Reflections on Plant and Soil Nematode Ecology: Past, Present and Future

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Abstract: The purpose of this review is to highlight key developments in nematode ecology from its beginnings to where it stands today as a discipline within nematology. Emerging areas of research appear to be driven by crop production constraints, environmental health concerns, and advances in technology. In contrast to past ecological studies which mainly focused on management of plant-parasitic nematodes, current studies reflect differential sensitivity of nematode faunae. These differences, identified in both aquatic and terrestrial environments include response to stressors, environmental conditions, and management practices. Methodological advances will continue to influence the role nematodes have in addressing the nature of interactions between organisms, and of organisms with their environments. In particular, the *C. elegans* genetic model, nematode faunal analysis and nematode metagenetic analysis can be used by ecologists generally and not restricted to nematologists.

Key words: ecology, future, history, interaction, management, molecular biology, nematology, plant, soil.

Looking Back at Vistas in Nematology

Compared to the nematode systematics, physiology, host-parasite interactions and disease management, nematode ecology is a fledgling discipline. At this time, some 50 years after the establishment of the Society of Nematologists (SON), endeavors to understand the ecology of plant and soil nematodes are more popular than ever and comprise an unprecedented part in research and teaching activities in the science of nematology. Looking back 50 years, Wallace (1962) focused his discussion on management of plant-parasitic nematodes through understanding of their microenvironment (e.g., attractants) and controls of their geographical distribution, the problem of race determination and mechanisms of survival and dissemination of free-living life stages. The last major assessment of the breadth, accomplishments and directions for nematological research by the SON summarized in Vistas on Nematology: Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists (Veech and Dickson, 1987) attests to the subsequent emergence of nematode ecology as a major research area of both practical and basic knowledge, and its importance in student training.

In that treatise, ecological research was dominated by three topics; interactions of nematodes with other organisms, nematode population dynamics, and nematodes as model systems. Ecological research was primarily concerned with the study of few nematode species in relation to functional groups of organisms such as arbuscular mycorrhizal fungi (Smith, 1987), rhizobia (Huang, 1987), plant pathogens (Sikora and Carter, 1987), viruses (Lamberti and Roca, 1987) and to other specifically defined groups of nematodes (Eisenback and Griffin, 1987). Understanding and methodological approaches to using nematodes as responders to land management or environmental conditions were in its infancy with dose-type bioassays using a single-species (Samoiloff, 1987). Interest in population dynamics of nematodes was focused on plant-parasitic nematodes, obviously because of the practical importance to disease management (Caswell and Roberts, 1987; Duncan and McSorley, 1987; Ferris and Wilson, 1987). Today, entire nematode faunae, both terrestrial and aquatic, are used to infer about conditions of food web status and function in managed and natural systems (Danovaro et al., 2009; Nagy, 2009; Neher, 2010).

The advent of ecology as an important subdiscipline in nematology was alluded to in Vistas. The study of interactions among organisms and between organisms and their environment is the core of ecology. Dr. van Gundy described prophetically, in context of plant disease management, the need for ecological research: "The impact of sustainability on nematology research suggests the need for a change in philosophy from a pure, narrowly focused disciplinary research approach to an interdisciplinary approach that takes into account the need to understand complex interactions occurring in many different components of agricultural systems over time" (van Gundy, 1987). The purpose of this review is to highlight key developments within the field of nematode ecology and to identify some emerging areas of research as driven by crop production constraints, environmental health concerns and technological advancements.

Past: Nematode Ecology – Early to Current

Early studies – Overgaard Nielson, Wallace, and earlier: Science advances with innovations in technology, often

simple, sometimes more elaborate. Consider the impact of the development of the microscope and the introduction of the Baermann funnel. Now, consider the present and future applications of the advancing molecular technologies. The enduring phraseology of Cobb (1915) at the beginning of the 20th century alerted the scientific world to the abundance and diversity of nematodes. Cobb, like most scientists, was standing on the shoulders of pioneers. Marine biologists began to recognize nematodes in the mid-19th century (e.g., Leuckart, 1849; Bastian, 1865; Villot, 1875; Von Linstow, 1876) and freshwater biologists later that century (e.g., Daday, 1897). Descriptions of free-living soil taxa appeared during the same eras (e.g., Dujardin, 1845). The early descriptive studies spurred a period of discovery of the abundance and diversity of nematodes (e.g., Bütschli, 1873; de Man, 1884; Filipjev, 1918; Cobb, 1915) and are well detailed by Overgaard Nielsen (1949), Paramonov (1962) and Filipjev and Schuurmans Stekhoven (1941).

