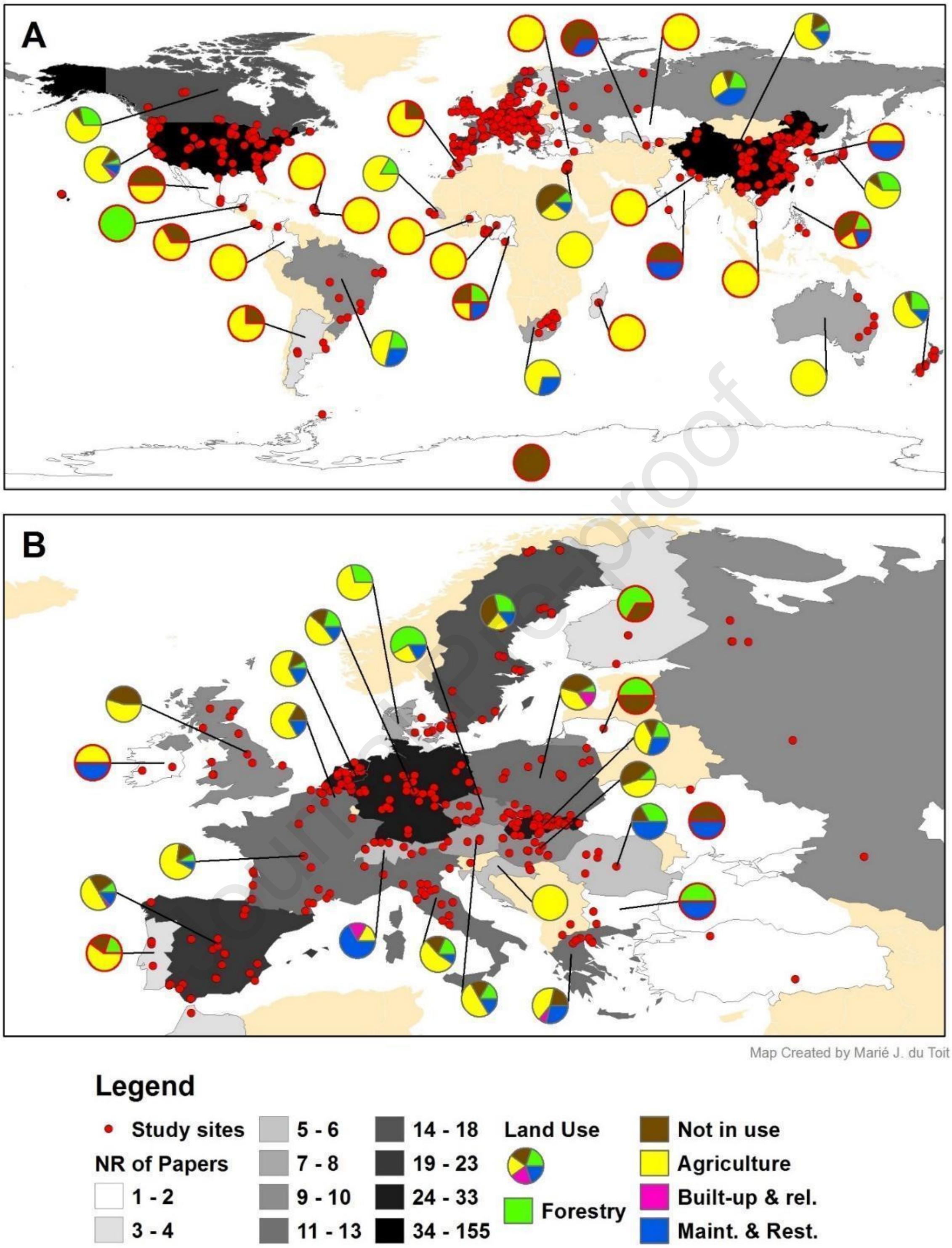

Figure 2: Map of the world (A) and Europe (B) to indicate the spatial distribution of study sites and the number of papers published in each country. Pie charts indicate the proportional representation of ***land use*** *categories focussed on in each country. Pie charts outlined in red indicate countries represented by less than five papers*

