TOOL WORKSHEET NO.7

Nematofauna

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DESCRIPTION OF THE INDICATOR

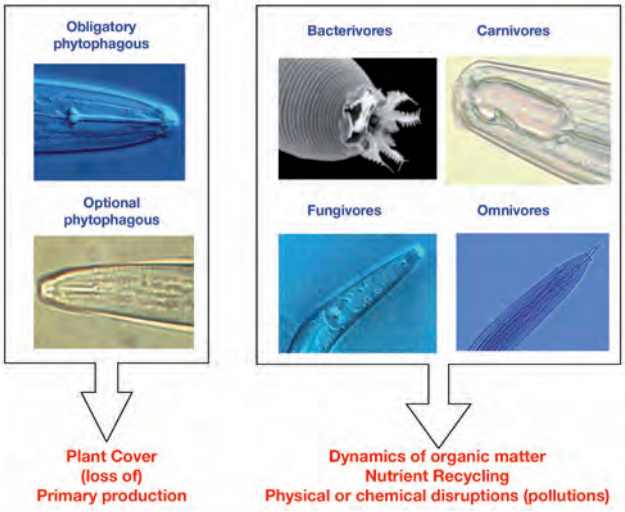
Name of the indicator: Nematofauna = the community of free-living and plant-parasitic nematodes living in the soil. Soil nematodes are microscopic worms (approximately 1 millimetre long).

Ecological role of the organism under test: These organisms are good bioindicators for many reasons:

- they are ubiquitous: present in all habitats, under all climates, all over the world;
- they are abundant: in ordinary soil there is about 1 million nematodes per m^2;
- they show great taxonomic and functional diversity (varied feeding habits, variable ability to colonise habitats) that can be summarised by the identification of the 70 main families;
- they play a key role in the soil foodweb, in particular, role of regulation of soil microorganisms;
- they are sensitive to habitat conditions and physical or chemical disruptions.

From a functional standpoint, nematodes can be differentiated according to their alimentary behaviour in the soil. Each of the trophic groups reflects one of the functionalities of soil:

- plant-parasitic nematodes (obligatory or facultative) tell us about the nature and state of plant cover and, on the risk of yield loss;
- microbivorous nematodes (bacterivores and fungivores) inform on the microbial compartment, dynamics of organic matter and nutrient recycling;
- nematodes from higher trophic levels (omnivores and carnivores) reflect physical or chemical disruptions of the habitat.



Type of indicator: Bioindicators of effect and impact.

DESCRIPTION OF THE SAMPLING METHOD

Norms describing the methods: Methods are standardised: NF EN ISO 23611-4 Oct 2011.[1]

Sampling plan and method: Nematologic analysis is conducted based on composite soil samples from 300 to 500 g. Soil sampling is simple (cores are taken in the 0-15 cm layer of the soil) and can be led by site managers. Sampling can be realised throughout the year; however, spring and autumn are the most favourable periods.

Storage and pre-treatment of samples: Analysis requires hiring a specialised laboratory, where soil samples can be sent to immediately after collecting. Samples have to be treated in the next 15 days after their collection, because nematodes need to be alive for analysis.

1 Page 1) NF EN ISO 23611-4 Oct 2011 STANDARD. Soil quality - Sampling of soil invertebrates – Part 4: Collection, extraction and identification of soil nematodes

Simplified description of the measurement method: At the laboratory, nematodes are extracted from soil samples using the elutriation technique (separation of nematodes from other soil particles by density in a water stream), followed by an active passage through a cotton-wool filter. Nematodes are subsequently counted under a binocular magnifier. After their fixation, they are identified (families and/or genera) using optical microscope based on the norm. This method helps building a table of the abundances of nematode tax a serving as a basis for diagnosis.

Laboratory work on soil samples: step of determination of the abundance by counting under a binocular magnifier

Measured parameters: On the basis of the composition and abundance of soil Nematofauna, several indices are calculated:

- SI: Structure Index which reflects the stability of habitat: the higher it is, the least disruptions there are in the soil. It depends on the relative abundance of several families (bacterivores, fungivores, omnivores and predators);
- EI: Enrichment Index which gives an indication of the dynamics of nutrients. This index is particularly of use in agro-systems. EI increases with the availability of nutrients, in particular nitrogen.
- Three other indexes, MI (Maturity Index), PPI (PhytoPhagous nematodes Index) and NCR (Nematode Channel Ratio) are also used to determine the effect of various disruptions / stress / soil practices.

Each soil is therefore characterised by the abundances of different functional groups of nematodes and by nematofaunistic indices.

