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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

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Nineteenth Regular Session

Rome, 17–21 July 2023

DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF SOIL MICROORGANISMS AND INVERTEBRATES THAT CONTRIBUTE TO BIOREMEDIATION OF AGRICULTURAL POLLUTANTS AND SOIL NUTRIENT CYCLING

NOTE BY THE SECRETARIAT

1. The Commission on Genetic Resources for Food and Agriculture (Commission) at its Seventeenth Regular Session adopted its Work Plan for the Sustainable Use and Conservation of Micro-organism and Invertebrate Genetic Resources for Food and Agriculture (Work Plan).[1] The Work Plan addresses microorganisms and invertebrates as functional groups and foresees that two of these groups will be addressed at each forthcoming session of the Commission. For the current session, the Work Plan foresees addressing soil microorganisms and invertebrates, with emphasis on bioremediation and nutrient cycling organisms and microorganisms of relevance to ruminant digestion.[2]

2. In response to the Work Plan, FAO commissioned the Austrian Institute of Technology to prepare a *study on the sustainable use and conservation of soil microorganisms and invertebrates that contribute to bioremediation of agricultural pollutants and soil nutrient cycling*. The draft study is contained in this document. Following review of the draft study by the Commission, it will be published as a Background Study Paper.

[1] CGRFA-17/19/Report, paragraph 95.
[2] CGRFA-17/19/Report, *Appendix E*, paragraph 14.

Documents can be consulted at www.fao.org

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DRAFT STUDY ON THE SUSTAINABLE USE AND CONSERVATION OF SOIL MICROORGANISMS AND INVERTEBRATES THAT CONTRIBUTE TO BIOREMEDIATION OF AGRICULTURAL POLLUTANTS AND SOIL NUTRIENT CYCLING

This document has been prepared at the request of the Secretariat of the FAO Commission on Genetic Resources for Food and Agriculture with a view to facilitating consideration by the Commission, at its Nineteenth Regular Session, of the sustainable use and conservation of soil microorganisms and invertebrates used for bioremediation and nutrient cycling. The content of this document is entirely the responsibility of the authors and does not necessarily represent the views of the FAO or its members.

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Executive summary

To protect the quantity and quality of agricultural soils, we need to conserve soil biodiversity

Soil hosts taxonomically highly diverse microbial communities and a huge variety of microscopic and macroscopic animals, as well as being the principal substrate for plant life. Successful agricultural production depends on the availability of soil nutrients in sufficient quantity and quality and on the presence of appropriate soil structure, all of which are strongly influenced by below-ground microbial and invertebrate activity. Land-use change and heavy use of agrochemicals in agriculture have been associated with a loss of functional and taxonomic soil biodiversity. The available evidence suggests that such losses have been massive. However, their worldwide extent has not been quantified. Climate change and the need to feed a growing world population pose major challenges. This implies the need for more sustainable agricultural practices and for efforts to conserve and restore soil biodiversity. Increased interdisciplinary and interregional efforts will be required.

Soil microorganisms and invertebrates have central roles in soil nutrient cycling

The transformation of dead organic material into soil organic matter and soil organic carbon is mainly brought about by microbial and invertebrate decomposers. Carbon is naturally sequestered in the soil through the activity of photosynthesizers, soil-bioturbator invertebrates and oxalate producers. The availability of nitrogen, phosphorus and potassium in the soil is a limiting factor for plant growth. Soil microorganisms fix atmospheric nitrogen and transform it into plant-available forms. Biomineralization of organic phosphorus into inorganic compounds is a biological process initiated by the enzymatic activity of microorganisms. The availability of potassium in the soil is connected to the presence of certain soil bacteria and fungi. Microorganisms that actively oxidize sulfur compounds to sulfate are highly important in the supply of this nutrient to plants. Overall, the cycling, bioavailability and biomineralization of all macro- and micronutrients are connected to the biological activities of soil organisms. Besides living freely in the soil, various microorganisms can be actively recruited from the rhizosphere soil by plants to colonize their inner root tissues. This results in a metabolically more profound plant–microbe relationship and is often crucial for proper plant development.

The natural occurrence, diversity and functional richness of soil organisms in agricultural systems are threatened by the application of excessive amounts of chemical fertilizers and by the absence of regenerative soil-management practices, and also because legislation on the protection of soil biodiversity is often lacking.

Naturally occurring soil organisms can be deliberately managed for agricultural purposes. For instance, the growth of microorganism and invertebrate populations can be promoted with biostimulants, or microorganisms can be used as soil inoculants in the form of biofertilizers.

Agricultural contamination in the soil can be bioremediated by soil microorganisms and invertebrates

Application of synthetic (mineral-based) fertilizers and chemical pesticides to maximize crop yields has become widespread in industrialized agriculture. Among other issues, this has led to the contamination of soils, food webs and food systems with heavy metals and persistent bioactive and ecotoxic substances, and hence to adverse effects on human health and the environment. Soil bacteria, archaea, fungi and earthworms have proved to be highly efficient in soil bioremediation applications. These organisms harbour a rich metabolic toolset that enables them to reduce the bioavailability, toxicity or concentration of harmful substances in the soil and groundwater. While it is possible to stimulate the native microbial and invertebrate communities already present in the soil in order to promote the degradation of a specific local contaminant (a method referred to as biostimulation), the more common approach is to isolate specific microbial strains from the contaminated site and cultivate them in the laboratory for subsequent use in soil inoculation campaigns (a method referred to as bioaugmentation). The development of microbial consortia with synergistic activities instead of single microbial strains represents a promising approach for inoculation campaigns aimed at enhancing nutrient cycling or bioremediation.

Successful conservation of soil organisms requires a combination of *in situ* and *ex situ* conservation approaches

Protecting soil biodiversity is a cornerstone of regenerative agricultural management, which includes many different, locally customized approaches that promote soil health and soil quality. Various management practices have proven capable of reversing the loss of soil biodiversity and helping to conserve native soil organisms, for instance maintaining soil cover (e.g. using mulch or cover crops), permaculture, tree crops and agroforestry (including silvopasture), diversified crop rotations, interseeding and reduced pesticide use. Fostering more widespread and more rapid adoption of sustainable agricultural practices requires better cooperation between farmers and land managers and researchers, engineers and legislators.

Appropriate soil microorganism and invertebrate conservation activities are required and need to be supported by appropriate guidelines – similar to the soil-health guidelines developed by FAO (*Voluntary Guidelines for Sustainable Soil Management* and *Protocol for the assessment of sustainable soil management*)[3] – that include well-defined key soil parameters, information on important indicator and core organisms, and carefully chosen quality standards that allow comparative assessment. *In situ* soil biodiversity protection in some cases needs to be complemented with soil regeneration programmes, involving, among other measures, the re-introduction of depleted or locally extinct soil organisms from *ex situ* collections. Microbial culture collections, however, face significant difficulties, including a lack of trained personnel and cutting-edge technologies for high-throughput cultivation, whole microbiome cultivation and propagation of currently uncultivable organisms. There is also a lack of coordination between collections.

Recommendations

Implementing the following recommendations would help overcome current hurdles to the conservation of soil microorganisms and invertebrates.

1. Guidelines and standard operation procedures for the definition of “healthy soils” need to be elaborated and used in comparative assessments of soil biodiversity. These and procedures need to include well-defined key soil parameters, which include biological parameters such as microbial/invertebrate taxa indicating soil health, and carefully chosen quality standards.
2. There is a need to develop consensus on (a) the most important soil functions, (b) parameters for inclusion in assessments of the effects that new agricultural methods have on soils, (c) key soil biodiversity parameters and (d) unified sampling, laboratory and analysis procedures for soil-biodiversity.
3. Recommendations on ideal soil conditions and on best practices and interventions in soil management in agriculture should be based on long-term observations made under a range of different environmental conditions and geographical regions.
4. The uptake of promising agricultural practices that are beneficial to soil biodiversity conservation needs to be supported by improving evaluation of their applicability and their ease of implementation and should consider potential undesired effects.
5. The functionality, standardization and maintenance of databases of soil-health parameters and soil-biodiversity characteristics at regional scales need to be improved.
6. Addressing the complex problems facing soil protection in agricultural systems requires scientific approaches that are interdisciplinary and involve a range of specialists, including environmental chemists, biologists, agronomists and taxonomists.
7. More and better coordination is needed among the numerous research activities and scientific networks working on the sustainable use and conservation of soil microorganisms and invertebrates.
8. Raising awareness and building capacities in soil biodiversity conservation through the education and involvement of farmers, as well as better dissemination and public outreach, are essential.
9. Already existing *ex situ* and *in situ* conservation initiatives need to be better coordinated and should also address the cultivation and conservation needs of understudied groups of soil organisms.

[3] FAO 2017. *Voluntary Guidelines for Sustainable Soil Management*. Rome. https://www.fao.org/3/i6874e/i6874e.pdf

10. Short-term and long-term goals for the conservation and sustainable use of soil organisms need to be identified and a priority list established among them.