A milestone in the ecology of free-living soil nematodes was the seven-year study in Denmark by Overgaard Nielsen (1949) on nematode faunae of different soils, their physiological ecology and inference to ecosystem services. Further notable ecological contributions emerged in the 1970s and 1980s (e.g., Nicholas, 1975). Centers of ecological study on nematodes were developed in Sweden (e.g., Sohlenius, 1973), Poland (e.g., Prejs, 1970; Wasilewska, 1970), Italy (e.g., Zullini, 1976), Germany (e.g., Sudhaus, 1981), and Russia (e.g., Tsalolikhin, 1976). In the US, there was a surge of activity in soil ecology around 1980 (Norton, 1978; Yeates and Coleman, 1982; Stinner and Crossley, 1982) and, in the same time period, a very productive program on the ecology of soil nematodes developed in New Zealand (e.g., Yeates, 1979). The studies of Ingham et al. (1985) stimulated interest in the positive contributions of free-living soil nematodes in nutrient cycling and agricultural productivity and the extensive review of nematode feeding habits by Yeates et al. (1993) provided a necessary foundational basis. A more detailed overview of historical developments is provided in Ferris and Bongers (2009).

Faunal analyses: The Maturity Index, based on the relative abundance of non-plant-parasitic nematode taxa was developed by Bongers and colleagues during the last decade of the 20th century as a measure of environmental disturbance. It evolved into a series of indices that emphasize different characteristics of the system. In essence, the MI series provide indicators of the state of ecological succession of a community whereby succession setback is reflected in lower MI values (Bongers, 1990; Bongers and Korthals, 1993; Bongers et al., 1997). Ferris et al. (2001, 2004) refined the concepts by defining the Enrichment Index and the Structure Index to provide higher resolution to effects on the soil ecosystems of enrichment, disturbance and contamination. Parallel and subsequent developments recognized that the magnitude of ecosystem services is measured not by the proportions of various functional guilds but by their biomass and activity levels. That led to development of nematode biovolume and assessments of metabolic footprints as measures of total biological activity and of ecosystem services performed by each functional guild (Yeates, 1988; Kimpinski and Sturz, 2003; Neher et al., 2004; Ferris, 2010). As an example, metabolic footprints provide metrics for the magnitudes of ecosystem functions and services provided by component organisms of the soil food web. Standardized metabolic activity levels, attributable to the abundance of nematodes performing various functional roles, are calculated from existing and accessible morphometric data (Fig. 1; Ferris, 2010).

Recognition of indicator potential: Nematodes have been recognized as good soil health bioindicators since the 1970s in both Europe (Prejs, 1970; Wasilewska, 1970; Sohlenius, 1973; Zullini, 1976; Sudhaus, 1981) and New Zealand (Yeates, 1979), and since the 1980s in the U.S. (Stinner and Crossley, 1982; Yeates and Coleman, 1982; Ingham et al., 1985; Freckman, 1988). Nematode community indices developed in the 20th century (Ferris et al., 2001; Fiscus and Neher, 2002; Ferris and Bongers, 2009) have been used to examine how various land management practices could impact soil health; a) Sánchez-Moreno et al. (2009) clearly depicted the difference in nematode faunae between organic vs non-organic, and till vs no-till using enrichment (EI) and structure indices (SI); and b) Wang and McSorley (2005) conducted a series of cover cropping and soil amendment studies in short-term row crops and concluded that most of these practices are only enhancing EI in a short-term. Interpretation of soil health condition by using nematode community analysis required a comprehensive analysis that included different nematode trophic groups, fungal-to bacterial-feeding nematode ratio, richness, diversity,
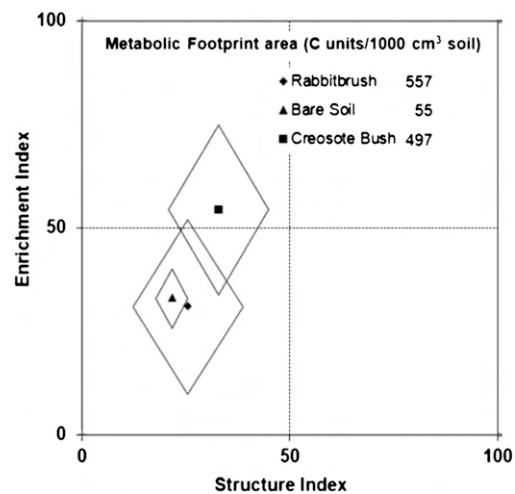

Fig. 1. Functional metabolic footprints of nematodes in depauperate and climatically constrained soil food webs of the Mojave Desert of California. Vertical axis of each footprint represents the enrichment footprint and horizontal axis the structure footprint. *Larrea tridentata* (creosote bush) and *Chrysothamnus sp.* (rabbitbrush), From Ferris (2010).

