

Using nematodes in soil ecotoxicology

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Abstract

Nematodes represent a very abundant group of soil organisms and non-parasitic species are important for soil quality and in the soil food web. In recent years, it has been shown that nematodes are appropriate bioindicators of soil condition and they are also suitable organisms for laboratory toxicity testing. The aims of this paper are to overview and critically assess methods and approaches for researching soil nematode ecotoxicology. In natural ecosystems, nematode abundance and community structure analyses were proved to be sensitive indicators of stress caused by soil pollutants and ecological disturbance. Community structure analyses may be approached from a functional or ecological point of view; species are divided into groups according to their feeding habits or alternatively the maturity index is calculated according to their ecological strategy. Many environmental factors have the potential to affect nematode community, which consequently results in high space and time variability. This variance is major handicap in field ecotoxicological studies because pollutant–nematode relationships are obscured. For prospective risk assessment of chemicals, several toxicity tests with nematodes were developed and are increasingly used. Sensitivity of these tests is comparable to tests with other soil species (e.g. enchytraeids, earthworms and springtails) while tests are less demanding to space and time. Most studies have focused on metal toxicity but organic compounds are almost overlooked. Endpoints used in tests were often mortality, reproduction or movement, but more sublethal endpoints such as feeding or biomarkers have been used recently too. Although there is an increasing amount of knowledge in soil nematode ecotoxicology, there is still a lot of various issues in this topic to research.

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1. Introduction

The soil is a crucial component of terrestrial ecosystems due to its key functions in fertility, decomposition processes, nutrient and energy flows. Most of these functions are performed or mediated by soil microorganisms and invertebrates. Unfortunately, there is an apparent deterioration of the soil due to chemical contamination, insensitive agricultural and forest management, erosion, and also as a result of contact with contaminated air and water. This situation has revealed increased interest in the protection of soil quality, which is now also included under European environment policy (European Parliament, 2002). Soil ecotoxicology forms scientific basis for effort to protect soil by studying the relationships between contamination and effects on important soil organ-

isms. The ecotoxicology research can be realized by monitoring the status of communities in real ecosystems or through the use of laboratory toxicity tests (bioassays). A wide variety of soil organisms have been used for both approaches including microorganisms, earthworms, enchytraeids, springtails, mites, and insects (van Straalen and van Gestel, 1993; van Gestel and van Straalen, 1994; Løkke and van Gestel, 1998; Cortet et al., 1999).

Nematodes are the most abundant metazoa in soil and their function is irreplaceable in soil food web. They interact closely with other soil organisms and their activity affects primary production, decomposition, energy flows, and nutrient cycling, especially nitrogen cycle (Sohlenius, 1980; Freckman and Baldwin, 1990). With their permeable cuticle they are in direct contact with xenobiotics in interstitial water and may be adversely affected. Regarding their significance in soil environment, nematodes should be considered seriously in research focused on effects of soil contamination. In the last 15 years, there has been a vast development of research into nematode

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ecotoxicology and ecology, both in field studies and laboratory experiments. The objective of this review is to summarize the use of nematodes in soil ecotoxicology and also critically assess some issues in this area.

2. Field studies

Research in nematode taxonomy and identification has very long tradition and there are over 15,000 species identified (Poinar, 1983). Many problems arise in estimating nematode populations in soils, as nematode distribution varies horizontally and vertically due to soil heterogeneity and there is also substantial fluctuation of parameters in time. Neher et al. (1995) found that population means and variance of the sampled area were similar when sampled using different strategies, patterns, directions and repetitions. On the other hand, community structure was associated with relatively large variance even within composite soil sample. The main objective of soil ecotoxicology in field studies is to research the causal relationship between pollution and biological parameters. An adequate and representative sampling procedure and sampling plan are hence crucial. Field study design should handle with natural (background) variability and should be able to separate this from effects to be examined. Sampling plan should be always site specific and consider the survey objectives. However, this is hard to achieve practically. Nematode extraction from soil samples is another critical step in field studies. Numerous extraction techniques were developed including Baermann incubation, centrifugal flotation with silica, sucrose, or $MgSO_4$, the Seinshorst elutriator, Cobb's decanting and sieving, the Whitehead direct method, and others (summarized e.g. in Freckman and Baldwin, 1990). As may be expected, they differ in total efficiency, as was proved in many studies (Persmark et al., 1992; Nagy, 1996; McSorley and Walter, 1991). It was also found that they vary in efficiency for different nematode groups and consequently, they may affect perception of the nematode community structure (McSorley and Walter, 1991; McSorley and Frederick, 2004; Neher et al., 1995).