Introduction

Soil is a natural substance consisting of solids, liquids and gases that occurs on the land surface.[1] It is the natural medium for the growth of terrestrial plants and has several biological functions. It is divided into horizons, or layers, and its lower boundary is arbitrarily set at 200 cm, where most biological activity and active pedogenic processes end. The uppermost soil layer is the thin organic horizon, which contains undecomposed or slightly decomposed debris and plant remains. Beneath this lies the biologically and chemically most important layer, the topsoil, which contains most of the organic material in the soil. The rhizosphere is the metabolic hotspot of the topsoil in terms of nutrient exchange, rhizosecretion, soil functional diversity and interkingdom ecological interactions.[2] The layer below the topsoil, the mineral subsoil, is reached by some plant roots and soil organisms.[3] The quantity and quality of topsoil are crucial for agricultural activities. According to the FAO Soil Portal, about 99 percent of the world's food supply derives from land-based production, with 50–70 percent of the land being devoted to agriculture.[4, 5]

Soil is not only the principal substrate for plant life but also a complex ecosystem, hosting taxonomically diverse microbial communities and many highly varied animals. A previous FAO report with a stronger focus on taxonomy[6] defined soil biodiversity as "the variety of life belowground, from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes." The soil food web concept involves all these complex communities of organisms and considers the dynamics and interactions that determine their roles in soil ecosystem functioning.[6]

Microbial soil biodiversity has been described as an important buffer against climate change in the soil, contributing to the rate of production and consumption of carbon dioxide (CO_2), methane (CH_4) and nitrogen (N_2), and increasing ecosystem resilience and sustainability.[7] The diversity of soil microorganisms is also positively associated with the concept of "One Health", which links the wellbeing of humanity to the health of other ecosystem components, including the soil.[8] Soil invertebrate diversity is a key mediator of several soil functions, as soil invertebrates participate in litter decomposition and control microbial populations through their multiple interactions with other soil organisms.[9]

Land-use change and intensive agriculture, both in tropical[10] and in temperate areas, have been shown to radically reduce functional and taxonomic soil biodiversity.[11] It has been suggested that loss of soil microbial diversity results in a significant decrease in the functional capacity of the soil with respect to processes such as nitrification and denitrification, greenhouse-gas (GHG) fluxes and pesticide mineralization.[6]

The importance of soil science, soil biodiversity conservation and sustainable soil management has been highlighted as crucial to the achievement of the Sustainable Development Goals (SDGs).[12] Several SDGs can be addressed by working towards sustainable agriculture and the conservation of the soil organisms that contribute to soil nutrient cycling and bioremediation. Improving crop yields through application of soil organisms in sustainable agriculture contribute to SDG 2, Zero Hunger. Improving bioremediation of agricultural contaminants and reducing pesticide use contribute to SDG 3, Good Health and Well-being. Various carbon sequestration methods that rely on microbial and invertebrate activity contribute to SDG 13, Climate Action. Protecting, restoring and promoting the role of soil microorganisms and invertebrates in all ecosystems, including agricultural lands, reduces biodiversity loss and contributes to SDG 15, Life on Land. Interdisciplinary work and intersectoral networks on soil biodiversity contribute to SDG 17, Partnerships for the Goals.

Following up on previous reports prepared for the Commission on Genetic Resources for Food and Agriculture, and acknowledging FAO's long tradition and technical work on the management of microorganisms and invertebrates in food and agriculture, the present paper responds to the need for a detailed assessment of the state of art in the conservation and sustainable use of soil microorganisms and invertebrates. It focuses on microorganisms and invertebrates contributing to nutrient cycling and the removal of contaminants from soils.

The study is based on an extensive literature review and summarizes current views on the taxonomy, conservation and exchange of these organisms. It highlights knowledge gaps, needs and challenges with regard to their sustainable use. In order to encompass the views of a wide range of stakeholders on knowledge gaps and critical issues related to the conservation and sustainable use of soil microorganisms and invertebrates, AIT circulated an invitation to complete an open online survey to several hundred researchers, institutions and organizations worldwide. Twenty-seven responses were received and evaluated. AIT also organized an online expert workshop entitled Status and Trends of Conservation of Soil Microorganisms and Invertebrates, with Emphasis on Bioremediation and Nutrient Cycling Organisms. Twenty-six international experts participated in three parallel sessions (i) nutrient cycling in soil; (ii) bioremediation in food and agriculture systems; and (iii) conservation of microorganisms and invertebrates, practices, policies and needs. The issues raised in the survey responses and at the workshop were taken into account in the drafting of the study.

Based on the vast literature reviewed during the preparation of this work, a table comprising the most important functional marker genes used in assessing the diversity of nutrient-cycling microorganisms is presented in Annex I. A detailed taxonomic list of important soil microorganisms and invertebrates participating in soil nutrient cycling is presented in Annex II. A list of functional marker genes used in assessing the functional diversity of bioremediating microorganisms, as identified during the literature review, is presented in Annex III. A detailed list of soil microorganisms and invertebrates used in the bioremediation of the substances under consideration is presented in Annex IV.

Chapter 1. Taxonomic and metabolic diversity of soil microorganisms and invertebrates that contribute to nutrient cycling and bioremediation

The importance of the multiple roles of below-ground biodiversity in both agricultural and built environments is well established scientifically and increasingly being recognized. Certain traditional, biodiversity-supportive farming methods have long made use of the beneficial functions of soil biodiversity without farmers' explicit knowledge of the underlying science. A growing number of reports and scientific studies dealing with soil biodiversity are being published.[3, 6] Notably, a recent survey that aimed to identify global hotspots of soil biodiversity found that, overall, these did not match hotspots for the biodiversity of other terrestrial taxonomic groups.[13] Above-ground biodiversity does not necessarily correlate with below-ground biodiversity, and high microbial taxonomic richness, high microbial community dissimilarity and high levels of soil microbiome ecosystem services each have their own hotspots, often in different regions of the world.[14]

1.1. Research tools of soil biodiversity ecology

In the past, our understanding of the diversity of soil organisms relied on quadrat sampling and manual counting or laboratory culturing and identification of isolates. Especially in the case of microbial life, these methods are highly biased towards cultivable organisms given that many microorganisms cannot be cultured under laboratory conditions. Introduction of molecular tools has made it possible to detect the genetic fingerprint of any organism with high accuracy and at greater resolution. Modern genomic approaches also focus on the variability of genes and functions rather than only on taxonomic richness. This is done by isolating all environmental DNA from samples obtained from different sampling sites and carefully choosing marker genes for the taxonomic group or the functionality targeted for exploration (Annex I). These marker genes are amplified using polymerase chain reaction (PCR) methods and then sequenced using next-generation sequencing technologies, thus allowing the organisms present to be identified. In general, specific ecological statistical models are used to infer information on whether a conservation intervention is needed for a given group of organisms.[11, 15]

Despite the extent to which they are represented in the soil biomass and their importance in various soil functions, only a fraction of soil microbes has been taxonomically described. New technological advances, such as matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and high-throughput sequencing, allow microorganisms to be rapidly identified and quantified. However, because of the difficulty involved in species-level identification, knowledge of soil microbial taxonomy remains insufficient at times. Moreover, an estimated 80–90 percent of soil microorganisms cannot be cultured with current laboratory practices,[16] despite the numerous efforts made to circumvent the limitations of classical cultivation strategies.[17] Metagenome-based estimates have shown that phylogenetically novel, highly divergent uncultured microbes with unknown functions dominate the soil ecosystem.[18] Thus, the status and trends of individual microbial species and even genera are mainly unknown.[19] Where invertebrates are concerned, although populations can be successfully quantified and identified with cost-effective methods, scientific literature on the large-scale spatial distribution and temporal population dynamics of below-ground invertebrate biodiversity is limited.[20]

Experimental findings on the decline of targeted microbial or invertebrate taxonomic groups due to changes in selected environmental factors or agricultural soil management practices are available.[21] However, the publications in question usually provide aggregated information on the abundance and species richness of populations or functional groups (e.g. earthworms, epigeic earthworms, nematodes or arbuscular mycorrhizal fungi), while species-specific temporal dynamics are less frequently reported.

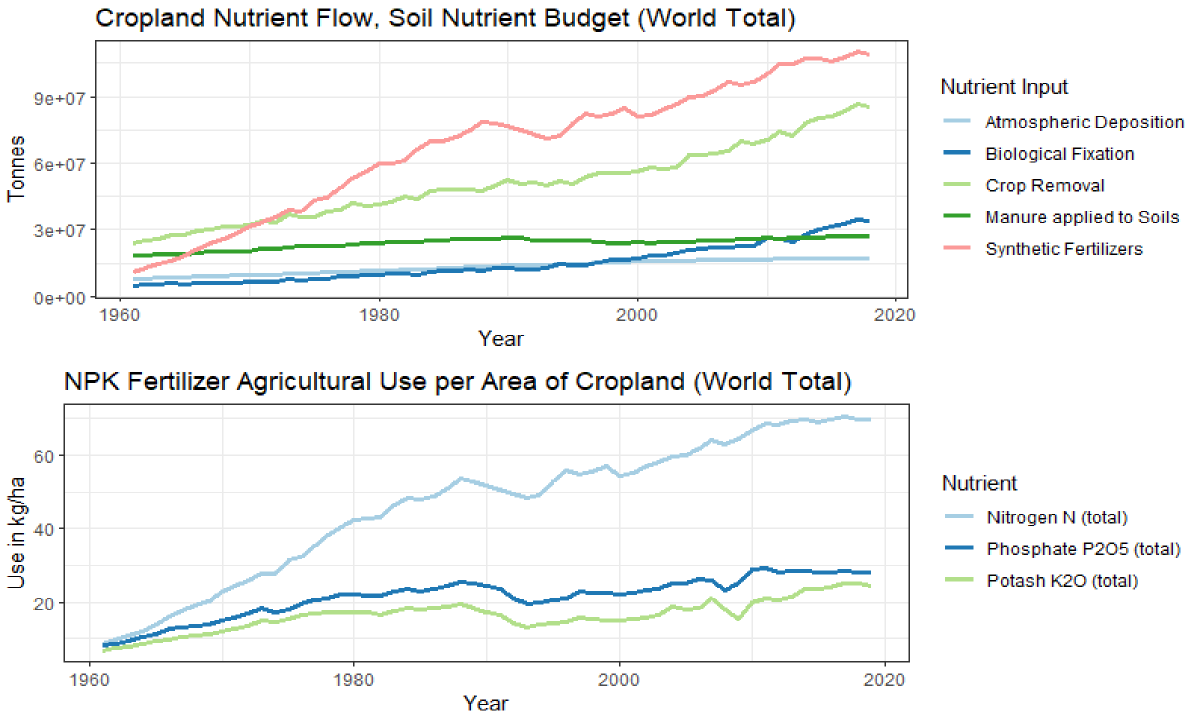
Mathematical models can help us understand complex ecological processes and predict how real ecosystems might change under certain condition. Modelling the extinction of soil organisms is a challenging task because of the complexity of soil microhabitats, the variability of the organisms' body sizes and the large size of their populations.[22] Furthermore, as existing ecological concepts cannot be applied to microorganisms, soil biota extinction models are currently limited to experimental findings from artificial microcosms[23] and cannot be readily scaled up and generalized.