dominance, maturity index (Bongers, 1990; Neher, et al. 1995; Neher and Campbell, 1996; Bongers et al., 1997) and more recently the EI, SI, channel index (CI) and basal index (BI) (Ferris et al., 2001; 2004). Failure to adapt a comprehensive view of these indices could lead to misinterpretation of nematode faunae. For example, nematode faunae in one-month old methyl bromide treated field plots were shown to have higher EI than non-fumigated fallow plots in one field trial (Wang et al., 2004a). However, other indices reflected that methyl bromide treated plots were highly disturbed, with low nematode diversity and richness, mainly dominated by two groups of bacterial-feeding nematodes, the Rhabdi-tidae and *Turbatrix*. At the turn of the century, issues are being raised on the service and disservice of nematode faunae (Ferris, 2010). A healthy soil food web should sustain nematodes with different life strategies and feeding behaviors ranging from fast-growing and fast-breeding bacteria-feeding nematodes at the bottom of the food chain, to slow growing, long generation and low fecundity predaceous nematodes at the top. Often, agricultural practices trying to ameliorate potential disservices result in unintended but long-lasting diminution of services (Ferris, 2010). One of the most commonly observed dis-services to nematode faunae in agroecosystems is the disturbance of omnivorous and predatory nematodes due to frequent soil cultivation, such as that reported by McSorley et al. (2007) when comparing soils from a nat-ural system vs agroecosystem located in close proximity. Another commonly reported reduction in service of nematode faunae is that observed in intensive farming systems with deep plowing followed by soil fumigation and heavy use of persistent herbicides, which increased EI, and decreased SI and channel index (CI) as reported by Wang et al. (2011) in a pineapple plantation in Hawaii.

PRESENT: ROLES OF NEMATODES IN ECOSYSTEM SERVICES

Nutrient mineralization: Nutrient mineralization is a consequence of two processes following the consump-tion of prey (bacteria, fungi, nematodes or other fauna) by nematodes. Ingested C is used for both respiration and assimilation whereas ingested nutrients (N, P, S etc.) are only used for assimilation. Generally the C: nutrient ratio of the nematode is larger than that of the microbial (bacterial and fungal) prey. The result is that the nema-todes ingest more nutrients than required, and the ex-cesses are excreted in a mineral or readily mineralizable form such as amino acids, NH_4^+ and PO_4^{-3} (Ingham et al., 1985; Bardgett and Griffiths, 1997). Calculations based on population sizes, and turnover rates show that nematodes can account for up to 25% of nitrogen min-eralization in the soil (Rosswall and Paustian, 1984; Hunt et al., 1987; De Ruiter et al., 1993). This contribution might be even greater in the rhizosphere (Griffiths, 1990). Recently, Neher et al. (2012) showed nematode faunae in the mineral horizons of pine soils explain as

much as 6.9 and 12.4% of variation in concentrations of NO_3^- and NH_4^+, respectively.

Besides contributing to N and P mineralization, the abundance of many free-living nematodes, especially bacterial- and fungal-feeding nematodes, also correlate with concentrations of many other soil nutrients in fallow field plots that were previously treated with yard waste compost or not (Wang et al., 2004b). Studies in controlled environments reveal that bacterial-feeders are more involved in N mineralization, whereas fungal-feeders are more involved in P mineralization (Ingham et al., 1985). However, contamination with Cu was al-ways correlated negatively with the number of bacterial-feeders, but positively correlated with the number of fungal-feeders (Pitcher and Flegg, 1968; Wang et al., 2004b). This is because fungal-feeding nematodes (Fu_2) were more tolerant to pollutants and other disturbance (Bongers and Bongers, 1998), and fungal-feeding nem-atodes only become prominent as recalcitrant substrates (high in lignin and cellulose) accumulate in the habitat (Bouwman et al., 1994). Conversely, involvement of predatory nematodes in nutrient cycling is most likely an indirect process following the pattern of population densities of their nematode prey. Although Yeates and Wardle (1996) suggested that roles of predatory nema-todes in nutrient mineralization are receiving increased recognition, Laakso and Setälä (1999) suggest that their mechanisms still require further studies. The abundance of three omnivorous nematode taxa (in Ironidae, Leptolaimidae and Dorylaimellidae) increased with bacterial-feeding nematodes and NO_3^- in disturbed and undisturbed pine forests in North Carolina (Neher et al., 2012). Omnivorous nematodes contributed more to N mineralization by direct release of N from prey and indirect through accelerated turnover and predation by microbial grazers than other functional groups in-cluding bacterial-feeding nematodes, protozoa, fungal-feeders (nemaotdes, mites, Collembola), plant-parasitic nematodes, and predators (nematodes, Collembola, mites) in abandoned fields (Holtkamp et al., 2011). In a study by Wang et al. (2004b) using a fallow field site, EI correlated positively, whereas CI correlated nega-tively with soil nutrient content but with a higher cor-relation coefficient value than that obtained from their corresponding individual nematode trophic group analysis, indicating a perhaps more sensitive indicator of these indices than the trophic groups. In contrast, limited correlation of SI with most nutrients suggests that the overall number of omnivores and predators are not as important in nutrient cycling in this fallow agri-cultural field.

Redistribution of other microorganisms involved in nutrient cycling: Nematodes are relatively immobile in soil, moving only centimeters from or to food sources over a few days (Griffiths and Caul, 1992) but are also easily moved across ecosystems either in river systems, flood water and irrigation water or phoretically on insects.

