Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
Spanish Journal of Agricultural Research 2010, 8(S1), S137-S143
ISSN: 1695-971-X

Development of soil quality metrics using mycorrhizal fungi

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Abstract

Based on the Treaty on Biological Diversity of Rio de Janeiro in 1992 for maintaining and increasing biodiversity, several countries have started programmes monitoring soil quality and the above- and below ground biodiversity. Within the European Union, policy makers are working on legislation for soil protection and management. Therefore, indicators are needed to monitor the status of the soils and these indicators reflecting the soil quality, can be integrated in working standards or soil quality metrics. Soil micro-organisms, particularly arbuscular mycorrhizal fungi (AMF), are indicative of soil changes. These soil fungi live in symbiosis with the great majority of plants and are sensitive to changes in the physico-chemical conditions of the soil. The aim of this study was to investigate whether AMF are reliable and sensitive indicators for disturbances in the soils and can be used for the development of soil quality metrics. Also, it was studied whether soil quality metrics based on AMF meet requirements to applicability by users and policy makers. Ecological criterions were set for the development of soil quality metrics for different soils. Multiple root samples containing AMF from various locations in The Netherlands were analyzed. The results of the analyses were related to the defined criterions. This resulted in two soil quality metrics, one for sandy soils and a second one for clay soils, with six different categories ranging from very bad to very good. These soil quality metrics meet the majority of requirements for applicability and are potentially useful for the development of legislations for the protection of soil quality.

Additional key words: arbuscular mycorrhizal fungi, ecological quality ratio, soil ecosystem services, soil quality, Soil Framework Directive.

Resumen

Desarrollo de indicadores de calidad de un suelo basados en hongos micorrícicos

A partir de la firma del Tratado sobre la Diversidad Biológica de Río de Janeiro en 1992, varios países han iniciado programas para la evaluación de la calidad del suelo, determinando la diversidad biológica tanto en superficie como subterránea. Dentro de la Unión Europea, los responsables políticos están elaborando una legislación para la protección y gestión del suelo. Por lo tanto, se necesitan indicadores que permitan valorar el estado de los suelos y reflejar los resultados de su gestión. Los microorganismos del suelo, particularmente los hongos micorrícicos arbusculares (HMA), viven en simbiosis con la gran mayoría de las plantas y son sensibles a cambios en las condiciones físico-químicas del suelo. El objetivo de este estudio fue investigar si los HMA son indicadores fiables y suficientemente sensibles de alteraciones en los suelos y pueden utilizarse para el desarrollo de normas de calidad, así como si cumplen con los requisitos para su aplicación por parte de los usuarios y de los responsables políticos. En este estudio se establecieron criterios ecológicos para el desarrollo de indicadores de la calidad para diferentes tipos de suelos. Se analizaron más de 250 muestras de raíces con HMA de diversos lugares de Holanda y los resultados se relacionaron con distintos criterios previamente definidos. Se obtuvieron dos conjuntos de indicadores para la evaluación de la calidad del suelo, uno para suelos arenosos y otro para suelos arcillosos, con seis categorías diferentes (de muy mala a muy buena). Estos indicadores de calidad del suelo cumplen la mayoría de los requisitos para su aplicación y son potencialmente útiles para la elaboración de normativas para la protección de la calidad del suelo.

Palabras clave adicionales: calidad del suelo, Directiva Marco sobre el suelo, hongos micorrícicos arbusculares, indicadores, ratio calidad ecológica, servicios de ecosistemas del suelo.

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Received: 02-10-09; Accepted: 27-04-10.

Abbreviations used: AC (percentage of arbuscules in plant roots), AMF (arbuscular mycorrhizal fungi), EQR (ecological quality ratio), HC (percentage of hyphae in plant roots), VC (percentage of vesicles in plant roots).

Introduction

The Treaty on Biological Diversity of Rio de Janeiro (Anonymous, 1992) has contributed to the interest worldwide for maintaining and increasing biodiversity. This increased interest for maintaining diversity and the awareness on sustaibility are not only focused in the most obvious above-ground environment, but also the below-ground soil environment is considered.

Soils are present worldwide in terrestrial and aquatic ecosystems forming the basis for human activities. In fact, the soil provides many of the services that humans draw from nature. The so-called ecosystem services provided by the soil can be used within certain conditions that reduce the risks of irreversible changes (Hanson *et al.*, 2008) and are linked to the concept of sustainability of the system. In this context, the soil ecosystem services that can be distinguished are: a) biomass production within the framework of agriculture and nature; b) flexibility of soil uses; c) providing an endurable environment and d) protection of the below- and above ground biodiversity.

Durable and sustainable soil ecosystem services require a good soil quality integrating the physical, chemical, and biological soil components and their interactions. This will need the reduction of inputs of chemical fertilizers and of crop protection agents, the decrease of soil dehydration, the management of beneficial soil organisms, factors leading to the elimination of soil degradation. A good soil quality determines agricultural sustainability, environmental quality and, consequently, plant, animal and human health (Bloem *et al.*, 2008).

Within the European Union, policy makers are working on legislation for the protection and management of good quality soils. Therefore, indicators are needed to monitor the status of the soils, these indicators can be grouped and developed in standards, like soil quality metrics. During the last decade, interest in the development of standards for soil quality is increasing (Bloem *et al.*, 2008). The interest is mostly focused on soil quality metrics that are applicable and based on soil indicators that can be determined rapidly and at a low cost.

How to asses soil quality is an intriguing and challenging question (Bloem *et al.*, 2008). The choice of indicative parameters for soil quality has not been resolved so far. Moreover, standards for soil quality, reflecting results on indicative parameters on monitored quality soils, like soil quality metrics, have not been proposed.

During the last decades, attempts have been made to develop tools for determining soil quality. Physico-chemical parameters were considered at first. Concentrations of nutrients, including nitrogen, phosphate and potassium, were used for determining the quality of a soil underlining its agronomic characteristics in order to assess whether the soil was good for planting crops or trees. These parameters consider the soil as a dead matrix and do not take into account the living parts of the soil. The biological soil components need to be taken into consideration to evaluate the quality of the soil as a whole system. The assessment of soil quality with biological approaches was initiated in 1992 in different countries, but little information has been exchanged or published in the international literature (Bloem *et al.*, 2008).

Soils contain abundant biological components including bacteria, protozoa, fungi, nematodes and earthworms. Generally, about 3,000 to 5,000 kg of soil living organisms occur in one hectare of soil (Bloem *et al.*, 2008). The abundance and diversity of micro-organisms in the soil provide information about the conditions of the surrounding soil. The soil micro-organisms, including bacteria, fungi, nematodes and earthworms, indicate whether physico-chemical conditions are suitable for their development on one hand, and on the other hand they fulfill key functions in the soil. Soil micro-organisms contribute to decomposition processes, nutrient cycling and improvement of soil structure.

Mycorrhizal fungi are a specific group of soil organisms that establish a symbiotic relationship with the great majority of plants. The fungal hyphae associate with roots of plants. Exchange of nutrients derived from the soil by the hyphae for carbon originating from the photosynthesis takes place in the mycorrhizal roots. Mycorrhizal fungi also contribute to the formation of soil aggregates and to the protection of plants against drought and root pathogens (Smith and Read, 1997).

Three major groups of mycorrhizal fungi can be distinguished: the ectomycorrhizal fungi, the arbucular mycorrhizal fungi and the ericoid mycorrhizal fungi. The ectomycorrhizal fungi mainly associate with trees. The arbucular mycorrhizal fungi (AMF) are found associated with the majority of plant species including herbs, grasses, most agricultural crops, shrubs, and a number of tree species. Ericoid mycorrhizal fungi are more specific and associate with heathland plant species (Smith and Read, 1997).

The three groups of mycorrhizal fungi are very responsive to the physico-chemical soil conditions.

Mycorrhizal fungi are very sensitive to soil disturbances, like eutrophisation by over-fertilisation, acidification, deep ploughing and other soil movements. The fungi respond rapidly to changes in the concentrations of nutrients in the soil, such as nitrogen and phosphate. Mycorrhizal fungi react to disturbances, while other organisms are not affected (Baar and Ozinga, 2007).

Arbuscules are the structures of AMF that are physiological active in the exchange of nutrients, water and carbon. Arbuscules respond to changes in the chemical composition of the soil and they are extremely sensitive to high phosphate concentrations in soils (Smith and Read, 2007; Baar *et al.*, 2008; Kahiluoto *et al.*, 2009). The mycorrhizal arbuscules disappear as soon as a soil contains high levels of plant available phosphate. Therefore, arbuscules are good indicators for changes in physico-chemical soil conditions. The diversity of AMF also reflects the situation of a soil, some AMF have been found under high nitrogen levels while others occur under low nitrogen conditions (Egerton-Warburton and Allen, 2000). AMF respond to the physico-chemical soil conditions and are indicative of the soil conditions. The indicative value of these soil organisms could be suitable for the development of standards of soil quality, described as soil quality metrics.

The development of applicable soil quality metrics requires indicators that can determine differences between bad and good soils. Other requirements are that the data to calculate the indicators are easy to determine and not costly to gather. Furthermore, soil quality metrics must be applicable in soil management assessments and have to provide repetitive results. Finally these indicators of soil quality must fit in policy making at national and international levels (Bloem *et al.*, 2006).

Considering the requirements for a soil quality metric, AMF seem to be good indicators of soil conditions, and the aim of this study is to investigate the possibilities for the development of a soil quality metric based on AMF. The following two hypotheses were tested: a) AMF can be used for the development of a soil quality metric, and b) A soil quality metric based on mycorrhizal fungi meets the requirements for its application.

Methods

For this study, the area of The Netherlands was selected for the development of soil quality metrics. The rational for this was that there is an increasing interest in The Netherlands in applicable soil quality metrics based on biological soil components.

For the development of a soil quality metric, several criterions were defined. Different categories were distinguished ranging from very good soil situations to very bad situations. A very good situation was defined as a soil where human influence was limited to as little as possible corresponding to references describing nearly undisturbed above- and belowground situations, indicating a soil with a well developed soil life and a high biodiversity of soil organisms. Other categories were distinguished, soils in the category «good» displayed a mild degree of disturbance caused by human activities and deviated slightly from the reference situation. At the other end of the soil quality metric, bad and very bad situations were distinguished with a high degree of disturbance caused by human activities. The boundaries of the categories were based on substantive ecological grounds. The values on the criterions were categorized between zero and one, the highest being the value for the very good referenced ecological conditions. The remaining values were divided by this, creating the «ecological quality ratio» (EQR). The EQR expresses the distance to the referential conditions. The ecological diversity below- and above ground that differs between various soil types was taken into consideration. This study was conducted for clay and sandy soils, because these are the most commonly occurring soil types in The Netherlands.

After defining the criterions, a literature study on the presence of AMF in The Netherlands was carried out (Limonard and Ruissen, 1989; Heijne *et al.*, 1996; Van der Heijden *et al.*, 1999, 2006, 2007; Dekkers and Van der Werff, 2001; Van der Heijden, 2001; Van der Heijden and Kuyper, 2001, 2003; Scheublin and Van der Heijden, 2006; Vergeer *et al.*, 2006; Galvan *et al.*, 2009). Additional data from the field were collected in the period from October 2007 to August 2009. Roots samples were taken from plants associating with AMF including *Holcus lanatus* L., *Plantago lanceolata* L. and *Trifolium repens* L. The samples obtained were washed and the roots were stained for microscopic analysis of the colonization levels of AMF (McGonigle *et al.*, 1990). This methodology was used to determine root colonization with the following structures: arbuscules, vesicles and hyphae.

In this study, 279 samples of arbuscular mycorrhiza roots were analyzed, 77 samples from clay soils and 202 samples from sandy soils. The percentage colonization of AMF in plant roots was determined and more specifically the percentage colonization of arbuscules, vesicles and hyphae. The obtained data were taken into consideration for the development of a soil quality metric. The distribution of the number of arbucular mycorrhizal taxa over the different soil categories was based on the observations in the samples.

Also, the AMF were related to the various requirements for an applicable soil metric with low costs. The requirements taken into consideration were: a) Sufficient data of the indicators to determine the differences between bad and good locations at the different soil types; b) Data collection of the indicators must be practical and rapid for low costs; c) Data collection has to provide repetitive results, also on the long term; d) Soil management measures possible based on the indicative values; e) The assessment of soil quality with AMF must be understandable for policy makers.

Results

The 279 samples of AMF from The Netherlands provided a basis for the development of two soil quality metrics, one for clay soils and another one for sandy soils (Table 1). For these soil quality metrics, different indicative mycorrhizal parameters were used, including percentage root colonization with arbuscules, the ratio of arbuscules and hyphae, the combination of arbuscules, vesicles and hyphae or the number of taxa (Table 1). These indicative parameters were considered individually, or in combination.

The highest percentage root colonization of arbuscles was 70%, and from that value, the remaining data were distributed over different categories. The distribution was based on their frequency in a certain category. A direct relationship between the percentage of arbuscules (AC) and the percentage of hyphae (HC) in plant roots was observed. The maximum value of this ratio was set at one, and the remaining ratios were distributed over the categories based on the corresponding frequencies. A linear relationship was not found between the percentages of root colonization of arbuscules (AC), vesicles (VC) and hyphae (HC), however, a non-linear relationship between these parameters was described in the formula (8 AC + VC + 2 HC)/1100. The maximum value of this ratio was set at one in a similar way as was done for the ratio of the percentage of arbuscules and the percentage of hyphae in plant roots. The soil quality metrics contain six categories of ecological quality ratio ranging from zero to one corresponding with the categories very bad, bad, inadequate, moderate, good and very good soil quality. The metrics reflect the occurrence of AMF within a category, giving qualitative references to the soil quality based on the occurrence of mycorrhizal fungi. The ecological quality ratio of 0.66 was found as the norm for Dutch clay and sandy soils with good ecological quality while the ecological quality ratio of 0.33 was the norm for bad soils. Very good ecological quality of clay and sandy soils can be found when the ecological quality ratio exceeds 0.83.

The results of the data indicate that soils with a good ecological quality with an ecology quality ratio of 0.66-

Table 1. The soil quality metric for clay and sandy soils based at 279 samples of arbuscular mycorrhizal fungi

	Ecological quality ratio					
	0-0.16 Very bad	**016.-0.33 Bad**	**0.33-0.5 Inadequate**	**0.5-0.66 Moderate**	**066.-083 Good**	**0.83-1.0 Very good**
Arbuscular mycorrhizal fungi in clay						
Arbuscules (%)	0-2	2-6	6-15	15-35	35-70	70-100
Arbuscule/hyfae	0-0.05	0.05-0.15	0.15-0.3	0.3-0.6	0.6-0.8	0.8-1.0
(8AC + VC + 2HC)/1100	0-0.12	0.12-0.20	0.20-0.32	0.32-0.63	0.63-0.79	0.79-1.0
Number of taxa	0-1	1-2	2-4	4-6	6-8	> 8
Arbuscular mycorrhizal fungi in sandy soil						
Arbuscules (%)	0-5	5-10	10-20	20-50	50-70	70-100
Arbuscule/hyfae	0-0.1	0.1-0.15	0.15-0.3	0.3-0.6	0.6-0.8	0.8-1.0
(8AC + VC + 2HC)/1100	0-0.1	0.1-0.15	0.15-0.3	0.3-0.6	0.6-0.85	0.85-1.0
Number of taxa	0-1	1-2	2-4	4-6	6-8	> 8

AC: percentage of arbuscules in plant roots. VC: percentage of vesicles in plant roots. HC: percentage of hyphae in plant roots.

Table 2. Requirements for arbuscular mycorrhizal indicators for soil quality. X indicates that the requirement can be met while the O indicates that not (yet possible)

Requirements for biological indicators for soil quality	Arbusculus	Arbusculus/ hyphae	(8AC + VC + 2HC)/1100	Number of taxa
Sufficient data distinguishing bad and good soil quality locations	X	X	X	X
Practical data collection	X	X	X	X
Rapid analysis for arbuscular mycorrhizal structures	X	X	X	
Rapid analysis for arbuscular mycorrhizal diversity				O
Repetitive results	X	X	X	X
Sampling and analysis for low costs	X	X	X	O
Soil management measures possible based on the indicative values for improvement soil quality	X	X	X	X
Understandable for policy makers	X	X	X	X

0.83 were the best quality soils present in The Netherlands while soils with a very good ecological quality have not been observed.

The AMF meet the majority of requirements for an applicable soil metric with low costs (Table 2). Data can be obtained easily; extension of data is possible, because collecting samples from the field is relatively easy. Analysis for arbuscular mycorrhizal structures including arbuscules, vesicles and hyphae is rapid. Data can be available within 24 to 48 hours. Sampling and data collection on the arbuscular mycorrhizal structures can be carried out at low costs, estimated at €80 per sample. The analysis methodology is repetitive providing similar results over a period of time. The analysis of arbuscular mycorrhizal taxa is at the moment the slowest step, it might take up to eight weeks and the costs are €300 to €400 per sample. These proposed soil quality metrics are understandable for policy makers because they are comparable to the quality metrics in use under the Water Directive Framework.

Discussion

The soil quality metrics developed for clay and sandy soils in The Netherlands have to be considered as a first step in the development of a methodology determining soil quality using biological soil components. Although attempts have been made for monitoring soil quality with soil micro-organisms, hardly any results have been published in the international literature (Bloem *et al.*, 2008). For The Netherlands, attempts have been made using a mixture of soil organisms, soil processes and soil functions for the development of a soil quality system (Bloem *et al.*, 2008). However, this system has not resulted in any applicable soil quality metric because the combination of soil parameters evaluated is complicated to interpret. Also, the costs of analysing a large combination of biological indicative parameters are high making the system unattractive for users. Limited progress has been made over the last decade developing quality standards for soils using biological soil components. The soil quality metrics for clay and sandy soils developed in this study are relatively simple, because they are based in just one group of soil organisms, the AMF. These fungi, occurring worldwide in more than 70% of the plant species, are extremely indicative of disturbances in the physico-chemical soil environment. AMF respond to changes in the soil nutrient concentrations, pH, organic matter content, soil structure and water availability (Smith and Read, 1997; Baar and Ozinga, 2007), meeting most of the requirements for indicators of soil quality. A weak point might be the detection of the fungal taxa, that is at the moment time-consuming and costly. Progress in molecular analysis methodologies will help to analyse the diversity of arbuscular mycorrhal fungi and allow rapid results for relatively low costs (Schüssler, 2009).

The soil quality metrics for clay and sandy soils developed for The Netherlands were based on observations on AMF in a variety of locations ranging from with little to with high human influence. The highest root colonization with arbuscules of 70% originated from nature conservation areas in The Netherlands. These areas, however, could not serve as undisturbed areas with hardly or no human influence. Human influences were quite strong in these nature conservation areas where management practices had been implemented to increase plant diversity. These areas could only serve as references of areas with little or no human influence. At locations with hardly any human influence, which still occur in other parts of the world, soils of very good quality can be found with colonization levels of arbuscules exceeding 70% (Smith and Read, 1997).

Therefore, the maximum 70% root colonization level of arbuscules observed in this study was related to the category good ecology quality. The advantage of this approach is that the standard for the best soil quality is not set at a too low level. A value of 70% root colonization of arbuscules indicates that soil improvement is still needed for the development of very good soil and that soil management measurements have to be taken. Such soil management measurements could consider, for instance, the reduction of nutrient levels in the soil, particularly nutrients as nitrogen and phosphate, and an increase of pH (Baar and Ozinga, 2007; Baar *et al.*, 2008).

The soil quality metrics developed in this study are comparable to the water quality metrics developed for the Water Framework Directive. The water quality metrics are also based on biological parameters enabling distinction between bad, good and maximum ecological quality ratio's. In the future soil quality metrics will be useful for legislating soil quality protection measures. For Europe, this could be the Soil Framework Directive that is currently under development.

These soil quality metrics in this study were based on almost three hundred root samples of AMF in The Netherlands. The soil quality metrics for sandy and clay soils can increase their reliability when based on a high number of samples. For the Netherlands, sampling from different locations in The Netherlands is an ongoing study and will continue to strenghthen the indicators already developed.

The obtained data provide a systematic relationship between the occurrence of AMF and soil conditions. As indicated by Estaun *et al.* (2002), the number of systematic studies on the relationship between abiotic soil variables and the development of AMF is still low. Therefore, the number of systematic studies on the relationship between physico-chemical composition of soils and the occurrence of mycorrhizal fungi at a larger scale, such as throughout Europe, should be investigated. The data derived from these studies will be indicative of soil quality parameters throughout Europe. This can result in the development of soil quality measures for different soil types applicable throughout Europe.

Acknowledgements

I am thankful to Screenfungi for providing the data of the mycorrhizal analyses obtained from 2007 to 2009. I appreciate that the expertise centre Triple E provided funding for this study.

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