INTERPRETATION OF RESULTS

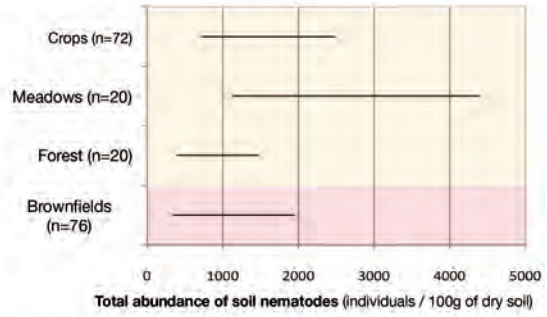


Figure 1: Variation range of total nematode abundance on sites of Bioindicateurs 2 program

Use of a global frame of reference:

A sole analysis of soil nematofauna can be used to characterise the biological functioning of soil on a site using a frame of referenced based on previous studies (ELISOL© frame of reference), continuously implemented by the results of new studies.

Use of a local frame of reference:

These studies of bio-indication reach full potential when data interpretation can be done in regard to a chosen situation of reference in the studied site. More generally, two types of studies are possible (1) synchronic studies: different modalities are simultaneously compared under the same pedo-climatic conditions, (2): diachronic study: a situation is studied at several successive dates to assess its evolution. The nature of soil, its climate as well as vegetation are major factors affecting biological communities; it is thus preferable to reduce the number of factors simultaneously varying in order to finely interpret data from analyses. For contaminated sites, the analysis of a control non-contaminated situation is preferable to be used as a reference situation.

EXAMPLE OF APPLICATION

Application on an issue related to a contaminated area: the METALEUROP site

The study was conducted in the contamination area of a former metallurgical plant which dust fallout led to an essentially pluri-metallic contamination of soils in the surrounding several kilometres.

In order to test sensitivity of soil nematofauna, in regard to the level of contamination, 4 woods (plurispecific plantations) differing in the level of contamination were studied (1) highly contaminated (main pollutants: [Pb]= 2500 ppm – [Cd]= 35 ppm), (2) moderately contaminated ([Pb]= 725 ppm – [Cd]= 13 ppm), (3) weakly contaminated ([Pb]= 325 ppm – [Cd]= 5 ppm) and (4) control ([Pb]= 50 ppm – [Cd]= 1 ppm).

As figure 2 shows, the abundance of three main types of soil nematodes, it demonstrates that:

- Abundance of all types of nematodes diminishes very gradually when concentration increases.
- Omnivorous and carnivorous nematodes, which are known for their sensitivity to pollutants, are present in very low densities for all three contaminated sites compared with the control site.

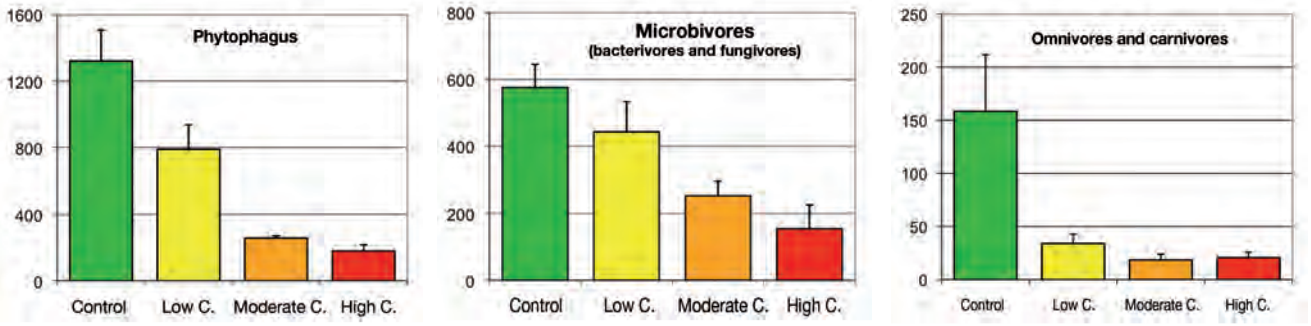
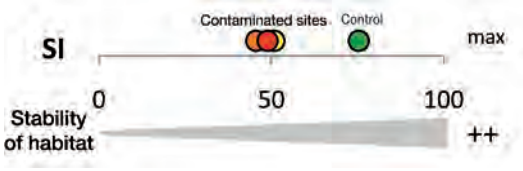


Figure 2: Abundance (individuals for 100g of dry soil) of three main types of soil nematodes

The Structure Index (SI) allows us to differentiate the control wood from contaminated woods (figure 3). In fact, the SI is higher for the control wood than for contaminated woods. This index, calculated by taking into consideration relative abundance of different functional guilds of nematodes, shows that the control wood hosts a nematode community that is more complex and where sensible taxons are present.

Figure 3: Structure Index (SI)



Summary of the "Contaminated site" case study:

The "Nematofauna" indicator is relevant for the evaluation of the state of soil in contaminated areas: the simultaneous analysis of abundance of different groups of nematodes and SI highlights the degradation of the biological state of soil when metallic contamination increases; this degradation is gradual only for specific parameters.

Application to an agricultural issue: the GOTHERON site

The studied agricultural area is an apple orchard in the Rhone Valley (INRA station) that has been cultivated for the last 5 years based on three practices: CONVENTIONAL (R: sustainable agriculture + mineral fertilisation), ORGANIC (B: organic phytosanitary products + organic amendments) and LOW INPUT (E: low phytosanitary inputs + mineral fertilisation). Furthermore, two cultivars are present on the test area: Smoothee , sensitive to scab (major apple tree disease) and Ariane, far less sensitive; Smoothee requires more phytosanitary treatments to ensure quality of production.