Based on the fact that nematodes themselves can phoretically transport bacterial and fungal spores, either attached to the mucus outer layer or sometimes as viable cells excreted by the nematode, this means that nematodes can redistribute and inoculate microbes into new soil patches. *Caenorhabditis elegans* will transfer the N_2-fixing bacterium *Sinorhizobium meliloti* to the roots of *Medicago truncatula* under the control of plant derived volatile chemical signals (Horiuchi et al., 2005). Bacterial-feeding nematodes were also vectors for four strains of beneficial rhizobacteria to wheat roots (Knox et al., 2003). Unfortunately, harmful bacteria can be transmitted in this way as well. For example, *Salmonella* can be transmitted by bacterial-feeding nematodes to fruits and vegetables in contact with the soil (Gibbs et al., 2005) and coliform bacteria into a municipal water supply (Locas et al., 2007).

System stewardship to enhance desired services: In many cases the soil system has been managed to improve the soil conditions for crop production without specifically targeting nematodes, but in which nematode faunae proved an invaluable tool to monitor the effect of the management. In an example from soil that had been under continuous arable production for several years, amendment with cattle slurry or municipal green compost was introduced to improve soil structure and fertility, or as a comparison of the grass-phase or arable phase of a rotation system (Griffiths et al., 2010). Nematodes were one of a suite of biological, physical and chemical measurements taken and used to create a multi-attribute model to define soil quality (Fig 2). Nematodes proved useful to indicate soil biological activity which was increased by the organic amendments, but there were trade-offs with increased environmental losses and compaction that off-sets the benefits (Fig. 3).

More desirable nematode community services would be those projected on Quadrate B in the EI-SI trajectory described by Ferris et al. (2001) (Fig. 1. i.e. when EI > 50%, SI > 50%) where the soil food web condition is low in physical disturbance and chemical stressors, high in nutrient enrichment, balanced in decomposition channels, and matured. As mentioned above, one of the restrictions in achieving maximum service of nematode faunae in agroecosystem is the disturbance of omnivorous and predatory nematodes due to frequent soil cultivation.
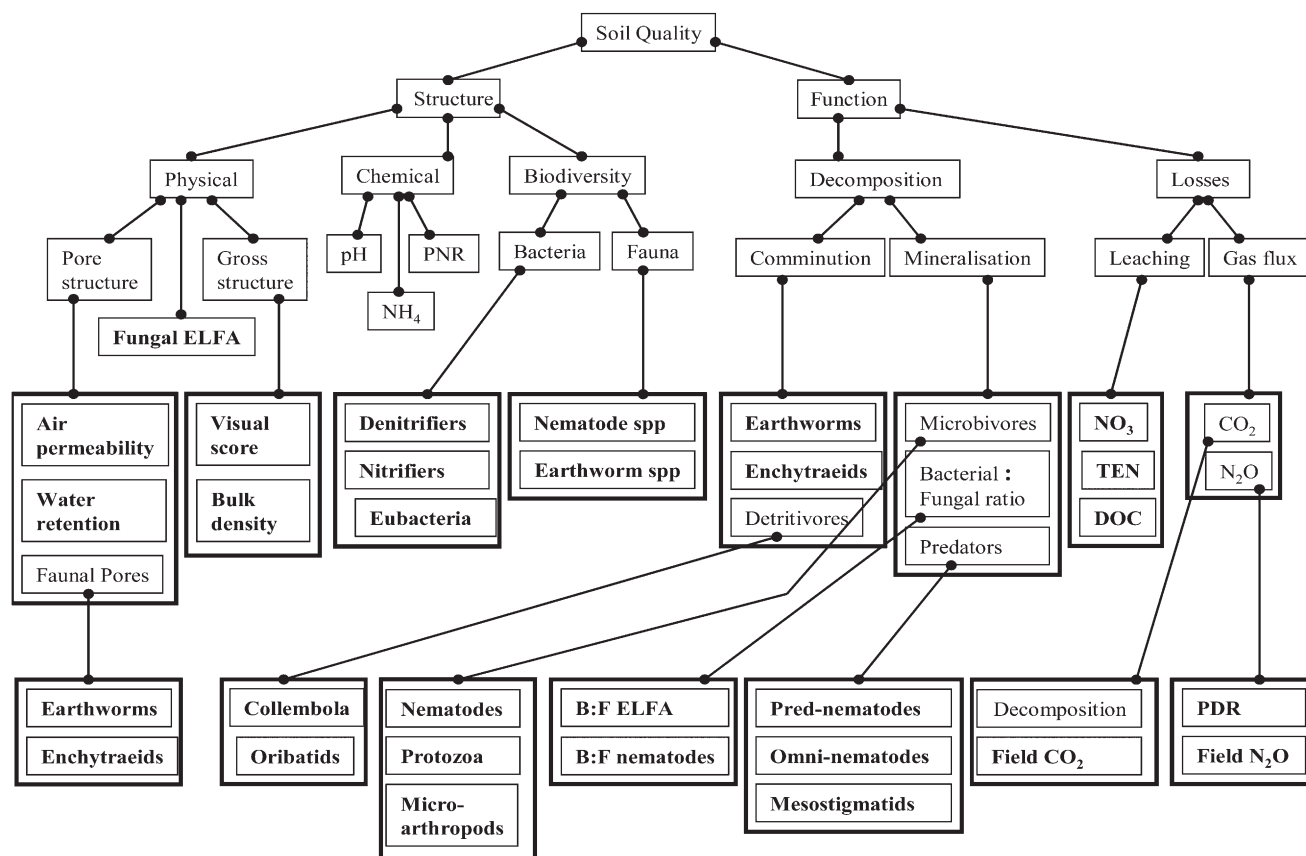

