Perspectives and limitations in assessing side-effects of pesticides on the soil microflora

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ABSTRACT

A desirable evolution towards sustainable agriculture requires taking into account potential harmful side effects of pesticides on the soil microflora. There are currently two main ways of monitoring these damaging consequences. In the past, size and activity descriptors of soil microbial communities were used. They have been criticised on the grounds that they are the net outcome of complex physiological and structural rearrangements of the soil microflora and lack the necessary sensitivity. Recent progress in analysis of microbial diversity at the species, metabolic, physiological, and genetic levels offers new promising alternatives. They have also some technical and methodological limitations.

Present State of the Art

Soil micro-organisms are critical determinants of soil production capacity, or soil fertility, in activating the turnover of organic matter and recycling nutrients for plants. They have also an environmental safe-guarding activity as they are prominent agents in the decontamination of soils and in the protection of air and water quality. More recently, it has been recognised that they have an evolutionary role in maintaining a gene pool from which new genotypes can emerge in response to selective pressures resulting from changes in land use or management practices [8]. As biocides, pesticides are a potential threat for soil micro-organisms and in the long term may alter their productive, protective and adaptive capacities. If sustainable agriculture is to be promoted and integrity of the gene pool maintained, it is necessary that the possible harmful side-effects of pesticides on the soil microflora be properly assessed. Moreover, from an ecotoxicological standpoint, the ubiquitous presence of micro-organisms and their intimate contact with the soil environment make them ideal monitors of soil pollution [4].

Government and international agencies are asking for tests that can be used in the framework of regulatory guidelines. Up to recent years, assays were designed to satisfy requirements for relevance to soil fertility, reliability of measurement and suitability for routine testing [5]. This approach has resulted in the selection of microbial assays with practical significance and high representativity, but with low sensitivity because of high redundancy. Risk is currently assessed through C and N mineralisation tests or biomass measurements and enumerations performed at the community level. The observed net changes result from a combination of physiological adaptation of some microbial species and rearrangements in the structure of natural microflora with some species proliferating due to enhanced tolerance to the environmental pressure. So, there is a need for additional assays that better evaluate ecotoxicological impacts of pesticides on the structure of soil microbial communities.

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New Perspectives

As already suggested [15], the term indicator refers to a test of a microbial population (monospecific) or community (plurispecific). A number of features or properties can serve as additional descriptors for the definition of indices of soil pollution or ecosystem perturbation (Figure 1).