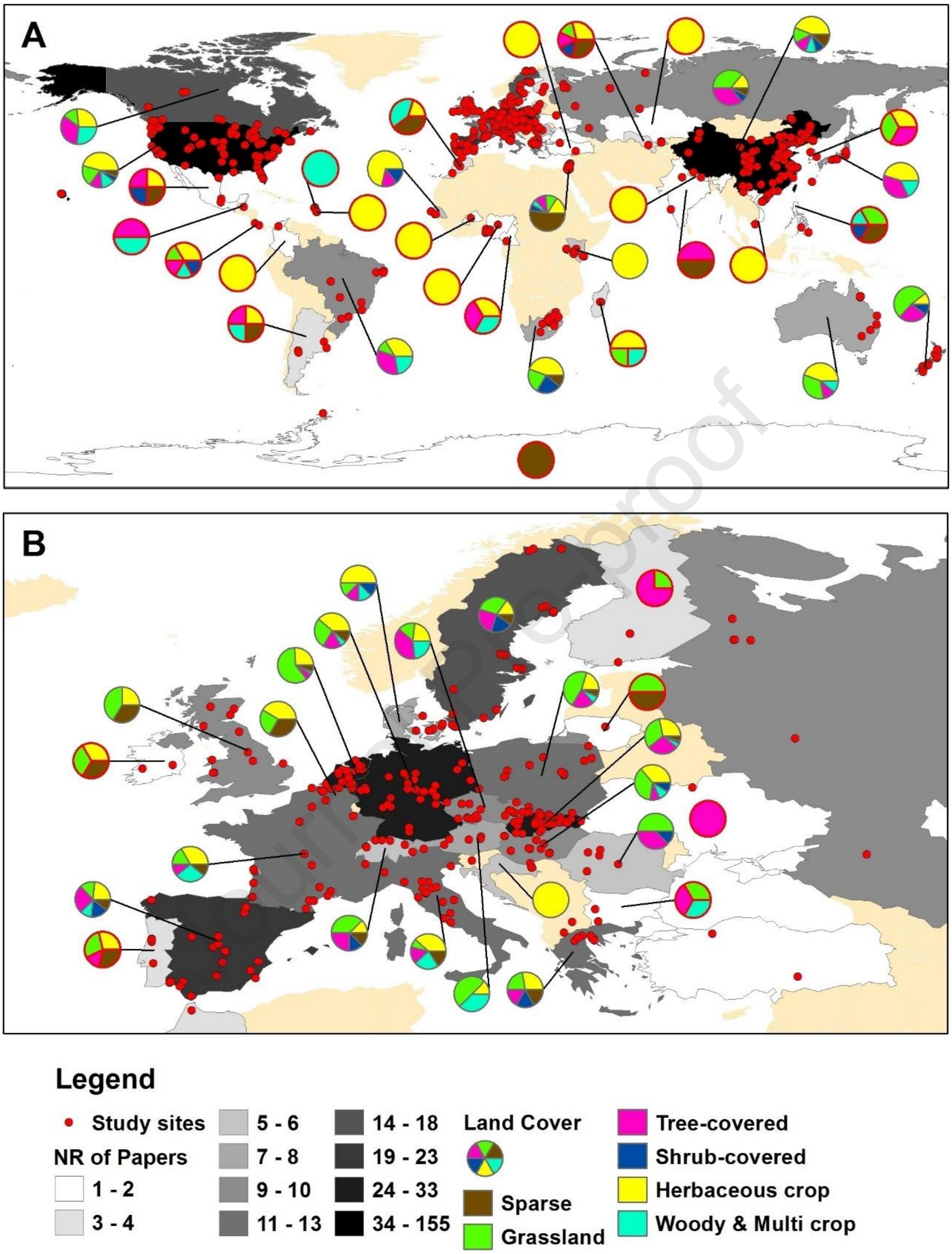

Figure 3: Map of the world (A) and Europe (B) to indicate the spatial distribution of study sites and the number of papers published in each country. Pie charts indicate the proportional representation of ***land cover*** *categories focussed on in each country. Pie charts outlined in red indicate countries represented by less than five papers*

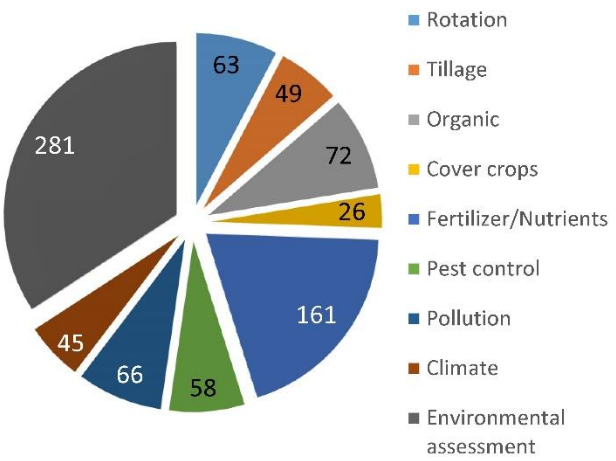

*Figure 4: Number of research papers with one or multiple study foci that applied nematode-based indices from 1993 – 2020. The study foci included crop **rotation**, soil **tillage**, **organic** food production, **cover crops**, **fertilizer** use and **nutrient** status, **pest control**, soil **pollution**, **climate**, and **environmental assessment***