1.2. Roles of soil microorganisms and invertebrates in nutrient cycling

One of the most remarkable soil functions that relies on soil biodiversity is the provision of nutrients for plant growth. To function properly and complete their life cycles, plants require 16 elements. These are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, copper, zinc, molybdenum, boron and chlorine. Nutrients in the soil have a specific vertical distribution that is controlled mainly by plant uptake and biogeochemical cycling.[24] As some elements, for example nitrogen, phosphorus and potassium, are limiting for plants, obtaining reasonable crop yields from soils under intensive agricultural use requires the use of external nutrients. According to recent data (Figure 1), the biggest contributor to the soil nutrient budget of croplands is still synthetic fertilization, although organic agricultural management practices relying on biological nutrient mineralization and nitrogen fixation via legumes and their symbiosis with nitrogen-fixing rhizobia are increasingly being used.

Conventional fertilizers can be environmentally harmful because of their energy-demanding production, the environmentally destructive mining of their constituents or their contamination with harmful substances such as heavy metals. A more sustainable and nature-based solution would be to make use of soil organisms involved in nutrient cycling or mobilization or to use management practices that favour natural nutrient cycles. Besides abiotic factors, such as precipitation, chemical leaching, the quality of bedrock and mineral content of local soils, biological processes are major determinants of the fate of elements required for healthy plant growth in the soil. The major roles of soil microorganisms and invertebrates in the cycling of most plant nutrients are described in the following subsections.

Figure 1. Cropland soil nutrient budget as nutrient flow by origin and total nutrient fertilizer agricultural use per area of cropland in the world from 1961 to 2019



Source: FAOSTAT data.

1.2.1. Carbon

Carbon is the base of all life on Earth. It has a complex global cycle that involves various organic and inorganic ecosystem components. Soil has a dynamic role in this cycle, interacting with atmospheric CO_2 levels, climate, land-cover changes and anthropogenic disturbances, and serving simultaneously as a carbon source and a carbon sink. Agriculture influences various aspects of the terrestrial carbon cycle, including the exchange and use of soil inorganic and organic carbon, methane and CO_2 emissions and carbon sequestration.

Soil consists of two pedologic carbon pools, the soil inorganic carbon (SIC) pool and the soil organic carbon (SOC) pool.[25] There is global consensus that SOC content is the main indicator of soil health and fertility and a primary quantitative measure of soil quality. SOC incorporates the organic residues, the C-rich products of the metabolism of various organisms and the carbon in the bodies of living organisms. It is influenced not only by the bedrock material but also by anthropogenic and biological processes. The total global stock of SOC as of 2017 was estimated at 680 PgC.[26]

The biggest source of soil carbon is the decomposition of dead organic material by various organisms. In areas with plant cover, the rhizodeposition of low molecular weight compounds (LMWs), such as simple sugars, amino acids and carboxylic acids, constantly supplies carbon to the SOC pool. However, some root-exudate compounds, such as dissolved organic carbon (DOC), phytosiderophores, vitamins and amino acids, do not persist in the soil over time. These labile soil carbon inputs regulate the decomposition of recalcitrant soil carbon by controlling the activity and altering the abundance of soil microorganisms, possibly via a positive priming effect that increases microbes' need for carbon.[27] Rather than consisting of a homogenous below-ground carbon pool, SOC stocks are vertically and geographically heterogeneous,[28] with the topsoil being the layer with the highest SOC content.

Below-ground biodiversity can contribute in various ways to global carbon fixation and to increasing SOC content. The carbon compounds released from roots or derived from decaying plant material represent a major input into the soil-food web and are readily accessible to heterotrophs. Heterotrophic decomposers, microorganisms and invertebrates alike, facilitate the breakdown of cadavers, plant litter and organic detritus to cover their energy, carbon and nutrient needs. Nutrient-rich green manure, vermicompost and traditional composts are all processed through the activity of heterotrophic microorganisms and invertebrates. Carbon sequestration in the soil is influenced by multiple biotic factors, including soil aggregation and bioturbation by the below-ground invertebrate micro-, meso- and macrofauna, and is an under-researched area.[9] A meta-analysis revealed that while earthworms are largely beneficial for soil fertility they accelerate decomposition rates and increase net soil GHG emissions, although determining their overall effects on GHGs will require further long-term field studies.[29] Diverse, metabolically different autotrophic organisms can also serve as carbon fixers. Photoautotrophic microorganisms living in the top layer of the soil contribute to the SOC pool by using CO_2 as the ultimate electron acceptor in their photosynthesis. Carbon sequestration and GHG mitigation through microalgal biochar in the soil is a promising application of algae in agriculture.[30] Moreover, cyanobacteria and Stramenopile algae are used in bioreactors to produce biofuels such as biogas, biodiesel and biohydrogen and thus reduce the use of fossil fuels in agriculture and transport.

The natural soil oxalate carbonate pathway provides an effective way of sequestrating carbon in which soil oxalate ($C_2O_4^{2-}$) is oxidized and carbonate (H_2CO_3) is generated. The pathway could be exploited as a low-cost method for atmospheric CO_2 capture in soils. Oxalate producers include plants, fungi from the class Agaricomycetes and Amoebozoa protists, which produce and accumulate oxalate in their outer "shells". Oxalate oxidation and degradation by oxalotrophs can effectively reduce atmospheric CO_2 concentrations both in natural and in agricultural soils while increasing SOC content.[31] Oxalotrophy is mainly an aerobic bacterial process[32] (Annex II) and is influenced by environmental pH.[33]

Another naturally occurring biological process with C sequestration potential that involves soil carbonate is microbially induced calcium carbonate precipitation (MICP).[34] In this slow process, bacteria serve as nucleation sites for carbonate precipitation: the negatively charged cell surface components attract Ca^{2+} ions. At present, bacteria that mediate MICP are not used commercially in agriculture. However, as it is an easily controllable mechanism, artificially induced MICP can rapidly produce high concentrations of calcium carbonate. As well as providing environmentally friendly biocomposition and biomaterial production, it could be used for capturing CO_2.[35]

Also relevant to efforts to reduce GHG emissions from the soil of agricultural fields are microorganisms' abilities to produce and utilize methane. Methanogenesis, the conversion of carbon-based organic matter into methane (CH_4) as a form of energy conservation is exclusively found among organisms from the Archaea domain. Methanogens are prevalent in waterlogged soils, such as those of paddy fields and peatlands, and are major contributors to agricultural GHG emissions.[36] The methane-producing abilities of archaea are used in biogas plants and in the treatment of wastewaters. Methane can be oxidized by methanotrophic prokaryotes, either aerobically using oxygen or anaerobically using alternative terminal electron acceptors.[37] Almost all methanotrophs are obligate methane and methanol utilizers. Methanotrophy is also closely connected to nitrification. Because of their enzymatic toolsets, nitrifiers can oxidize methane and methanotrophs can contribute to nitrification.[38, 39] Methanotrophs occur naturally in soils, including in agricultural fields.[40] They could be added to paddy fields to mitigate methane efflux.[37]

Methanol (CH_4O) is naturally produced by plants from pectin polymers and can be found in gaseous form in the atmosphere and in the soil at the site of origin.[41] Methanol is the second most significant organic compound in the atmosphere after methane. It has a high carbon turnover, plays an important role as a hydroxyl radical sink and increases harmful tropospheric ozone concentrations.[42] Soil microorganisms play a role in the biological oxidation of methanol to CO_2 and therefore have a direct impact on the concentration of atmospheric methanol.[43, 44]

1.2.2. Macronutrients

Nitrogen is an essential component of nucleic acids and amino acids in all organisms. Terrestrial microorganisms fix atmospheric nitrogen and transform the multiple oxidation states and chemical forms of nitrogen present in the soil.[45] Nitrogen availability in the soil is a critical limiting factor for plant development, as nitrogen is a crucial component of chlorophyll molecules. Plants can only utilize inorganic forms of nitrogen, such as nitrate (NO_3^-) and ammonium (NH_4^+). Nitrogen mineralization or ammonification is the process through which the enzymatic activities of microorganisms decompose organic material and release ammonium.

Nitrogen deficiency leads to major crop losses, and therefore use of synthetic nitrogen fertilizer is crucial for profitable crop production in most soils. Use of synthetic nitrogen fertilizer has been increasing in the last decade (Figure 1), and according to current data, crops fertilized in this way feed at least half the world's population.[46]

The Earth's atmosphere consists of 78 percent dinitrogen (N_2), which is an inert gas that is reduced to ammonium by diazotrophic microorganisms found in the soil or in association with plants. Diazotrophs can belong to various groups of prokaryotes. A recent study on the detailed phylogeny of diazotrophs identified 325 bacterial genera that contained the six *nif* genes needed for the formation of nitrogenase; these belonged to the phyla Actinobacteria, Aquificae, Bacteroidetes, Chlorobi, Chloroflexi, Cyanobacteria, Deferribacteres, Euryarchaeota, Firmicutes, Fusobacteria, Nitrospira, Proteobacteria, PVC group and Spirochaetes and came from various environments.[47] Biological nitrogen fixation is a promising substitute for, or complement to, chemical nitrogen fertilization in farming.[48]

Nitrogen-fixing rhizobia belonging to a wide range of genera and species establish symbiosis with legumes. The rhizobia inhabit nodules in the root of the legume and very efficiently fix nitrogen. They are well researched and have been widely used as biofertilizers in agriculture for decades (Annex II). In addition to legumes, actinorhizal plants also have nitrogen-fixing nodules, in this case exclusively with symbiotic *Frankia* species.[49]

Ammonia can be oxidized by microbes to nitrate via nitrite (NO_2^-). Ammonia-oxidizing microbes comprise chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA). AOA dominate the nitrification process in acidic soils, while AOB dominate in neutral, alkaline and nitrogen-rich soils.

Denitrification is the process through which ammonia is converted into gaseous dinitrogen. Because it requires a large amount of organic matter, this process is limited to the topsoil. It occurs more rapidly in waterlogged soils. Denitrifiying prokaryotes are very taxonomically diverse.[50] However, they are not typically used in agriculture but rather in treating manure or sewage.

Invertebrates have indirect effects on soil nitrogen cycling. Microbivorous protozoa and nematodes help to release organic content from the soil microorganisms they feed on, and control the population of their prey organisms .[51] It has been shown that the presence of earthworms can increase the levels of nitrogen mineralization.[52] Together with other macroinvertebrates, earthworms contribute to the formation of below-ground macroaggregates, and increased nitrification occurs in the casts and burrows formed by these organisms.[53]

Phosphorus (P) is a crucial element in nucleic acids and in adenosine triphosphate (ATP) and is hence needed in large amounts by all organisms. A shortage of phosphorus in the soil can delay crop maturity, reduce flower development, lower seed quality and decrease crop yield. Phosphorus is therefore considered a major limiting element for plant productivity.

Phosphorus exists in several forms in the soil. Inorganic phosphorus (Pi) makes up 35–70 percent of soil phosphorus and is the form of phosphorus preferred by plants; unavailable forms are bound as minerals or adsorbed to soil particles. Organic phosphorus (Po) makes up 30–65 percent of soil phosphorus and is mainly accessible to other organisms.[54] The main forms of Po in the soil are phosphate monoesters (i.e. inositol phosphates such as phytate), phosphate diesters (i.e. nucleic acids and phospholipids) and phosphonates.

Biomineralization through microbial activity occurs when organically bound phosphorus is converted into forms of Pi that are available to plants.[55] These Pi forms are called orthophosphates, and include $H_2PO_4^-$ and HPO_4^{2-}. Soil pH, which is influenced by microbial activities such as the secretion of organic acids, determines the immobilization/mobilization of organic and inorganic phosphorus compounds and minerals. Furthermore, plant-available phosphorus can be released from organic phosphorus compounds via microbial enzymatic activities or the release of chelating compounds. Inorganic phosphate solubilization in the soil results from the capacity of some microorganisms (e.g. *Gluconacetobacter, Rhizobium, Klebsiella* and *Aspergillus*, see Annex II) to dissolve mineral phosphate, which is mainly mediated through localized acidification and presumably (i.e. this has not been proven) by the production of siderophores (complexing agents with high affinity for iron).[55] Several plant growth-promoting bacteria (PGPB) show enzymatic Po solubilization activity in the rhizosphere. Various bacterial strains have also been found to produce organic acids or organic acid anions to solubilize Pi from tricalcium phosphate.[56]

Aside from bacteria, microbes with potential to increase phosphate acquisition by plants include below-ground fungi, especially mycorrhizal fungi.[57, 58] Uptake of Pi by plant roots is an active metabolic process limited by uptake capacity and the availability of Pi in the soil. Root clusters are a root modification, affecting the whole root system, that enhances nutrient uptake from soil.[59] Certain plant-associated bacteria may induce or promote root cluster formation [60]. An additional strategy is the formation of arbuscules in the root where mycorrhizal fungi scavenge large amounts of Pi from the soil and deliver it directly to the cortical cells in the root or release phosphorus in the intracellular space in the root tissue.[58] Also, several non-mycorrhizal fungal species such as Aspergillus or Penicillium spp. have been found to contribute to higher Pi availability either by producing organic compounds with carboxylic groups [61], such as oxalate and low-molecular-weight organic anions (LMWOAs), which interact with mineral surfaces containing phosphorus, or by producing extracellular phosphatases (Annex I). In many cases, studies screening for the Pi solubilization potential of isolates are done only *in vitro*, with media supplemented with insoluble tricalcium phosphates, which has been shown to be an inappropriate universal selection factor for actual phosphorus biomineralization.[62]

Some microorganisms in the soil accumulate phosphorus in the form of high-polymeric inorganic polyphosphates (PolyPs) and have plant growth-promoting potential.[63] Among plant growth-promoting fungal and bacterial strains (Annex II) some of the most popular biologicals on the market are products containing organisms with phosphate mobilization and phosphate accumulation potential. Some lichens may also have potential as phosphate-solubilizers.[25] A study on the availability of soil phosphorus in the presence of earthworms found that concentrations of plant-available soil phosphorus were increased by earthworm activity, although the factors involved remained unknown.[64] Similarly to their role in soil nitrogen cycling, invertebrate microbial feeders can influence the composition of the microbial community and thereby affect the availability of phosphorus.[52, 65, 66]

Like nitrogen, potassium (K) is a macronutrient required in large quantities by plants during their main vegetative growth period. It also protects plants against various abiotic stresses, as it maintains their physiological cellular ion concentrations. Soil potassium can be bound in many different minerals, adsorbed to the surface of soil particles or exist as free cations in the soluble fractions of the soil. Potassium is absorbed in the form of K^+ ions along the entire root surface of higher plants and is used to maintain the osmotic potential and turgor of all cells. Potassium fertilizers contain organic potassium compounds (such as potassium citrate), potassium chloride (KCl) or potassium sulfate (K_2SO_4).

As substantial amounts of potassium can be bound to soil particles, potassium-solubilizing microorganisms[67] may be promising biofertilizers. Root-associated microorganisms, such as root endosymbionts and microorganisms living in the rhizosphere, can increase the availability of K^+ in the soil solution and support plant uptake and transfer through interaction with the plant, for example by inducing gene expression of K^+ transporters in plant cells.[68] Strains with this kind of activity have so far been found among the bacterial genera *Bacillus, Pseudomonas, Klebsiella* and *Arthrobacter* and among fungi of the Ascomycota phylum, such as *Aspergillus fumigatus* or *Torulaspora globose.*[69] Among the fungi, both mycorrhizal[70] and non-mycorrhizal endophytic fungi[71] have been found to improve potassium nutrition.

Sulfur (S) is another important macronutrient and is highly interwoven with the cycling of other nutrients.[72, 73] There are several oxidation stages and natural forms of sulfur. The most oxidized state is sulfate (SO_4^{2-}), which is also the form available to plants. In the soil, sulfur is predominantly bound in organic compounds (So) or present in inorganic forms (Si), for example as elemental sulfur or as sulfate in the form of gypsum or adsorbed to soil particles.[74] Soil pH and cation presence play a crucial role in determining the amount of sulfate adsorbed to soil or available to plants in the soil solution. Sulfate deficiency can reduce yield and crop quality.[75, 76] An increasing number of studies report widespread sulfur deficiency in crop production.[75, 77, 78] The sulfur fertilizer products currently used in agriculture are different types of sulfate fertilizers or mixtures of So and Si in organic fertilizers. Depending on the oxidation state of the form of sulfur present in the fertilizer, either organisms with reductive sulfur metabolism or organisms with sulfur oxidation capability are activated.[79] Microorganisms that actively oxidize sulfur compounds to sulfate are highly important for the provision of sulfate to plants.

Magnesium (Mg) is a central component of chlorophyll in plants, and therefore magnesium deficiency affects photosynthetic metabolic pathways and leads to stunted growth and yield losses. Most Mg^{2+} ions in the soil are present in solution or adsorbed to clay or organic-matter particles. Magnesium deficiency is rare in natural soils but can occur if the crop's magnesium demand is high (citruses, tea and sugar cane are the crops most often affected) or if improper fertilization practices are used in highly intensive farming. Some regions, such as the tropics and subtropics, as well as China, are particularly affected by magnesium deficiency in agricultural soils.[80] Excess Mg^{2+} can also be a problem, as it can interfere with calcium uptake by plants and microorganisms. Where the availability of soil magnesium is low, arbuscular mycorrhizal fungi may help plants obtain a sufficient amount of magnesium from the rhizosphere.[81] The geographical distribution and potential risks of magnesium deficiency require more research, as do magnesium fertilization practices.