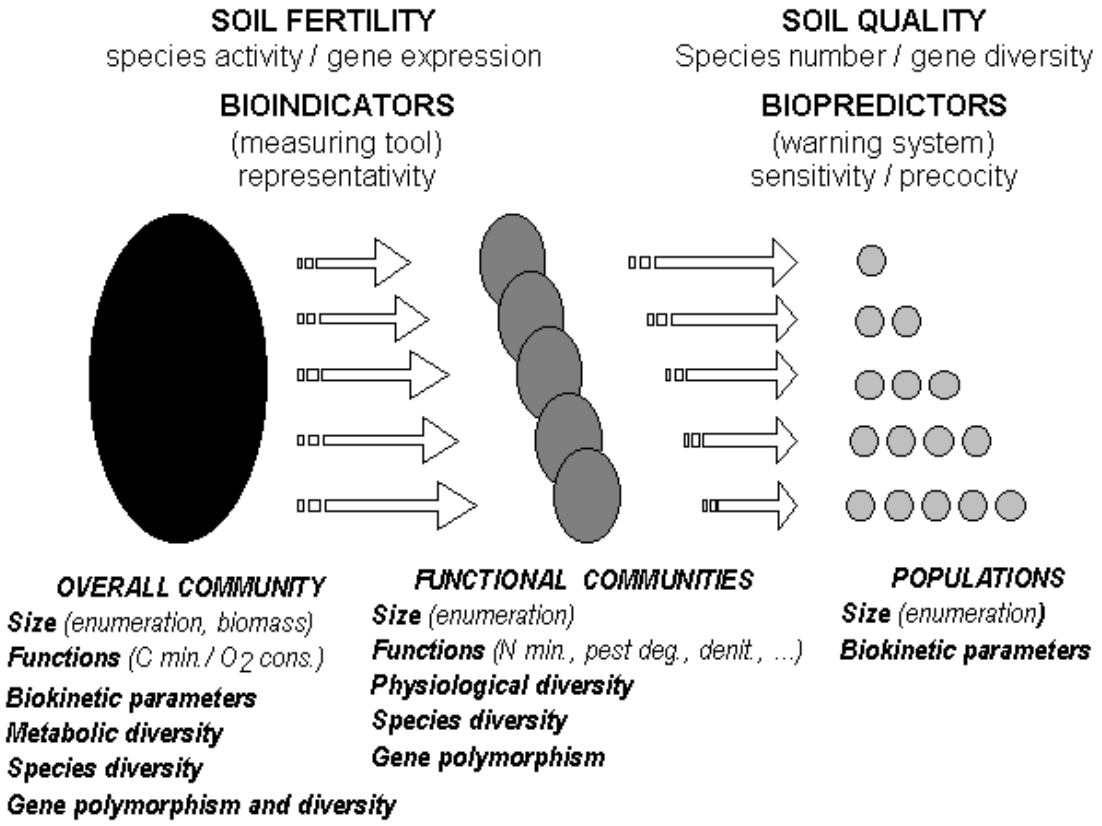

Fig. 1. Different types of microbial bioindicators based on different degree of organisation and possible descriptors that can be used for the definition of indices of ecotoxicological disturbance.

Two criteria, the level of organisation of the test microbial group on the one hand and some constitutive or functional descriptor on the other hand, can be combined to construct a framework for selecting indices as a function of a double questioning: are we interested in maintaining soil productivity or in safe-guarding soil biological quality? do we want to measure existing damage or predict and protect the soil microflora from undesirable side-effects?

For a long time, soil microbiology has accepted two basic simplifying concepts: the microbial community as a whole should be considered as an appropriate bioindicator and the functional aspects, "not the description of its individuals, are of prime importance" [6]. This statement assumes that the integrative value of overall functional indices makes them more relevant from an operational point of view as compared to more analytical structural indices. But, new attitudes are necessary for maintaining the integrity of the different ecological roles played by the soil microflora. For instance, the increasing awareness of the

soil as a source of genetic novelty and as a repository of genetic resources which can have a potential biotechnological value [13] has stimulated recent interest in the definition and evaluation of soil quality. Defining the concept of soil quality has proved to be a difficult exercise. However, there is a general agreement in considering that soil microbial diversity is a key property of what constitutes a high-quality soil. Progress needs to be made in the methodology used to describe microbial reservoir, and to estimate how microbial diversity can be modified by the presence of toxicants. From a practical point of view, the identification and the monitoring of microbial species showing particular sensitivity or resistance to toxicants of different chemical nature would be of particular interest in defining biosensors with the potential to be used as early warning systems for predicting and preventing more acute and extended ecological alterations.

It has been assumed that high microbial diversity would facilitate tolerance to the input of pollutants and resilience after they have disappeared. The flow of energy through different pathways ensures that systems will continue to operate under stressed conditions. Biodiversity can be seen as an upper-level element of regulation which ensures functional stability and confers robustness to the soil system [9]. Structural changes that affect microbial communities in response to chemical stress can be regarded as early and sensitive records of ecosystem disturbance. There is still limited evidence demonstrating the potential use of microbial diversity measurements to assess side-effects of pesticides on the soil microflora [2]. We also need additional progress in the analysis of the soil microbial community to answer questions such as: is there an optimum level of biodiversity for agricultural soils [9]? Is there a lowest threshold level of biodiversity below which productive and ecological functions of the soil microflora could be irreversibly altered? A prerequisite is the understanding of the causal relationship between structure and activity of microbial communities [1]. This relation is likely to be more successfully investigated using microbial physiological groups which have an intermediate level of structural complexity and show limited capacities for repair of externally induced failure in their ecological function. Nitrifiers, denitrifiers, pesticide degraders which are represented in the soil by very low numbers of different members are examples of this category of bioindicators which could have the potential to be quite reactive to toxicological stimuli. Since they show lower biodiversity and live under permanent nutrient stress, oligotrophic communities have also been postulated to be highly sensitive to toxicants [5].

Limitations

Limitations in data acquisition

There are a number of new analytical methods that can be used to evaluate microbial diversity at the species, metabolic, physiological, and genetic levels which cover different and complementary aspects of microbial diversity. Combinations between different biological “preparations” [intact natural microbial communities, culturable or extractable micro-oganisms, extractable DNA] and different analytical procedures give differently biased pictures of the structure of the soil microflora. Two categories of limitations can be derived, some at the methodological level others at the technical level.