It has been frequently concluded that not total nematode abundance but community structure and diversity are the most sensitive traits to environmental stress (Yeates and Bongers, 1999). Although many keys exist for classification to particular species, such detailed identification is not necessary for the evaluation of soil nematode community structure. Ecological and ecotoxicological researches request to consider nematode function and ecological importance predominantly. Nematodes are allocated a functional group (guild), mostly according to their feeding strategy (Yeates et al., 1993): (1) plant feeders, (2) fungal feeders, (3) bacterial feeders, (4) substrate ingesters, (5) animal predators, (6) unicellular eukaryote feeders, (7) dispersal or infective stages of animal parasites, and (8) omnivores. The morphology of the nematode stoma is important marker for allocating a particular trophic group. Nematodes in different trophic groups exhibit varying sensitivity to soil contaminants (Korthals et al., 1996b; Korthals et al., 2000; Bongers et al., 2001). Ecotoxicological field studies

have been focused mainly on heavy metal soil pollution. Effects on the nematode community were assessed after 10 years of exposure to different levels of copper and pH in agroecosystem (Korthals et al., 1996a). Effects were studied of elevated heavy metal content in soils as a result of sludge application (Weiss and Larink, 1991; Yeates et al., 2003) or through the use of timber preservatives (Yeates et al., 1994; Bardgett et al., 1994).

A sensitive bioindicative approach of soil condition is based upon life strategies of nematodes. Nematode species are categorized with colonizer–persister (cp) values (Bongers, 1990). The cp scale corresponds to r–K strategy: cp 1 (colonizers, opportunists) are r-strategists, cp 5 (persisters) are K-strategists. Furthermore, diversity is expressed using the following indices: Maturity Index—MI (Bongers, 1990, 1999), Plant Parasite Index—PPI (Bongers, 1990), PPI/MI ratio (Bongers et al., 1997), or the Sum Maturity Index (Yeates, 1994). MI is calculated as the (weighted) mean cp-value for all individual nematodes in a representative soil sample with the exception of plant parasites (Bongers, 1990, 1999). The PPI, calculated separately, includes plant feeding nematodes and is referred to as a sensitive index of ecosystem development (Bongers, 1990; Yeates, 1994). The value of MI ranges from 1 (polluted or eutrophicated ecosystems) to approximately 4 (undisturbed conditions). The MI has been shown to be a sensitive indicator of soil recovery following environmental disturbances (Ettema et al., 1998), and variations in agricultural management (Freckman and Ettema, 1993; Neher and Campbell, 1994). The MI has also been used many times for measuring pollution-induced stress, especially by heavy metals (Korthals et al., 1998, 1996b; Yeates and Bongers, 1999; Yeates et al., 2003). Maturity indices, which differentiated among sampling sites better and more efficiently than trophic diversity indices or measures based on populations of individual trophic groups, may be appropriate for use in a regional and/or national monitoring programs (Neher et al., 1995). In some studies, classical measures of diversity (e.g. Shannon index) have been also successfully used to characterize nematode community changes under stress (Háněl, 2003; Smit et al., 2002; Yeates and Bongers, 1999; Wasilewska, 1997).

The soil environment is a very complex ecosystem with many factors affecting soil biota. According to Sarathchandra et al. (2001) and Háněl (2003), differences in nematode abundance and diversity arise mainly as a result of soil management practices. It has been found, that nematode communities are highly variable and differ significantly among microhabitats with different substrate structure, or biotic and abiotic conditions (Lazarova et al., 2004) and large variance must be expected even within composite soil sample (Neher et al., 1995). In field studies, high variability in biotic parameters is inherent, usually present, and has to be considered seriously, when effect of soil pollution is to be studied. Moreover, the investigation of nematode–pollutant relationship in field studies can be strongly complicated by influences of soil environment. For example, soil nematode communities may become stressed by a number of environmental factors (e.g.

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