Plants readily absorb calcium ions (Ca^{2+}) from the available liquid soil fractions and use them in their cell structural elements and as intracellular messengers. Calcium deficiency can induce oxidative damage, stunted growth and yield loss.[82] Soil calcium concentrations are closely connected to the cycling of other nutrients. Excessively high soil nitrogen content can lead to lower calcium concentrations,[83] and excessive soil salinity levels can induce calcium deficiency in crops, an outcome that can be avoided by sufficient watering.[84] Calcium is also connected to calcification and carbonization processes. In general, few organisms directly influence the concentration of free Ca^{2+} in the soil. However, bacteria participate in calcification (MICP), which is discussed above in the paragraphs on carbon cycling.

1.2.3. Micronutrients

Copper (Cu) is an important microelement, but if it is present in the soil in large quantities it can cause major problems by creating toxic patches for plants and other organisms.[85] The forms of copper that are bioavailable to plants are Cu^{2+} and Cu^{+}. In the soil, it can form complexes with both organic and inorganic ligands. Most of the studies conducted on soil copper focus on toxicity and bioremediation using various organisms rather than on the natural mineral cycle. Copper deficiency in plants leads to stunted growth and yellowing,[86] while copper toxicity can lead to similar symptoms.[87] Copper deficiency is usually remedied by adding copper sulfate fertilizers to the soil, and some microorganisms can increase copper solubility in the soil and the plant environment.[88] Some other bacteria can reduce the bioavailability of copper or form various complexes with minerals.[89] This is discussed in more detail in Section 1.3.1. Other microbes can induce copper tolerance in plants.[90]

Iron (Fe) is an essential element and occurs predominantly as ferric (Fe^{3+}) oxides in soils, the most common mineral form being goethite. Plants prefer to absorb ferrous iron (Fe^{2+}) or complexed ferric ions.[91] Iron deficiency in agricultural fields can lead to major losses, as iron is needed for the formation of chlorophyll and important enzymes. On the other hand, excess Fe can lead to soil toxicity[92] and to toxic accumulation in soil invertebrates.[93] Plants have their own strategies for making iron more available. These involve secreting phytosiderophores or utilizing plasma membrane-bound iron reductases in their roots.[94] Some microorganisms, for example some nitrogen-fixing bacteria, are capable of solubilizing iron from mineral oxides[95, 96] by producing mineral-solubilizing enzymes (siderophores), while iron-oxidizing bacteria can oxidize Fe^{2+} into Fe^{3+}.[97]. Iron oxidation in the soil is key to the natural cycling of iron. However, knowledge of the contribution of microbial and other biological processes is limited.

Zinc (Zn) is an important trace element for all organisms. Soils and agricultural fields are particularly vulnerable to zinc deficiency, and this can adversely affect plant metabolism, reduce protein synthesis, disrupt hormone production and result in underdeveloped root systems. Plants usually absorb zinc as Zn^{2+} ions or in the form of organic ligand–zinc complexes.[98] Where soil pH is high, $ZnOH^{+}$ ions can also be taken up by the roots.[99] Zinc-solubilizing microorganisms can increase bioavailability by excreting compounds that create chelates with minerals that contain $Zn_3(PO_4)_2^{-}$.[100] Some plant growth-promoting bacteria have been found to be able to ameliorate excess zinc toxicity.[101]

Chlorine (Cl) is mainly found in the soil solution as chloride ions (Cl^{-}) and is used by plants to regulate their carbon-dioxide uptake and photosynthesis. Microorganisms are key players in the chlorination of soil organic matter.[102] The environmental importance of this process and the specific organisms that contribute to it are largely unknown.

Manganese (Mn) is important for enzyme synthesis and healthy chloroplast formation in plants. It is also a regulatory bottleneck in carbon turnover, as manganese-peroxidase catalyses the oxidative decomposition of carbon in the soil.[103] Manganese-solubilizing bacteria in the rhizosphere could potentially be used as agricultural bioinoculants, as they can boost crop yield by increasing the bioavailability of manganese via reductive processes.[104] These microorganisms can be important in manganese-deficient sandy, dry and calcareous agricultural soils. Under anaerobic conditions some soil bacteria and archaea couple their growth to the reduction of manganese.[105]

Molybdenum (Mo) is an important enzyme cofactor for microorganisms and plants. It plays a major role in nitrogen fixation in some diazotrophs, as it is part of the molybdenum-nitrogenase enzyme.[106, 107] Molybdenum exists in a soluble form in the soil but is rare and is highly susceptible to leaching, as it can bind to tannins in the topsoil.

Boron (B) is an essential non-metal micronutrient that is absorbed by plants as boric acid ($B(OH)_3$, H_3BO_3). Microorganisms can increase the amount of plant-available boron in the soil by digesting organic complexes. The role of boron in the soil is still largely unknown. High boron levels caused by the use of desalinated seawater irrigation in agriculture have been found to negatively affect soil bacterial and fungal communities and key soil enzymatic processes.[108]

1.3. Roles of soil microorganisms and invertebrates in the bioremediation of major agricultural contaminants

Various agricultural practices, for instance excess use of fertilizers (including animal manure) and pesticides, amendment with solid wastes and irrigation with sewage, are leading to soil pollution. Traditional methods of soil remediation include landfilling, leaching, excavation and disposal, and physico-chemical cleaning. Bioremediation is the process whereby living organisms breakdown, remove, alter, immobilize or detoxify chemicals and physical wastes from the environment, converting them into an innocuous state or reducing their concentrations to levels below limits established by regulatory authorities.

Methods of soil remediation include phytoextraction (uptake of contaminants), phytofiltration (removal of contaminants), phytostabilization (immobilization and stabilization of contaminants), phytovolatilization (conversion of pollutants into volatile form and their gaseous release), phytodegradation (enzymatic degradation of pollutants), phytodesalination (take-up of salts by halophytic plants) and rhizodegradation (breakdown of contaminants in the rhizosphere by microorganisms), and they can all use plant-associated microorganisms to degrade xenobiotics.[109, 110] Phytoextraction can be a time-consuming method, and several applications are needed to achieve proper decontamination of the soil. Microorganisms can be used either on site, in combination with plants and/or invertebrates, or in bioreactors and confined spaces containing excavated soils.

Soil pollution is discussed in detail in the FAO and Intergovernmental Technical Panel on Soils report *Status of the world's soil resources,*[111] which identifies agricultural soil contaminants and their origins, and in the FAO publication *Soil pollution: a hidden reality.*[112] The present study focuses on the microorganisms and invertebrates with the potential to bioremediate heavy metals and pesticides in soils (a full list of organisms is provided in Annex IV).

1.3.1. Origin and site of heavy-metal and trace-element contamination and their bioremediation

The use of some agrochemicals, leaded gasoline in agricultural vehicles, wastewater irrigation and fertilization with manure and sewage sludge have resulted in heavy-metal contamination of soils. Heavy-metal pollution can impair plant metabolism and reduce plant productivity. Heavy metals may derive from several groups of chemical elements and are categorized by their density, atomic number and chemical properties. They can accumulate in some edible parts of plants and can therefore threaten human health. Conventional chemical fertilizers or fertilization techniques can be a source of heavy metals and of natural radionuclides, such as potassium, uranium and thorium.[113] Heavy metals contained in animal manure, silage, various types of compost or untreated sewage water used for irrigation may contaminate soils and groundwater.

Several technologies can be used to remediate sites contaminated with heavy metals. The traditional approach of using physico-chemical methods can be expensive and in some cases involves dangerous radiation or chemicals.[114] Bioremediation is a modern, safe, low-cost and relatively eco-friendly alternative that is particularly suited for removing low concentrations of pollutants. The term bioremediation refers to *in situ* biological treatment that uses soil microorganisms and is primarily used to degrade organic contaminants, including petroleum hydrocarbons, solvents, and pesticides, and to transform species of trace elements to reduce their availability.[115] Biosorption (sorption with biological material) using microorganisms allows heavy-metal decontamination without the generation of toxic sludge or secondary pollutants[116, 117]. Biosorption can be performed with both living and dead microbial biomass.[118] The use of dead cells has the advantage that they can be easily stored in powdered formed and hence do not need to be maintained under the specific growth conditions needed by living microorganisms. While bioaccumulation (accumulation of the pollutant in the organism) is an active process that depends on microbial metabolism and is partially reversible, biosorption is a metabolism-independent, reversable process that does not require much energy input or ideal respiratory environments. Another method of bioremediation is to use organisms that can transform the toxic forms of a pollutant into non-toxic and less-mobile forms.

The ideal way to obtain good microbial candidates for bioremediation is to collect on-site samples and isolate heavy-metal resistant strains with the specific genetic toolset needed to transform the polluting agent (Annex III).[119] Alternatively, some organisms can be stimulated to grow on site. The introduction of bioengineered or non-native microorganisms into the soil is questionable, even at contaminated sites, although they offer a fast and easy way of treating sewage sludge or sewage water in closed systems where sterilization or termination of the organisms is possible before the bioremediated material is used in the field. All bioremediation involving the use of live organisms should be subject to a proper evaluation of risks to human or animal health or to the local ecosystem, and addition of anything to the soil should be carefully controlled.

The success of bioremediation and the removal rate of contaminants also depend on a set of environmental factors (e.g. pH, temperature and SOC content) specific to the respective contaminants.[120] Many heavy-metal bioremediation methods are directly connected to the regulation of the pH of the environment, as enzymatic or ionic charges make the process easier or harder. The mobility and availability of most metals in the soil depend on microbial processes.[121] Numerous native soil bacteria contribute naturally to the reduction of toxicity levels by excreting exopolysaccharides that absorb heavy metals.[122]

Anaerobic sediments and paddy soils provide microorganisms with environmental conditions that differ from those in “regular” agricultural soils, and different microbial metabolisms dominate in them. For example, anaerobic sulfate-reducing bacteria can precipitate heavy metals as insoluble sulfides.[123] Some plant-associated microorganisms can also act as enhancers or complementors of the phytoremediation or phytoextraction of trace elements.[124] A study on the potential of earthworms in the removal of heavy-metal contamination[125] observed a decrease in levels of arsenic, cadmium, copper, chromium, mercury, nickel, lead and vanadium. Many of the microorganisms and earthworms added to the soil – on site or in tanks – to reduce the bioaccumulation or bioavailability of toxic substances can also simultaneously increase plant growth, soil fertility and nutrient availability. Complex multiple heavy-metal pollution at one site is also common and often occurs together with organic hydrocarbon contamination.

Arsenic (As) is a typical highly toxic pollutant with four oxidation states, among which the inorganic species arsine and arsenite are the most toxic. The main sources of arsenic contamination are mining (e.g. for coal or gold), quarrying and the use of some herbicides and fungicides in agriculture. Use of fossil fuels also releases considerable amounts of arsenic into the environment, where it then disperses relatively quickly through groundwater movements. Arsenic is present in groundwaters and soils around the world, and it can be taken up and accumulated by crops, especially by edible and medicinal mushrooms, which are considered to be hyperaccumulators,[126] and by rice (because of the flooded field conditions in which it is grown).[127] Its presence in plants can lead to stunted growth and lower yields. In humans, arsenic compounds are deposited in the skin, lungs and kidneys, where they cause reactive oxidative stress and damage DNA functions and mitochondrial respiration. These effects can lead to multiple severe conditions.

Removal of arsenic through expensive and laborious physical-chemical methods that generate toxic wastes can be replaced with the use of microorganisms to bioremediate arsenic pollution through reduction, oxidation, intracellular bioaccumulation or methylation.[128] This can involve microorganisms that either complement phytoremediation by affecting plant metabolism[129, 130] or are able to biosorb, biotransform[131] or biomineralize arsenic directly in the soil.[132] Several arsenic-resistant bacteria that can colonize the rhizosphere of plants and participate in the bioremediation of the contaminant have been isolated from contaminated soils.[133, 134] Some fungi in the rhizosphere[135-137] and soil algae[138] are also capable of reducing arsenic accumulation and ameliorating toxicity in crops by modulating the antioxidant enzymes in the plant. The use of invertebrates in the bioremediation of arsenic in agriculture has not been explored in any depth, but some studies have shown that using earthworms in contaminated soil may have positive effects.[125]

Cadmium (Cd) is a persistent toxic metal that accumulates in the food chain and in individual organisms because of its chemical similarity to zinc. Crops take up and store cadmium in their tissues, leading to direct human exposure. Long-term exposure to cadmium may lead to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central and peripheral nervous, and respiratory systems.[139] Cadmium is reported to be able to negatively affect the uptake of nutrients,[140] leading to stunted plant growth.

The main agricultural sources of cadmium contamination are phosphate fertilizers[141, 142], organic fertilizers (e.g. wastewater or sewage sludge) that have not been properly treated, and fuel combustion.[143]. Studies from all around the world report persistent cadmium contamination of agricultural fields[144-147], with the most industrialized and agriculturally intensified countries probably being those worst affected. The source of phosphorus fertilizer can also affect the cadmium levels of an agricultural region. For example, in the European Union (EU) higher cadmium contamination levels are found in the west, where phosphate rock fertilizers are imported from North Africa, than in the east, where practically cadmium-free Russian magmatic phosphate rock[148, 149] is used.

The most commonly used method of decreasing the bioavailability of cadmium in soils is to add organic amendments, such as compost, biochar, manure, rice husk or saw dust. Organic amendments increase the soil's organic-matter content, which forms complexes with cadmium and thus reduces the bioavailability of the contaminant. However, these practices do not decrease the actual cadmium concentration in the soil and hence do not reduce the potential to pollute groundwater.[144] Cadmium can be removed through phytoextraction or bioremediation. Bioremediation of cadmium can be done using microorganisms,[143] for example various types of bacteria (e.g. *Bacillus, Pseudomonas, Salmonella, Bifidobacterium, Serratia, Rhodobacter, Pantoea* and *Enterobacter*), algae and fungi (e.g. *Trichoderma, Aspergillus, Fomitopsis, Penicillium, Mucor* and *Cladosporium*) (full list in Annex IV). These organisms are usually cadmium resistant, capable of biosorption or bioaccumulation, and suitable for application in a variety of conditions and environments. Some bioremediating strains may have plant growth-promoting effects.[143, 150, 151]. In addition to the use of such organisms in fields, they can be also used to treat wastewater.[152]

Another nature-based approach that can be used to reduce cadmium in the soil is the application of biochar. The sorption properties of biochar are related to its large surface area, high cation exchange capacity (CEC), alkaline pH and surface functional groups. Meta-analyses have shown that cadmium reduction in plants can be expected when biochar is applied to soils that have low pH, coarse texture and intermediate levels of organic carbon content.[153]

As noted above, small amounts of **copper (Cu)** are essential for plants and other organisms, but excess concentrations in the soil can lead to problems. The main origins of copper in agricultural fields are fungicides (copper sulphate),[154] pig manure[88] and the use of inadequately treated wastewater for irrigation. Fields or orchards near abandoned or active copper mines are especially affected. Some plants are very tolerant of copper contamination. However, as copper enters the food chain, it accumulates and leads to animal and human pathologies. Higher than normal soil copper concentration alters the composition of the bacterial and fungal community and decreases concentrations of the key soil enzymes, urease, invertase and cellulase.[155]

Methods for bioremediating excess soil copper include bioimmobilization[156] and bioaugmentation-assisted phytoextraction.[157, 158]. Alkaliphilic bacteria may be able to raise the pH of the soil by one or two units, making copper biologically unavailable to plants. An ethylenediamine tetraacetic acid (EDTA) leaching technique involving the use of the earthworm *Lumbricus terrestris* has been found to effectively immobilize the copper in the soil.[159] Recent studies have found that a combination of earthworms and ectomycorrhizal fungi increases the phytoextraction rate of copper,[160, 161] which highlights the potential of bioremediation systems based on multiple organisms.

Mercury (Hg), a non-essential heavy metal, is a widely used industrial product and an active ingredient in many pesticides. Mercury pollution is primarily driven by anthropogenic emissions, which greatly exceed those from natural geogenic sources.[162] The mercuric ion (Hg^{2+}) readily adsorbs to soil particles. Soil microorganisms can methylate mercury to yield highly neurotoxic methylmercury (MeHg, CH_3Hg^+),[163] which can be further methylated to dimethylmercury.[164] Sulfur- and iron-reducing bacteria[165] and methanogenic archaea[166] are involved in this process. Given the high levels of mercury contamination in Asian soils and the anaerobic environment of the flooded fields used for rice production, methylmercury exposure is especially high in people who consume rice and rice-derived food daily.[167] Several bacteria can convert the toxic forms of mercury into non-toxic mercury compound[168] (Annex IV) through their mercuric reductase and organomercury lyase activity (Annex III).

Nickel (Ni) is an essential micronutrient. However, at high concentrations and through bioaccumulation in some tissues it induces leaf chlorosis and inhibits plant development by reducing cell-division rates.[169] Humans can develop severe allergic reactions to nickel and nickel alloys when they are inhaled, consumed in high volumes or come into contact with the skin.[170] Agriculture contributes to nickel contamination of the soil through the use of conventional fertilizers and sewage sludge.[171] High nickel concentrations in the soil can inhibit microbial processes and lower nitrogen-fixation rates.[172] Because of their sensitivity to high nickel concentrations, microorganisms have developed several mechanisms for detoxification[173] (see Annex IV for a list of organisms used in nickel bioremediation).

Lead (Pb), a highly dense, toxic heavy metal that used to be a major fuel additive until it was slowly phased out in the 1980s and 1990s in Japan, Europe and North America[174] and the establishment of the Partnership for Clean Fuels and Vehicles by the United Nations Environment Programme in 2002.[175] However, aviation fuel still contains lead additives, which can affect agricultural regions where planes are used to spray pesticides,[176] mainly causing atmospheric gaseous contamination and groundwater and soil contamination when spilled. In recent times, the use of fertilizers, pesticides and sewage sludge fertilization/irrigation has become the main source of lead in the soil. Lead disrupts the uptake of plant nutrients and seed germination and causes various neurological issues in mammals.[177] Some classical physicochemical remediation methods are inefficient because of the relatively low concentration of lead in the soil.[178] Various lead-tolerant bacteria, fungi and algae can effectively immobilize this heavy metal.[179] Microbial bioremediation of lead is possible through exopolysaccharide biosorption by bacteria[180] and biosorption by fungi.[181, 182]

1.3.2. Origin and location of pesticides and their residues in agricultural soils and their bioremediation

Pesticides are defined as substances or mixtures of substances, intended for preventing, destroying, or controlling any pest causing harm or interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products.[183] The toxic effects of now-banned pesticides still have negative effects on soil biodiversity[184] and are considered a threat to human health. According to data from FAOSTAT, despite the decline in the use of some individual active pesticide ingredients, there was a steady rise in the agricultural use of pesticides in the agricultural areas between 1990 and 2020 worldwide (Source: FAOSTAT data.). The EU Pesticide Database contains 1 481 active substance records, out of which 452 are approved for use.[185] On a global scale, the use of agricultural pesticides is expected to grow.[186] Problems with contamination continue even in regions where there are widespread and strict bans on the most toxic/hazardous pesticides. For example, in the EU, the frequency and intensity of the contamination of fruit and vegetables with hazardous residues increased between 2011 and 2019[187] and some banned pesticides are still in use.[188] In response to concerns about this massive use of harmful pesticides, the European Commission's Farm to Fork Strategy, published in 2020, aims to half the usage of pesticides by 2030.[189]

Other regions are even more vulnerable. For example, East Asia is one of the highest risk regions in the world, with a large number of ecotoxic active ingredients in use and a high assessed pesticide risk score.[186] A concerning finding is that 31.4 percent of the world's high pesticide pollution risk areas are in biodiversity hotspots.[186] Given the ability of pesticides to accumulate and disperse, not only agricultural fields but also bordering areas face the risk of losing biodiversity and important ecological functions. The half-life times given for the new generation of pesticides correspond to particular laboratory conditions, but these can greatly differ from real-life scenarios[190]. The comprehensive study conducted by Tang *et al*.[186] identified the following countries as having high pesticide-pollution risk, high water scarcity and high biodiversity: Argentina, Australia, China, Ecuador, India, Mexico and South Africa.

Even where efforts are being made to reduce pesticide use, soils remain contaminated with the residues of banned pesticides. These require removal, preferably through bioremediation. Approaches to the banning of harmful or potentially harmful compounds vary around the world. For example, Brazil, China and the EU are slowly phasing out some outdoor pesticides whose use is still permitted in the United States of America.[191] Each year, FAO and WHO, under the Codex Alimentarius Commission's Codex Committee on Pesticide Residues, organize a Joint Meeting on Pesticide Residues to evaluate current guidelines on pesticide residues in food.[192] They also developed the International Code of Conduct on Pesticide Management, published in 2014.[193]

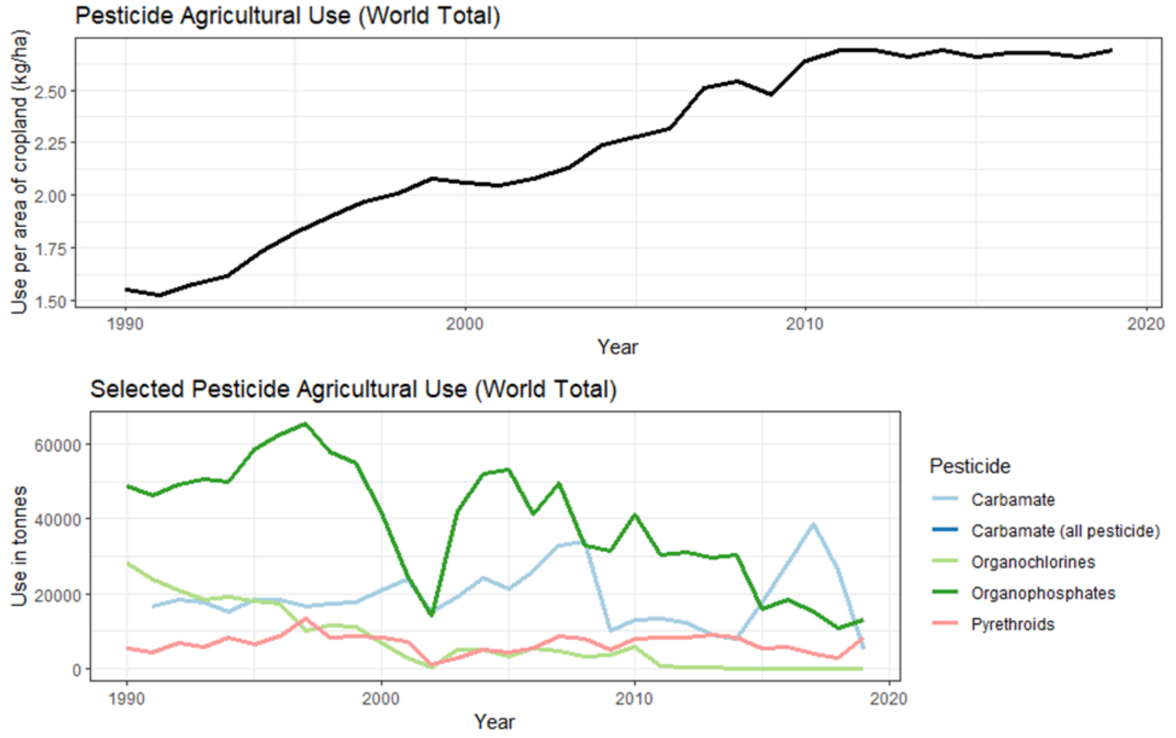
Organochlorine and **organophosphate fungicides and insecticides** are highly toxic, volatile and cause persistent widespread contamination. They have been proven to have several negative effects on human health[194] and animal health, and to disrupt soil microbial biodiversity[195] and soil functions because of their antimicrobial and insecticidal effects.[196-198]. Their high persistence and low biodegradation rate mean that even after being banned, as they have been in several countries, they remain present in the environment. Furthermore, some of these compounds are still in use in a number of developing countries, and residues can be detected in surface waters and in crops all around the globe.[199-202] Despite their high toxicity, phytoremediation and microbial remediation approaches are known to extract, degrade or immobilize organochlorine and organophosphate contamination.[203] This is particularly significant, as these pesticides are known to be highly persistent in the environment. Among the soil microorganisms capable of withstanding these pesticides in the soil, various fungal [204] and bacterial[205] species can degrade them. However, commercially widely available and viable bioremediation products are still under development.[205]

Carbamates are esters of carbamic acid with different substituents and are mainly used as insecticides, herbicides and fungicides. They have lower toxicity and environmental persistence than organochlorines and organophosphates, although they can cause neurological and endocrinological problems in humans, are highly soluble in water and spread easily as groundwater contaminants.[206] Carbamate bioremediation approaches using organisms such as fungi [207, 208] and bacteria[209] isolated from carbamate contaminates soils have been developed. However, the extent to which these are used in bioremediation of polluted soils is not known.

Pyrethroid insecticides, such as deltamethrin, are the synthetic analogues of pyrethrins from chrysanthemum and are widely used for various industrial and public purposes. For decades they were the safer alternative to organophosphate pesticides. However, their widespread and careless use combined with their slow biodegradation led to the pollution of aquatic environments[210] and soils. Thorough ecotoxicological assessments of their effects on all terrestrial organisms are not available, but these pesticides have toxic effects on terrestrial invertebrates[211] and neurotoxicological effects on vertebrates in high doses.[212] Bioremediation using several pyrethrin-degrading microorganisms is possible.[213] These microorganisms can degrade pyrethroids in the soil via various pathways involving the action of specific pyrethroid hydrolase enzymes (Annex III). However, the actual extent of use of microbial bioremediation approaches to deal with soil pyrethroid pollution is not known.

Lichens can decrease the bioavailability of soil contaminants, as shown in an experiment in which the lichen *Peltigera canina* was used to treat dichlorodiphenyltrichloroethane (DDT) contaminated soil and had positive effects on the development of zucchini planted in the treated soil.[214]

Figure 2 Trends in global pesticide use 1990 to 2020



Source: FAOSTAT data.

Chapter 2. Threats to the biodiversity of microorganisms and invertebrates that contribute to soil nutrient cycling and bioremediation

Soil biodiversity is threatened by a number of factors that can accelerate local species extinctions or changes to the biota.[6, 14, 19, 215-218]. Potential threats include failure to comply with relevant legislation and biodiversity guidelines, inadequate controls and inspections on the use of substances and practices that can harm biodiversity,[187] and insufficient consequences for the misuse of such substances and practices or for illegal import of living organisms and biological materials. Direct threats such as damaging and unsustainable agricultural practices and intensification,[219] alien species invasions,[220] disruption of soils by antimicrobial agents and genetically modified organisms, and the effects of climate change on below-ground ecosystems, are discussed in the following paragraphs.