The basic nature of the biological material to be investigated is probably one of the most serious limitations. It has long been recognised that in the soil environment culturable micro-organisms represent no more than 1 to 5% of what can be seen under the

microscope. Moreover, the composition of the culturable fraction of the soil community is likely to be dominated by r-strategists or opportunistic organisms taking advantage of nutrient concentrations in culture media which greatly exceed those that are commonly found in soils. Direct extraction of microbial cells [3] or DNA [15] from soil has been presented as a solution to the problem of representativity by eliminating the selective isolation and cultivation steps. It is not certain that much gain is to be expected from these alternative approaches. No unbiased calibration techniques are now available to control the efficiency of extraction procedures which select for the more abundant or available free-living species and for community members at physiological states which make cell lysis easier [12]. Moreover, processing of extracted material for the recognition of the different species that make up the sample community is often based on PCR amplifications of families of homologous gene sequences which are selected either to reflect species diversity [16S rRNA gene] or gene polymorphism. Although PCR is a powerful tool, there is now evidence that this procedure contributes to increase the bias in the profiling of microbial communities. This results from the preferential amplification of some sequences which bind preferentially with the primers [11] or the formation of chimeric molecules [16].

Another source of inconsistencies in the description of microbial diversity comes from the definition of the species concept, which has yet to be found suitable for general use with bacterial and fungal communities [10]. This is particularly true when trying to differentiate between strains, based on a set of phenotypic characters. For the purpose of inventorying species and quantifying microbial diversity from a collection of isolates, significant progress has been made with the advent of molecular techniques. For environmental applications, technical progress needs to be made to fill the tremendous gap between the visible and the hidden parts of biodiversity. Moreover, in the context of a functional approach of natural ecosystems, it is appropriate to ask the following questions: should the species concept be adapted when the elementary functional units of the soil system are often represented by microbial associations? Should taxonomic distance and the level of metabolic activity of the functional units have additional significance as weighting factors for the quantification of biodiversity?

Limitations in data interpretation

Changes in soil microbial properties in the presence of pollutants are generally more easily perceived in controlled laboratory conditions. Detection of functional defects and structural shifts at one place and on one occasion have no real meaning but for the sample under investigation. The question is: what is its representativity? The answer is in the definition of an appropriate sampling design. The actual significance is to be appreciated by reference to natural temporal and spatial variations which condition the distribution of the microbial species and processes in natural environments. It has been argued that the background of naturally-occurring depressions which soil micro-organisms have to suffer should offer the framework for the interpretation of ecotoxicological data [7]. This ecological concept for the assessment of side-effects of agrochemicals on soil micro-organisms would be of particular value when considering spatial and temporal effects which control the variability of the species composition of the microflora. This structure of the microbial community is likely to be highly dependent on the fluctuations of the soil's fundamental characteristics and properties. As demonstrated in macroecology, a prerequisite to address the question of the significance of the ecotoxicological impacts is to search, or define, observation scales

which can reveal specific levels in spatial organisation and temporal succession. Those levels of organisation that show stable variation probably reflect the presence of specific predominating microbial associations that tend to respond functionally to local conditions. It can be expected that appreciation of the tolerance and resilience of these al functionally relevant combinations would give an overall view of the reactivity of the soil microflora to agrochemicals or other toxicants.

Limitations

Because micro-organisms are directly exposed to soil pollution, microbial assays can provide early evidence of changes that, in the long term, can affect soil fertility and, more generally, soil quality. As compared to functional descriptors of the overall metabolic activity which have good representativity but low sensitivity due to high redundancy, the description of different aspects of microbial diversity can contribute to the definition of alternative potentially more sensitive indices of soil pollution. Overcoming some methodological and technical limitations is required to obtain more reliable data on the shifts that affect metabolic patterns and species distributions of the targeted natural communities taken as bioindicators of ecological impacts. Consideration of physiological groups comprising a small number of species may offer the additional possibility of linking biodiversity to function. Finally, the interpretation of microbial indices of soil pollution must refer to natural temporal and spatial variations and take account of scale effects which can reveal stratification in space organisation, regionalisation and temporal succession.

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