FIG. 2. Structure of attributes defining soil quality. The basic attributes were measured in the field and are in bold, while the aggregate attributes form the stages leading to soil quality. Attributes within a bold box aggregate to form a higher level attribute. Thus: Earthworms and Enchytraeids combine to give Faunal pores; Faunal pores, Water retention and Air permeability combine to give Pore structure; etc. It should be noted that Decomposition occurs twice as it contributes to both Function and Losses. The abbreviated attributes are: PNR – potential nitrification rate; PDR – potential denitrification rate; ELFA – ester linked fatty acids; TEN – total extractable nitrogen; DOC – dissolved organic carbon. Nematodes contribute to both the decomposition and biodiversity attributes of soil quality (from Griffiths et al., 2010).
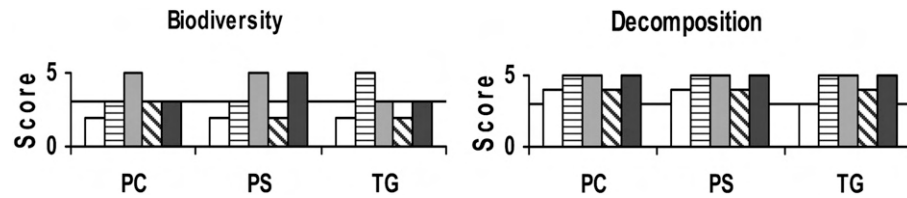
F<small>IG</small>. 3.   Score values from a soil quality multi-attribute model, for those attributes to which nematodes contributed, see Fig. 2, at field sites with added compost (PC) or slurry (PS) or in the grass phase of a grass – arable rotation (TG), sampled in May 2006 (□), Oct 2006 (▩), April 2007 (▨), June 2007 (▨) and Sept 2007 (■). A score of 3 means that the attribute was unaffected by the treatment (<15% different from the control treatment) while a score >3 indicates an improvement in the attribute and <3 a deterioration in the attribute (from Griffiths et al., 2010, derivation of score values are explained within).

Several attempts have been made to use cover crops in combination with conservation tillage practices to reduce soil disturbances and increase the abundance of soil organisms in higher positions in the soil food chain. However, these studies generally found that long-term conservation tillage (more than two years) is required before increased abundance of omnivorous or predaceous nematodes can be observed (Hanel, 2003; Minoshima et al., 2007; Sánchez-Moreno and Ferris, 2007; DuPont et al., 2009; Marahatta et al., 2010). However, what is puzzling is that incorporating a green manure such as sunn hemp (*Crotalaria juncea*) at 1% of soil weight in a greenhouse pot experiment increased the abundance of predaceous and omnivorous nematodes during eight weeks of yellow squash (*Cucurbita pepo*) growth (Wang et al., 2003). The challenge is to find a cover crop that can generate a high amount of residues in relatively short period of time and to maintain this green manure residue in the field over a cropping season. In experiments with cover cropping in a strip-till living mulch system, periodically clipping the living mulch to serve as surface organic mulch, Wang et al. (2011) were able to enhanced SI just in two cucurbit cropping cycles (i.e. < two years). Research is needed to mitigate further disturbance of agricultural practices on nematode faunae.

F<small>UTURE</small>: C<small>HALLENGES AND</small> O<small>PPORTUNITES</small>

Marriage of microscope and thermocycler – Mullis meets Cobb: Taxonomic studies of nematodes in North America go back to Nathan Cobb (1859-1932), the father of American nematology. During his career, Cobb described more than 1000 species of nematodes, terrestrial and marine alike, using microscope, slide, and photography techniques developed by him, later followed by others. This deeply rooted use of microscopy and morphology still features prominently in the field of nematode taxonomy. The microscope and morphology will continue to play a major role in taxonomic analyses of the future, but studies will be routinely augmented by high resolution molecular approaches. It is the expressed hope of many taxonomists and ecologists that the integration of approaches will help overcome the "taxonomic impediment" that limits the taxonomic contribution to studies

of biodiversity, systematics and ecology (Ebacha et al., 2011). It is well-documented that a significant gap exists between the number of described nematode species, and the total number of nematodes believed to inhabit the planet. Now enter applications of molecular methods widely used after the publication of Vistas on Nematology with the introduction of Taq polymerase in the polymerase chain reaction (PCR) technique by Kary Mullis (Mullis et al., 1986). The result has prompted some researchers to suggest that the most expeditious solution to removing the impediment of classical systematics is to forgo classical morphological approaches entirely, and use Molecular Operational Taxonomic Units (MOTUs) in place of formally described species. This suggestion has been widely criticized as an attempt to replace the historically and conceptually rich content inherent in Linnaean taxonomic practices with a "theoretically vacuous technology" (Wheeler 2004; 2008). While this debate may seem acrimonious to some observers, the outcome has to be considered positive in light of recent taxonomic studies in nematology that combine morphological and molecular approaches. In ecological terms, however, there remains a vital need to relate presence and abundance of particular nematode taxa, however quantified, to particular soil attributes or processes.