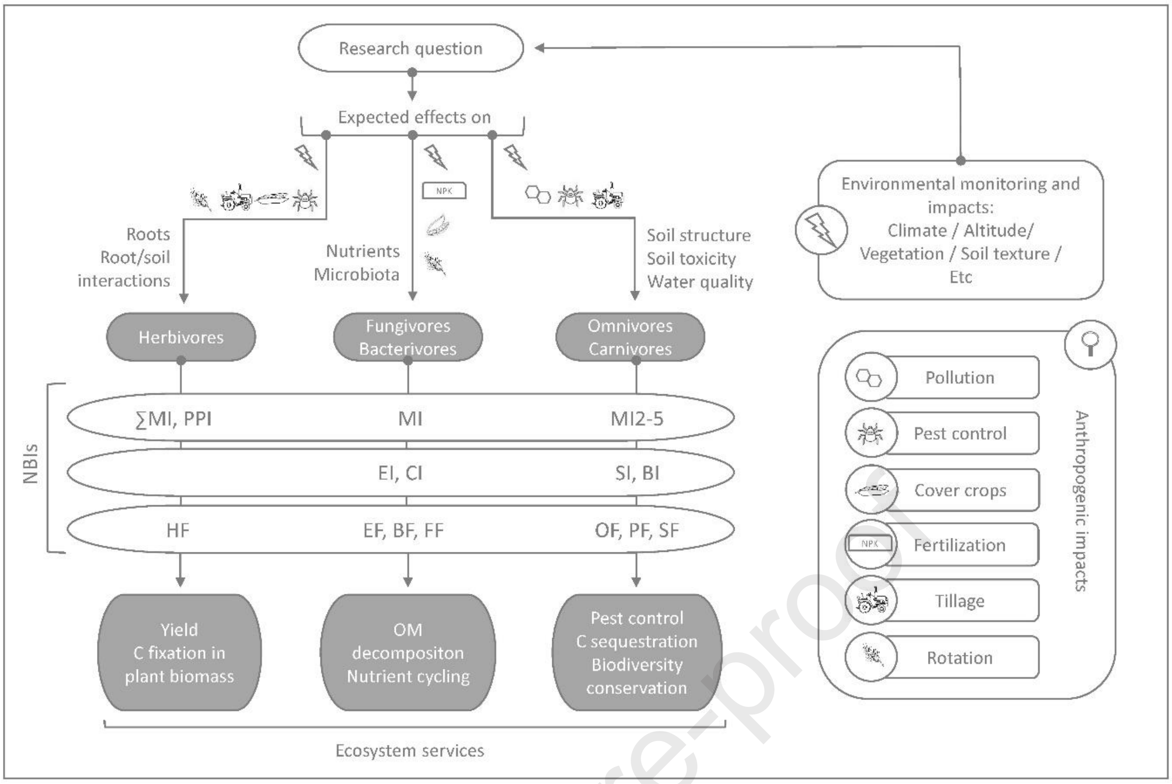

Figure 5: Diagram showing the process of choosing NBIs appropriate to the research question and underlying hypotheses. Environmental factors and natural and anthropogenic impacts affect different components of the (agro)ecosystem, including plants and their roots, soil nutrients and the microbiota, and/or soil structure and the soil chemical environment. When such components are perturbed, different nematode functional guilds are affected. Such changes are subsequently assessed by different nematode-based indices (NBIs), indicators of several ecosystem services. Nematode-based indices are abbreviated as follows: Maturity Index (MI), Maturity Index 2-5 (MI2-5), Plant-Parasitic Index (PPI), Sigma Maturity Index (∑MI), Enrichment Index (EI), Structure Index (SI), Channel Index (CI), Basal Index (BI), and Metabolic Footprints (MFs), including the Enrichment Footprint (EF), Structure Footprint (SF), Herbivore Footprint (HF), Bacterivore Footprint (BF), Fungivore Footprint (FF), Omnivore Footprint (OF) and Predator Footprint (PF). Further developments as diversity-weighted biomass or diversity-weighted MF are available (Ferris and Tuomisto, 2015), but not included in this graph

HIGHLIGHTS

- Nematode-based indices (NBIs) represent a powerful toolset of soil ecosystem status.
- Application of some NBIs increased exponentially on a global scale.
- Selection of NBIs must be focus-orientated and based on sound hypotheses.
- Development and calibration of molecular-based NBIs should be prioritized.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: