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Soil microbes and Soil Organic Matter dynamics

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Introduction

Soil microbial communities are main drivers of SOM transformation

Microorganisms are the largest group of soil-borne organisms in terms of both number and mass. As soil is their habitat, any changes in the soil physical state and the addition of any materials – whether raw organic and inorganic materials or chemicals – to the soil, will affect their activity and real functioning, among them the transformation of organic materials. Soil Organic Matter represents the main source of energy for soil microbiota, through a wide variety of compounds differing in energetic value and linked to different biochemical pathways of transformation. This complex system of energy and matter fluxes requires and/or selects a composite structure of microbial communities. Thus, besides the key role of SOM on soil structure and, as consequence, on water dynamics, root development and resistance against erosion, improving SOM content in Mediterranean regions may act also as preservation of soil biodiversity, considered in general as the variety of the life below ground.

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The role of soil microbial communities and ecosystem services

The majority of soil functions and ecosystem services furnished by soil are related to the presence of a complex system of organisms and their trophic chains, with important non-trophic effects. Soil biota, together with the diverse forms and functions of plant roots, have important roles in delivering ecosystem goods and services, most related to agriculture, such as: organic substrates decomposition by primary decomposers, regulation of nutrient cycling, pathogens control and defence, soil formation, maintaining and promoting soil structure, gaseous exchanges and C sink, depollution by bioremediation processes, plant development. All these could be quantified for their economic value.

It has been estimated that at least 80% of the ecosystem services can be linked to the effective functioning of soils on Earth. Soil has a biological value in its own right: it is an important habitat and gene reservoir for an enormous variety of microorganisms and larger soil dwelling animals; soil provides services through its role in storing, degradation and transformation of solid materials, such as nutrients and also the contaminants that are applied through animal and human activities or aerial deposition; soil provides services through its role in regulating the flow of water and rainfall in the water cycle.

Agricultural practices might have strong impacts on soil conditions and the life of soil organisms in general. Several factors indirectly affect the soils, including the abiotic non-living background and the soil-biological status, the living entities, as well. Among the non-living parameters environmental- and non-environmental factors are originating, such as the weather, the radiation, the temperature and/or the hoeing, the irrigation, the mulching, etc., respectively. The living parts are consisting of the microorganisms, as the key-components of any ecosystem functioning. The bacteria and fungi, the most well-known microorganisms are responsible for the vast bulk of decomposition, and also make up the largest part of the biomass in soil. Many of the essential transformations in the nitrogen, sulphur, phosphorus and other element cycles are mediated by microbes. These roles are important in re-establishing function and biodiversity in ecosystem restoration. Bacteria are the most abundant microorganism group in soil and can attain concentrations of more than 10^8 cells per gram of soil. A soil bacterial community consists of many populations (subgroups of one specific type of organisms), each with a characteristic response to certain environmental conditions or human-induced biotic and abiotic stresses.

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The soil microorganisms:

- drive processes including soil formation, nutrient cycling and nitrogen fixation, the breakdown and retention of organic matter and the maintenance of soil structure;
- include the underground life stages of many valuable insects, such as pollinators and pest predators;
- are food for other soil-fauna elements (i.e. nematodes), which are part of the larger soil-food web and indirectly able to feed the wildlife, such as mammals and birds;
- breakdown chemical contaminants and pathogens, helping to protect water quality and restore contaminated soils;
- include symbiotic soil fungi on which many plants highly depend;
- are potential source of pharmacological compounds. Many advances in medicine and pharmacology are based on developments in soil science, such as the discovery of streptomycin from soil-borne actinomycetes.

Soil microbial biodiversity: Close relationship between soil type, climate and microbial communities

The biodiversity of microorganisms is a basic requirement of soil functioning. Each physiological group of microorganisms has its own capacity and special strategies of surviving in the soil. Not all microorganisms of the groups have the same energy and/or capacity for doing that. There is a great environmental influence on the development of those special characteristics. Changing any of the influencing factors might affect also the composition and the general activities of the whole bacterial communities.

The ability of soil to provide ecosystem services is dependent on microbial diversity, with 80-90% of the processes in soil being mediated by microbes. There still exists a knowledge gap in the types of microorganisms present in soil and how they contribute to specific soil functions. The influencing environmental factors might have also a large variability in their abundance and composition (quantity and quality). Examples of these influencing “stress factors” are: the temperature, pH (soil acidity and salinity), water and/or oxygen potential …etc. Soil properties like structure, water, air and nutrient availability, and also the organic matter availability, etc. all affect bacterial/microbial community structure in soils. Because physical disturbances in soil are known to impact soil properties greatly, they also impact the abundance, community structure and activity of soil microorganisms. Thus, land-use management practices, like application of different tillage systems, might present themselves as case studies for soil microbial diversity studies in relation with soil organic matter content.

On microbial level, soil biodiversity can be defined as richness of species (i.e. the number of individuals belonging to different "groups" denominated taxa) and evenness of each species (i.e. their distribution within the same taxa). The composition of communities (that is, the set of microbial species present in a given environment) can vary over time as a result of changes that occur in the microenvironment or by the action of microorganisms that are part of it, or that are placed in it, and/or because of climate, topological, biochemical and anthropogenic changes. In addition, many microorganisms can maintain the same composition within a community, but change certain metabolic processes with functional and ecological consequences. The microbial diversity is largely related to land management, so the bacterial communities being the most represented are also the most studied in order to predict the fertility of agricultural soils; the soil fungi are less studied, although they represent a large part of the microbial biomass, they are involved in fundamental processes such as the degradation of organic residues and have a primary role in C sequestration.

The main obstacle that limits research in this area is the difficulty of growing soil microorganisms in vitro, making it impossible to study them. The possibility of studying soil microbial communities is given by new molecular techniques that allow characterization of even the non-cultivable organisms.

A collection of cases of study representing different correlations between vegetation type and soil characteristics, or distribution of soil microbial biomass within different soil reference groups, or thematic maps of soil biological fertility were reported for some Italian regions (Benedetti et al. 2013).

Feedback effects by crops at the rhizosphere interface

Many types of microorganisms work well with plants, helping them by increasing the level of available nutrients. This can enhance the production of vegetables and fruits by a great deal. Some microorganisms, like for instance the mycorrhizal fungi help plants take up nutrients, especially phosphorus and other elements to the roots or shoots. Additionally, microbial inoculums defend plants against diseases by competition or antimicrobial properties.

The soil volume at the soil-plant interface, the rhizosphere, is the most active portion of soil with a gradient in chemical, biological and physical properties changing both radially and longitudinally along the root. It was estimated (Newman, 1985) that roots can release from 10 to 250 mg C /g root produced or about 10-40% of their total photosynthetically fixed carbon. The organic forms of C released (e.g., low molecular weight organic acids) have the most influence on the chemical, physical and biological processes in the rhizosphere (Jones et al., 2009) and their composition and amount is influenced by many factors including plant type, edaphic and climatic conditions, soil fauna, nutrient deficiency or toxicity. Also different tillage systems and crop residues management may impact on the long term level. After 10 years of crop rotation (at conventional or zero tillage, with residue removal or retention in soil), the preferential microbial utilization of carbon sources was found when crop residue was retained. On the other hand rhizosphere soil microbes significantly increased the utilization of most carbon sources by applying zero tillage (Yang et al. 2013).

Among major functions, root exudates act as nutrient scavengers (e.g. Fe and P), agents of invasiveness (i.e. allelopathy) or as chemical signals to attract symbiotic partners (e.g. rhizobia and legumes) or the promotion of beneficial microbial colonization on root surfaces (e.g. *Bacillus subtilis*, *Pseudomonas florescence*) (Bais et al. 2004). Legume-Rhizobia Symbiosis is the most extensively studied, due to the importance of nitrogen fixation process. Mycorrhizal Fungi are also important for plant nutrition, as majority of these associations are beneficial both to the host plant and the colonizing fungi. Mycorrhizas assist plants in obtaining water, phosphorus and other micronutrients (e.g., Zn and Cu) from the soil and in return receive carbon from the plant.

There are two broad categories of mycorrhizal associations with plant roots:

1) the ectomycorrhizas occur mainly in the roots of woody plants (i.e. forest trees) and form a dense hyphal covering (fungal sheath or mantel) over the root tip but do not penetrate the cell walls;

2) in contrast, the arbuscular mycorrhizas fungal hyphae grow into the root cortex and enter the cells forming highly branched structures which increase the contact area between the fungus and the plant for materials transfer (nutrients to the plant and carbon to the fungus).

Unlike the ectomycorrhizas, the arbuscular mycorrhizas are wholly dependent on the plant for their carbon and when associations occur, both arbuscular mycorrhizas and ectomycorrhizas might demand up to 20-40% of the total photosynthetically fixed carbon the plant produces, however fortunately in many instances the required carbon is less than 10%!

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Plant Growth Promoting Rhizobacteria (PGPR) are organisms that, after being inoculated on seeds, could successfully colonize plant roots and positively enhance plant growth. Plant growth promotion can be obtained by the release of plant growth stimulating compounds (e.g. phytohormones such as auxins or cytokinins) and improvement of nutrient uptake (particularly the hardly available Fe), and it can also occur indirectly by control of pathogens (biocontrol) via synthesis of antibiotics or secondary metabolite-mediated induced systemic resistance (van Loon et al., 1998; van Loon 2007).

Use and regulation of rhizobia, mycorrhizae and rhizobacteria are among practical methods for managing biological resources to improve soil fertility. Positive effects on soil organic C conservation are also expected.

Exogenous OM may impact on soil microbial consortium (both structure and functioning)

Soil-borne microorganisms play an indispensable role from the aspect of the metabolic cycle, keeping up the natural cycles of carbon and other elements. Organic residues ending up in the soil can be decomposed in the presence of air (aerobic process) or without air (anaerobic process). Aerobic microorganisms actively decompose organic matter into nutrients if the soil moisture and air content as well as temperature are in the favourable range, while anaerobic microbes thrive in wet soil with no aeration. The so-called obligatory aerobic microbes can die without oxygen while facultative aerobic species survive without oxygen but they definitely need oxygen for effective metabolism. The "quality" of exogenous organic materials added to soil is also important, as not stabilised biomasses or presence of xenobiotics above certain levels may negatively impact on soil microbial communities' structure and functioning.

Effects of crop management

Intensive soil disturbance results in wasting the soil's humified and non-humified organic materials by boosting aerobic microbial respiratory processes, deteriorating thereby the soil's structure, reducing soil aeration and impeding the uptake and utilisation of nutrients.

'Crop focused' tillage practice led to the degradation of soil-structures and disregard soil-borne microbial activities. The protection of soil quality in cropping could contribute to restoring degraded soils and could help maintaining a useful equilibrium between organic matter decomposition and breakdown through

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reasonable controlling of microbial activity. Soil-damage caused by tillage-induced compaction and also the soil loosening – might facilitate detoxification processes in soils. Nutrient-availability, therefore can be assessed and evaluated more exactly by clarifying the microbial responses.

Activation processes: managing soil microbial resources

OM addition to soil loads the system with new microbial consortium depending on the quality of the input materials (manure, compost, etc.). What is its fate and the effects on the system capacity to build and store new SOM? It is possible to shift the balance between catabolic and anabolic activity toward the latter?

Detecting the abundance and activity of soil microbial communities

It might be important to follow the changes in the microbial communities:

- the numbers of microbes,
- evaluation of enzymatic activities,
- assessment of community composition and
- the dominant physiological groups in the soils.

It is expected that any shifts in bacterial and fungal communities associated with land-use differences will be directly related to the effects of that land-use change, so a pattern can be found by comparing the microbial composition of intact and farmed soils.

Measurement of the microbial community indicates the status of the soil system, and can help us to evaluate the possible countermeasures in soil management, to enhance the quality of agricultural or polluted soils.

A large body of methods for assessing soil microbial abundance, activity and/or community diversity is available in literature. The selection of most suitable methods should be driven case-by-case according to the objective of investigation: e.g. monitoring on territorial scale (basin, region, etc.) or solving specific problem on a limited area will require different approaches. A hierarchical scale of microbiological, biochemical and molecular parameters was proposed to assess soil quality through defining the biological fertility of soil (Bloem et al. 2006). Among the assessment of soil-fertility, the microbial abundance

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estimations of the N-fixing and P-mobilizing microbial counts are the most numerous. If those biofertilizer microbes are missing, or not working efficiently in the soils, the use of microbial inoculation products might serve as a potential solution of increasing the soil-health.

Soil management and soil quality: evolution of microbial communities

Soil quality is the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality and also to support human health and habitat. Soil quality concept and definition are therefore strictly linked to the specific land use and function, therefore there cannot exist an absolute and univocal condition of optimum in "soil quality".

The specific biological properties of soils enable them to be indicators of soil quality, since their abundance and the dominant bacterial groups can tell us just what type of soil are we looking into. For example, if the microbial community shows a great number of phosphorus-mobilizers, it can mean that the soil is somehow limited in the available phosphorus for the growing crops. Also, bad soil conditions decrease the number of microorganisms and their enzymatic activity in general, which is unfavourable, since these enzymes take part in soil formation. The quality of soil therefore is highly dependent on the microbial community, and vice versa.

Role of microbes in promoting SOM stabilization against soil physical degradation

Numerous microorganisms are known to live in soils. The main types of them are the bacteria and the fungi. Bacteria are mainly consisting of only one cell, which size is about 5 micrometer. The bacteria might be quite numerous in most of the soils; the cultivable amount of the total heterotroph CFU-s (colony forming units) is known to be about only 1-6% of all microorganisms. Typically many more bacteria, 10^8 can be found in each gram of soils, in comparison with the microscopic fungi found at about 10^4. A main feature of bacteria is to have an outer extracellular layer around of their cells. The role of this specific layer is to protect them from environmental stresses, such as drought and/or high temperatures…etc. They also need protection against several other stress-factors (pH, heavy metals, acidity, salinity…etc). The extracellular material of bacterium cells, which are consisting of sticky materials, are mainly responsible for the aggregate formation, the aeration and finally the structuration of the soils. The

extracellular materials might also act by cementing the humus substances and stick them to the soil particles, which might be stabilised in this way and resist against the fast degradation in the soil.

Beside the bacteria, microscopic fungi have a role also in the soil-structure stabilization.

Two types of fungi are known:

- the yeasts, which also single cell-like forms as the bacteria. They are the typical fermentative microorganisms in the soil. During this process they produce alcoholic substances from the easily available sugars;

- the moulds, which have long hyphal structure that can create a network in the soils. Such hyphae can be also efficient in the aggregate formation together with the bacterial polysaccharides.

For developing stabile soil-structure both the bacteria and also the microscopic fungi are needed.

Managing microbial resources in soil to contrast biological erosion and to detoxify soil

Soil erosion and pollution can damage soil quality and can prohibit the soil from possible agricultural usage. Since microorganisms can adapt to different environmental stresses, including heavy metals or other type of contaminations, they have been used in soil remediation practices for many years. Some pollution can be highly controlled, and some can be completely removed from the soil system, because microorganisms degrade the pollutants or might use them as energy and/or C and N-sources in the soils.

Most of the microorganisms need oxygen to their growth and survival. Those so called aerobic microbes are active only in favourably loosened and aired soil. In such circumstances the toxic substances that have accumulated in the compact 'sealing' layer break down and decomposition makes nutrients available as well. For this reason, loosening – eliminating compaction – qualifies as a detoxification process from the aspect of microbes and microbial life.

To manage this phenomenon properly, soil microbes have to eliminate the source of the damage in the soils. They, however, often require some "assistance" from the scientists to do the degradation processes perfectly. The most important amendment could be the addition of nutrients, water and/or other

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beneficial additives (i.e. some salt or micronutrients…etc.). Some microbial populations may be better at degrading certain pollutants, while others just live in the background, so we have to focus on the enrichment of groups that can help improving soil quality. After analyzing the microbial background of the soil several possible microorganisms or consortiums (one or two microbial groups working together) can be selected, and tested for increased activity or returning them to the soil enriched in inoculums.

Fertilization issues

Soil is a typically open ecological system, in which the input and output of materials and energy are practically constant. If the soil is low in nutrients, and the native microbial community can no longer revitalize the system, it is also possible to use microbes originating from elsewhere, where they have not yet lost their functionality and activity. These inoculums usually contain a mixture of bacteria and fungal species and can be available commercially. There is a wide selection of useful microorganisms (both bacteria and fungi), that can be used not only for the remediation of certain pollutions, but also for improving the fertility of nutrient poor soils. The specific microbial inoculums, which can be introduced into the soils called:

- biofertilizers, consisting of microbes that are able to replacing the most frequent N,P,K fertilizers by their biological activity (biological N_2-fixation, P-mobilization, K-solubilisation),
- biopesticides, as biocontrol agents, that are able to control the presence and activity of the so-called soil-borne plant pathogenic fungi and bacteria and other pests (e.g. nematodes, insects).

Those microbial inoculums can serve as environmental friendly products in contrast with the artificial inorganic fertilizers, known as soil acidifiers basically on a long-term level.

Protecting typical agricultural products in a sustainable global market

Typical agricultural products are defined according to European Regulations through appropriate labels (DOC, DOP, GPI). The respect of production requirements should theoretically protect also the production territories although in some cases no specific regulation is dictated on environmental sustainability (e.g. soil protection). Beyond these products there are many which are not recognized due to the local scale production, however they represent an important challenge to increase agro-biodiversity through campaign of conservation of local varieties together with the agronomic techniques adopted according to the rural tradition. Each plant species in the soil releases some root exudates also as a result of climatic

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and environmental characteristics, which will attract a specific microbial population. This will create the edaphic microenvironments that constitute food webs associated with specific plant. This implies that both plant germplasm and soil microbial biodiversity should be preserved in the case of typical foodstuff productions. The Italian *Guidelines for conservation of genetic resources for food and agriculture* (Mipaaf 2012, **www.reterurale.it**) dictates rules for both *in-situ* and *in-factory* soil microbial resources conservation.

In the case of « terroirs », the link between soil-grapevine-microorganisms-wine qualities is well established. In the Mediterranean region, especially in the more arid areas in the south, there is a dramatic problem of water supplying during the long summer drought: improving soil organic matter content could help to increase water retention in soil, which should avoid or mitigate water stress to plants and favour grapes yield. On the other hand the quality of organic fertilization should be assured, as the composition of exogenous organic materials added could negatively impact on the eco-physiological response of plant to the organic matter supply.

The preservation of native soil biodiversity should be an overriding concern when planning crop management and fertilization in the area of typical productions.

Provisional list of « ready-to-use » or forthcoming possible actions

Following the Treaty on Biological Diversity of Rio de Janeiro (CDB, 1992), many governments have been establishing national or regional programmes to sustain biodiversity and preserve the related functions supporting life. In Italy, the Ministry of Agriculture and Forestry Policies in 1999 adopted a National Plan for Agricultural Biodiversity. In this framework *Guidelines for conservation of genetic resources for food and agriculture* were published in 2012 (Mipaaf, **www.reterurale.it**).

A compendium of information on the great diversity of life in the soils across Europe is represented by the first European Atlas of Soil Biodiversity, published in 2010 by Joint Research Centre's Institute for Environment and Sustainability of EC (**http://eusoils.jrc.ec.europa.eu**). The main objectives were to inform the general public, policy makers, land managers, teachers and the general scientific community of the unique characteristics of life in soil and raise awareness of its environmental importance and global significance, together with the challenges offered by soil biodiversity conservation in preventing biodiversity loss due to climate change.

Conclusions

The goal to improve the organic matter content in soils of the Mediterranean regions and securing soil functionality and soil fertility should take into consideration preservation of soil biodiversity, including microbiota.

A decision tree for organic matter management should take into account the ecosystem services provided by soils, that are driven by soil microbiota. Therefore it should be firstly considered the following:

- The soil microorganisms are key living entities in the soils. The best practice is to consider them living, which means that we should keep the nutrients, the appropriate water and the air content in the soil. The aerobic bacteria will need all of those three parameters. Among the nutrients it is the humus substances, which are providing the available nutrient sources for the higher plants with the contribution of the soil microorganisms.
- The intensive soil disturbance results in wasting the soil's humified and non-humified organic materials by boosting aerobic microbial respiratory processes, deteriorating thereby the soil's structure, reducing its aeration and impeding the uptake and utilisation of nutrients. 'Crop focused' tillage practices are assumed to have led to the degradation of soils of originally favourable structures by disregarding soil-borne microbial activity. Laying emphasis on the protection of soil quality in cropping systems could contribute to restoring degraded soils and could help maintaining a useful equilibrium between organic matter decomposition and breakdown through reasonable controlling of microbial activity.
- The quality of organic inputs should be considered as a key factor in preserving the soil microbial resources and the ecosystem services provided, which in turn have an economical value to be preserved, as well as the food web chain to produce both healthy food and typical foodstuffs need to be protected.
- Microorganisms that can degrade pollutants as energy sources or help the soil-plant system by increasing the nutrient availability or removing metallic contaminants is highly necessary in order to manage polluted soils in an environmental-friendly way.

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