The vital linkage between DNA databases and species delimitation: Most studies of nematode faunae that employ DNA approaches use nucleotide sequence as a surrogate for species identity. There are two essential requirements for establishing the validity of this substitution. First, the species boundaries as determined through a comprehensive species delimitation process must be well understood with respect to the genetic marker. Secondly, the results generated by analysis of the genetic marker should be consistent with those species boundaries and apply to all members of the species. Unfortunately other than for select groups of plant-parasitic and bacterial-feeding nematodes few species satisfy both requirements. By necessity, early molecular diagnostic work used a few exemplar specimens to represent the species in comparative studies. Often, these exemplars were sent by a working taxonomist without associated morphological or physiological characterization and no provision for vouchers.

Consequently, misidentifications or previously un-recognized genetic variation may have entered the DNA databases with little recourse for correction.

Identification of nematode species using molecular tools can only be as good as the reference databases. These databases must be based on a solid foundation of good taxonomy. For nematodes, the wide use of SSU and LSU rDNA diagnostic markers have resulted in relatively well developed DNA sequence reference databases (e.g. ~7000 sequences for SSU; van Megen et al., 2009) covering many of the major phylogenetic clades. Implicit in studies using the SSU or LSU genes as diagnostic markers is the assumption that the nucleotide sequence of these genes provides sufficient resolution to recognize species. Given the wide range of potential modes of speciation in nematodes, exceptions to this assumption most likely exist. Similarly, it is occasionally assumed that nematode species will be characterized by a single SSU or LSU sequence. Within species variation has been documented for both genes. An acknowledgement of intraspecific variation, in part, underlies the use of MOTU cut-off values, which are designated levels of similarity that constitute within group (MOTU) membership. Of course, differences in rates and modes of speciation argue against genetic distance as a criterion for species membership and the application of a set cut-off value for species designation or any other taxonomic level. Researchers using a molecular barcode approach for nematode biodiversity assessment must be aware that MOTUs may recognize a species, a collection of species, or a subgroup within a species (Powers et al., 2011).

Applied potential – ecosystem analysis and diagnostics: An increasing requirement for environmental monitoring, within Europe the EU Soil Strategy (COM 2006a) describes the European Union (EU) soil policy in general terms, and the draft Soil Framework Directive (COM 2006b) proposes legally binding elements of that policy. Thus, there will be consequences for lack of implementation of soil indicator and monitoring schemes. Soil invertebrates are recognized as useful indicators as most are highly sensitive to disturbances and nematodes have been used as indicators of overall ecological condition because of the wide range of feeding types, and the fact that they reflect the succession stage of the systems in which they occur (Freckman, 1988; Bongers, 1990; Neher, 2001; Chen et al., 2010). Two, Europe-wide projects evaluating soil biological indicators both ranked nematodes highly as an important indicator of soil status (Bispo et al., 2009; Ritz et al., 2009). In increasing use of molecular biological tools to describe the nematode community (Wilson and Kakouli-Duarte, 2009; Chen et al., 2010) can only aid the integration of nematodes into national and international monitoring schemes. An additional outcome in the years to come from such undertakings will be a wealth of nematode data for meta-analyses (i.e. Mulder and Vonk, 2011).

Nematodes as Ecological Models: Many of the important recent advances in understanding of genetics, evolution, developmental biology and host-parasite interactions have involved the use of model organisms, including the *r*-strategist bacterial-feeding nematode, *Caenorhabditis elegans*. These studies are usually conducted in the laboratory allowing for control of environmental conditions and treatments, replication and ease of setup compared to field experiments. In the case of *C. elegans*, the studies are done in culture whereas assemblages of nematodes with other organisms are easily conducted in soil and aquatic microcosms. Application of the *C. elegans* model to address ecological questions is really just beginning.

Several toxicity tests with nematodes have been developed to determine the risk of chemicals to biota. Studies have been primarily concerned with finding lethal endpoints of metals and organic compounds in single species cultures, aqueous solution, soil and sediment dose bioassays (Sochová et al., 2006). Nematodes used in the laboratory bioassays are bacterial-feeding, opportunistic soil nematodes of *cp*-value life-history strategies 1 (e.g., *C. elegans* and *Panagrellus redivivus*) and 2 (e.g., *Plectus acuminatus*). *C. elegans* genetics clearly provides an advantage in toxicology tests as toxin effects can be directly linked to gene activities allowing for determination of sublethal effects (Martinez-Finley and Aschner, 2011).

That species within a community have different sensitivities to toxins and stressors is perhaps an advantage of nematodes as compared to other taxa in conducting environmental health studies. Ordination of nematodes into c-p groups has proven useful to predict sensitivity of taxa to stressors. For example, the survival of nematodes of high c-p groups in soil is reduced by chemical stressors, including nitrogen fertilizers (Kimpinski and Welch, 1971; Sohlenius, 1990; Yeates and King, 1997; Sarathchandra et al., 2001), swine slurry (Mahran et al., 2009), metals (Zullini and Peretti, 1986; Korthals et al., 2000; Georgieva et al., 2002), soil acidification (Dmowska, 1993), and nematicides (Smolik, 1983). An extension of such tests for acute toxicity assays involves subjecting nematode faunae extracted from soil to various stressor levels in solution. Using this approach, Kammenga et al. (1994) reported no variation in sensitivity of taxa within a nematode community to Cd and to pentachlorophenol whereas taxa of c-p groups 4 and 5 were shown to be more sensitive to Cu (Bongers et al., 2001) and to NH_4^+, NO_2^- and osmotic tension of solutions (Tenuta and Ferris, 2004).

Availability of numerous and convenient standardized culture bioassays allow nematologists and non-nematologists alike to take advantage of the *C. elegans* genetic model (Nass and Hamza, 2007). An exciting recent application of the *C. elegans* genetic model is to address ecology and evolution questions of broad concern to biologists. Examples of applications include a *Yersinia*

pestis-C. elegans model to show biofilm-mediated interactions between bacteria and predatory invertebrates (Darby et al., 2002), the advantage of sexual reproduction to increase developmental flexibility (male or hermaphrodite) of progeny under changing resource conditions (Prahlad et al., 2003), that starvation stress induces adult diapause as a means of survival and dispersion (Angelo and van Gilst, 2009; Kim et al., 2009), and modification of foraging strategies in response to environmental conditions (Boender et al., 2011). Other examples of recent use of the *C. elegans* model include showing that growth at high densities resulted in genetic changes to pheromone receptors (McGrath et al., 2011) and that sexual reproduction in *C. elegans* allows coevolution to survive against the pathogen *Serratia marcescens* (Morran et al., 2011).

POTENTIALS AND PREDICTIONS

Leaps in biology are associated with conceptual and technological innovation: While traditional approaches to nematode identification are sufficient for taxonomic work or studies of a few species and individuals, this approach becomes prohibitive in large scale ecological studies involving numerous samples each loaded with highly abundant and diverse taxa. The exploration of prokaryotic faunae using next generation sequencing (NGS) has proven indispensable in studies of prokaryotic diversity. As a result, current projections of biodiversity are orders of magnitude higher than previously thought (Sogin, 2009). Following the advances in the prokaryotic community, the NGS approach was developed for the assessment of nematode diversity. Because differences between prokaryotic and eukaryotic taxa (e.g., copy number of rDNA repeats, sequence variation between rDNA copies, number of cells) can skew biodiversity assays, a set of proof-of concept experiments with artificially assembled communities was conducted to establish a clear understanding between sequencing tags and nominal species using pyrosequencing of SSU and LSU rDNA diagnostic loci. Nematode faunae consisted of nematode species of known identity (morphology and sequences of diagnostic loci) and density and represented different phylogenetic clades, feeding habits, and sizes of body, and included distant as well as closely related species. (Porazinska et al., 2009, 2010a). The expectation was to recover sequencing tags of all nematode species in abundance relationships observed at the organismic level.

Results strongly supported the use of the NGS approach for nematode diversity assays as the patterns observed at the level of sequencing tags consistently and repeatedly matched the patterns at the level of species and individuals. Despite the general agreement between these two data sets, the results also indicated potential problems for precise sequence data interpretation. For instance, one individual of different nematode species can produce a significantly different number of sequencing tags (2 to 400), therefore the relationships among rare (tail) species could be impossible to deduct.

Experiments to better understand the rRNA copy numbers are needed to improve our inferences about abundances. But the results also indicated potential issues with recognizing diversity because tandem repeats within species and individuals are far from identical. Out of all sequencing tags matching a particular species, only ~50% were identical to the consensus reference sequence, about 35% of tags differed from the reference sequence by 1-2 bp, and the remaining tags differed by more than three bp. This variation points to the presence of a fundamental difference between specimen-based barcodes derived by traditional molecular methods (a consensus sequence) and metagenetic-based barcodes derived by NGS (a cloud of dominant and slightly variant sequences). Using these two concepts (i.e., blast-searching sequencing tags against consensus reference databases) indiscriminately can lead to erroneous overestimation of diversity by several orders of magnitude. Potential presence of closely related species in environmental samples can further complicate the insight about abundances as some of the variant sequences within a cloud could be shared between species and, thus, their origin would remain unresolved.

Specimen-based barcoding based on single-organism PCR typically generates a consensus sequence representative of the individual. Consensus sequences can then be clustered into MOTUs at various similarity levels to tie back to classical taxonomic categories. MOTUs within 97% similarity in Prokaryota, for instance, are typically considered the same species (Vandamme et al., 1996), but the exact boundary cutoffs for microbial eukaryotes are not known and likely vary among different taxa. Clustering methods applied to metagenetic-based barcode clouds typically produce multiple MOTUs per species at any preselected cut-off level leading to inflated estimates of diversity at any taxonomic characterization (Porazinska et al., 2010b). The presence of, highly predictable distribution patterns of MOTUs and their frequencies, however, allows to link these back to biological species (Porazinska et al., 2010b).