The Enrichment Index (EI) shows that under CONVENTIONAL practices, more nutrients are available in the soil than with the two other types of practices. The Structure Index (SI) demonstrates that the LOW INPUT practices ensure the development of a trophic micro-chain that is more complex and longer (more stable soil) than with the two other sets of practices.

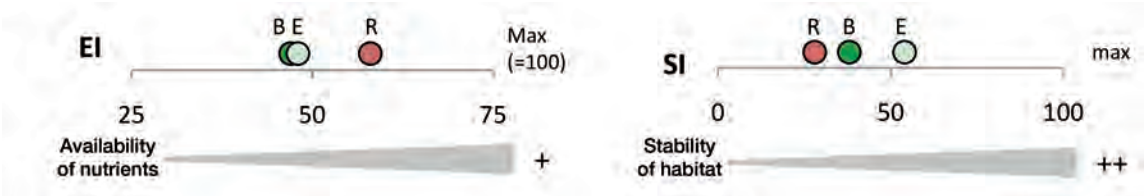


Figure 4 : Enrichment Index (EI) and Structure Index (SI)

Smoothee cultivar and the practices of its associated orchards lead to more plant-parasitic nematodes and less living nematodes (except with CONVENTIONAL practices) than Ariane cultivar, as shown by figure 5. The increased use of pesticides, with Smoothee cultivar which is sensitive to scab, has a measurable negative impact on soil life: decrease in abundance of micro-regulators and increase in bio-aggressors.

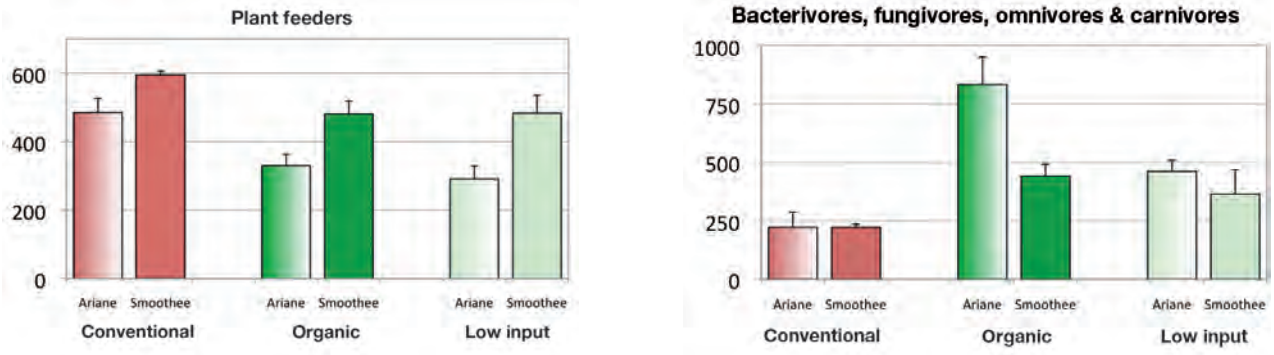


Figure 5: Abundances (individuals for 100g of dry soil) of soil plant parasitic nematodes and free nematodes (= bacterivores, fungivores, omnivores & carnivores)

Summary of the "Agricultural site" case study:

The "Nematofauna" indicator enables the quantification of effects of several types of practices (conventional, organic and low input) in orchard as well as practices related to apple tree cultivar on the soil functioning. The simultaneous analysis of abundance in different groups of nematodes, EI and SI highlights modifications of the functioning of the soil. Therefore, this indicator can be used as a tool for decision-making when evaluating agricultural practices.

INTERESTS AND LIMITS OF THE INDICATOR

Limits:

1 Need of analysing freshly sampled soils: the first step of analysis has to be done within the 15 days following soil collection, on soils preserved under good conditions, because nematodes must remain alive.

2 In the event of a study of multiple-impact situations (uses x practices x types of soil…), as different factors influence soil organisms, it can be difficult to identify explicative factors. In this case, studying a control situation is recommended to have a basis for comparison.

Advantages :

1 One single analysis provides a lot of information, which integrates all factors impacting the soil, and tells us of the different functions of soil: (1) global level of biological activity, (2) availability of nutrients, (3) stability / level of disruption of soil, (4) risk of dammages by plant-parasitic nematodes on crops, (5) size of the microbial compartment.

2 These indicators are operational and easy to interpret because, for each parameter, the variation range is documented and they show great value "diagnosis of the functioning of soil" for cost due to easy implementation of the method.

ELISOL ENVIRONNEMENT *is an innovative company founded in 2011 which values results of scientific research in the field of soil biology. The company conducts soil quality studies based on soil organisms and, in particular, nematodes; it also is a laboratory of phytonematologic analyses and a training institute specialised in soil biology.*

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