This proof-of-concept was later tested on real environmental samples from a tropical rainforest in Costa Rica previously described using the traditional approach (Powers et al., 2009; Porazinska et al., 2010c). The results and conclusions about nematode species diversity were nearly identical, and provided further evidence supporting the use of NGS at a fraction of labour and cost of the traditional methods.

Climate change effects/expectations: Concerns over loss of biodiversity and climate change were addressed in a simulation model for C and N transfers among grassland plants and soil biota (Hunt and Wall, 2002).

There appeared to be considerable flexibility (compensatory activity) within the soil food web consistent with the concepts of functional redundancy, although the stabilising mechanisms could not be ascertained, which leaves some uncertainty in the real world outcomes. A generalization regarding plant responses to elevated CO_2 is increasing N limitation as plant growth potential from increased CO_2 might outstrip soil N supply. However, the Hunt and Wall model clearly indicated that the soil food web (i.e., increases in fungal and faunal biomass) increased N availability to offset potential N limitation. Bacterial-feeding nematodes in the model accounted for 60% of faunal mineralization, yet if bacterial-feeding nematodes were deleted from the model (i.e., extreme biodiversity loss) the changes within the food web (i.e. more bacteria, fewer fungi, and increases in other bacterial-feeding fauna and reductions in nematode predators) were such that plant growth (net primary production) was unchanged. Another modelling approach was applied to a forest system but this time combining the effects of elevated CO_2 and UV-B radiation. In this case, there were negative effects on fungal-feeding nematodes and omnivores (Kuijper et al., 2005).

These models have some resonance with experimental data from FACE (free air CO_2 enrichment) systems in that elevated levels of CO_2 actually decreased nematode abundances in deciduous and coniferous forest soils (Neher et al., 2004), while Li et al. (2007) noted an interaction between elevated CO_2 and levels of N fertilization in a wheat system. They observed increases in omnivores and predators and changes to several ecological indices (i.e., MI, SI, NCR) (Li et al., 2007). Results from grassland systems tended to be system-specific. For example, in a seminatural temperate grassland, Sonnermann and Wolters (2005) only saw an effect on root-hair feeders and predators, which increased and then decreased over the three years of the study. The effect of elevated CO_2 increased the abundances of the root-feeder *Longidorus elongatus* over a nine-year study in a sheep-grazed pasture on sand but other root-feeders were unaffected and other nematode tropic groups and taxa not or marginally affected (Yeates et al., 2003; Yeates and Newton, 2009). Ayres et al. (2008) reported a neutral response of herbivores in three grassland systems, despite a large increase in root production, which they attributed to simultaneous antagonistic mechanisms.

Effects of altered soil temperature and moisture have been studied in fewer experiments. Papatheodorou et al. (2004) saw no synchronization between nematodes and their food resources generally, but the nematode response to altered conditions was taxa dependant. Similarly, Bakonyi et al. (2007) noted that *Cephalobus* and *Plectus* were associated with dried plots, while *Cervidellus, Ditylenchus, Eudorylaimus, Seinura* and *Thonus* were favoured in experimentally warmed plots.

Drying and warming effects on the soil nematode community were most pronounced in bare soil, less so in soil under poplar, while no significant effect was found in soil under *Fescue*.

New insights into ecological principles: Besides metagenetic sequencing, there are several other molecular techniques being developed to assist in nematode community analysis in the last two decades. For example, denaturing gradient gel electrophoresis (DGGE) was used to assay for nematode species richness in soil (Foucher and Wilson, 2002). This has been further developed and subsequently used for the comparison of nematode faunae in soil (Takemoto et al., 2010). Since one of the main purposes of developing molecular approaches for nematode faunal analysis is to reduce time and labor of skilled taxonomists used for a conventional morphological approach, a group from UK and Ireland are developing directed terminal-restriction fragment length polymorphism (dT-RFLP) for rapid assessment of soil nematode faunae (Griffiths et al., 2006; Donn et al., 2011). An alternative approach, taken by a Dutch group, is to quantify the abundance of key nematode taxa by qPCR (Neilson et al., 2009) based on an updated nematode phylogeny (Van Megan et al., 2009). Ability to quantify nematode abundance in each key feeding guild is critical for performing nematode faunal analysis. Thus, development of rapid assessment of soil nematode faunae should involve developing methods that allow the calculation of nematode community indices.

Another area of interest in nematode ecology is determining or confirming the feeding preference (trophic group) of certain nematodes. Current categorization of nematode feeding groups relies mainly on nematode morphology (Yeates et al., 1993). Future research on nematode ecology could also use molecular techniques and stable isotope chemistry (Moens et al., 2005) to assist in determining or confirming the trophic groups of some ambiguous species.

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