2.1. Damaging and unsustainable agricultural practices

One of the leading causes of global biodiversity loss is agricultural intensification.[219, 221, 222] Agricultural practices, such as crop rotations, tillage and fertilizer use can have huge effects on the local SOC pool and on atmospheric CO_2 concentrations.[223] While tillage may dry the soil and the associated use of heavy machinery may increase soil compaction,[224] a lack of sufficient studies across a range of regions, continents and environments means that its impacts on soil biodiversity are not fully understood. It has been suggested on several occasions that tillage may disturb underground fungal connections[225] and decrease bacterial diversity.[226] A study conducted in various parts of Europe found that intensive rotations in land use reduced the biodiversity of soil microorganisms and invertebrates and reduced the complexity of soil food webs compared to grasslands and farms with medium intensity soil management.[11] The study also found that soil animals under intensive rotations had smaller body mass than those in grasslands.[11] However, a study in China found that conversion of natural habitats into agricultural land did not affect the functional community composition of soil nematodes; however, agricultural practices might lead to the loss of rare and specialist taxa in low-latitude areas.[227] The findings raise the need for more research in areas at various latitudes. A study in Switzerland analysed the root and soil microbiome of no-till, conventional tillage and organic fields, and reported reduced microbial network complexity and lower abundance of the keystone mycorrhizal taxa belonging to the orders Glomerales Paraglomerales and Diversisporales in fields under conventional agricultural intensification (tillage and chemical fertilizer inputs).[228]

A recent FAO report lists the effects of land-use intensification, tillage, improper irrigation, pesticide use, fertilization practices, microplastics and crop diversification on the soil microbiome.[229] It concludes that tillage can shape soil microbial communities and negatively influence soil functioning and that treated wastewater irrigation can have direct and indirect effects on the composition of the soil microbiome and various negative effects on its functioning. The authors reviewed the results of a range of studies on the effects of pesticides on soil microorganisms and found that, in addition to the numerous studies referring to the disruptive impacts of pesticides on the soil microbiome, some conflicting results had been reported. In some cases, organochlorine pesticides were found not have a strong impact on soil ecosystems – or soil microbiomes were found to be highly adaptive to the introduction of chemical substances. The report also draws attention to recommendations for policymakers regarding the need to increase support for research, development and innovation, particularly to address the widespread knowledge gaps that exist in some crucial areas. The points highlighted include the need for research on the components and definition of healthy a soil microbiome, for research on the soil microbiome to be expanded from laboratory conditions to the field, for international and interdisciplinary studies linking various elements of microbiome research and for collaborative efforts with other sectors.

Mendes *et al*.[230] proposed that the "intermediate disturbance hypothesis", which suggests that diversity tends to increase after a moderate disturbance event and that an equilibrium state harbours lower diversity,[231] is valid in agricultural soils. However, other studies have reported higher levels of soil microbial biomass carbon and higher soil enzymatic activity in soils with low disturbance.[232, 233]

Increasing above-ground diversity, for example growing several types of plants together rather than a monoculture, leads to an increase in soil nutrient content, plant biomass[216] and below-ground diversity.[234] The increase in plant productivity in species-rich plant communities relies on plant–soil feedbacks[235] and the functions of the soil and plant microbiomes.[236] These processes are negatively affected in monocultures. Long-term monoculture has been shown to reduce the biodiversity of several components of the soil biota,[237, 238] such as the diversity of nodulating and nitrogen-fixing rhizobial symbionts of legumes.[5]

Crop diversification from monoculture to species mixtures increases annual primary productivity and leads to higher yields, although the use of varieties bred for maximum performance in monoculture will not have the same outcome in a diversified field.[239, 240] Most studies of continuous cropping[241] and long-term monocultures (including annual and perennial crops) report that they lead to loss of soil fertility, increases in the number of plant pathogens, decreases in soil enzymatic activity and changes to soil microbial diversity.[242, 243] Long-term continuous cropping systems have been found to negatively affect soil nematode communities and to decrease soil fertility and nutrient replenishment, potentially leading to lower crop productivity and loss of profit.[244]

Plants can also have legacy effects on the soil microbial community, i.e. effects that continue after the plants are no longer present. The legacy effects of intensified crop production on soil microbiomes can be negative, and there is therefore a need for innovative sustainable agricultural management that creates positive above- and below-ground legacies.[245] An experiment on the legacy effect of monoculture found that it persisted for over six months.[246]

World agricultural demand for nitrogen, phosphorus and potassium fertilizers is slowly but steadily rising,[247] with a few regional differences. Africa needs to import K_2O fertilizer, while the other nutrients can be supplied from local sources. North America's demand for nitrogen and phosphorus is higher than can be met without imports. Latin America and the Caribbean and South Asia are the regions whose soils have the worst NPK fertilizer status, and they are highly reliant on imports from other countries. West Asia, Eastern Europe and Central Asia export fertilizer. East Asian fields are short of potassium, and the region is therefore dependent on imports. Central and Western Europe depend on the import of phosphorus fertilizers. Western Europe also has high demand for nitrogen fertilizer, while Eastern Europe needs to import potassium fertilizer.[247]

The production of traditional chemical nitrogen fertilizers requires a lot of energy and fossil fuels, and excess nitrate can be leached into ground and surface waters, and this can have negative effects on health if the water is used for drinking.[248] Thus, increasing demand for this kind of fertilizer cannot be met without increasing environmental damage. Heavy, long-term application of nitrogen fertilizer has been shown to increase soil nitrification by changing the AOA and AOB soil communities.[249] Overall, it has been shown that AOA have a more important role than AOB in acidic agricultural soils.[250, 251]. The composition of the microbial communities involved in the soil nitrogen cycle is also affected by the type of land use. An analysis of such effects on ammonia and nitrite oxidizers in different soils suggests that disturbances alter the distribution of active nitrifier communities and can alter the physicochemical properties of the soil.[252]

The big demand for phosphorus in agriculture is met with non-renewable phosphate rock fertilizers, which both threatens groundwater reserves and means that the future supply of phosphorus fertilizer is insecure.[253] Agricultural activities and excess fertilizer runoff are key sources of excess nitrate and phosphate in water reserves and eventually lead to the eutrophication of surface waters.[254, 255] A study of the different effects of fertilization practices and soil amendments on the concentration and form of mercury in rice found that conventional phosphate (calcium superphosphate), manure and rice straw fertilization increased the abundance of major mercury-methylating prokaryotes and thus led to the formation of dangerous methylmercury in the grains.[256]

Soil fumigation with biocides prior to planting crops used to be widely practised as a means of combating plant diseases and weeds.[257] However, due to its high costs, varying efficiency and potential negative ecological effects, other methods such as cover crops as green manure with biofumigation effects have been introduced.[258, 259] Different fumigation methods differ in terms of the amount of residue they leave and in terms of the toxicity of the chemicals used. Residue levels in the final product depend on the oil content of the plant tissue, the solubility of the chemical applied and the number of treatments.[260, 261] Even after washing and cooking, some fumigants remain present in the food or wood product and can even change the aromatic profiles of oily seeds.[262] Soil fumigation damages soil biodiversity, negatively affects the physicochemical characteristics of the field[263] and reduces the abundance of bacterial taxa taking part in soil nitrogen cycling.[264] It has been proposed that chemical fumigants could be replaced with biofumigants. However, because of their basic mechanism of decreasing the diversity of taxonomic groups of disease-causing microorganisms, biofumigants seem also to have a negative effect on all soil-fungal diversity and not only on pathogens.[265] Agricultural soils where microbial diversity has been reduced by intense fumigation may provide better conditions for pathogen survival because competition is reduced.[266, 267]

2.2. Invasive species, antimicrobial resistance genes and genetically modified organisms

The intentional and unintentional introduction of non-native earthworm species from agricultural vermicomposting technologies, fishing or animal feed into the native soils of several continents is likely to have led, and still be leading, to the decline of native terrestrial worm diversities. Some studies suggest that earthworm invasion leads to shifts in the composition of soil bacterial communities[268] and a significant decline in soil invertebrate diversity and density.[220] A long-term study on the effect of the presence of invasive earthworms on native plant productivity found that forest soils with greater worm diversity as a result of invasion were shallower and had reduced microaggregate proportions, which disrupts the fertile seed-bed and makes the forest more prone to invasion by non-native plant species.[269] These effects could negatively affect soil ecosystem functions and services and above-ground biodiversity over time.[270] The exotic-invasive epigeic earthworm *Aporrectodea trapezoides* almost completely eradicated the once-common species *Driloleirus americanus* in some relict prairies in North America.[271] Accidental introduction of other alien soil invertebrates into new continents, for example the introduction of the successful earthworm predator the land planarium *Bipalium adventitium* to North America,[272] further endangered native earthworm diversity.[273] The introduction of natural but non-native microorganisms into agricultural fields has failed on multiple occasions,[274, 275] while in some cases the introduction of non-native microorganisms and invertebrates has led to irreversible changes to soil biodiversity.[276-278] A known case in which invasion led to fungal extinction is the rapid spread of the pathogenic Ascomycete fungus *Hymenoscyphus pseudoalbidus* in Europe, which resulted in a massive decline of the native decomposer fungus *Hymenoscyphus albidus* in the soil.[279] CABI provides a comprehensive and well-curated online invasive species compendium.[280]

The main sources of antibiotic resistance genes (ARGs) in the soil are the application of animal manure as fertilizer and irrigation with human wastewater. ARGs can persist for as long as two years in the soil after manure has been applied.[281] Their concentration in agricultural fields rose significantly during the period from 1940 to 2010 (i.e. during the decades that followed the invention of antibiotics), as shown, for example, by a study of soils in the Netherlands.[282] More recent long-term studies with three[283] and ten[284] years of follow-up to manure and sewage-sludge (biosolid) fertilization of fields found increased levels of persistent ARGs in agricultural soils each year and that concentrations persisted even after the manure and biosolid application stopped. A meta-analysis of ARG prevalence in the environment identified sulfonamide and tetracycline ARGs as the most researched and reported examples of ARG presence in farm and field environments worldwide.[285]

Antibiotics in agriculture pose a major threat to native soil microbial biodiversity in fields and therefore potentially also to the prevalence of plant-root symbionts of soil origin.[215] Antibiotics and ARGs contribute to the development of multidrug-resistant bacterial strains in the environment.[286]. A study of irrigation with wastewater from pigpens found that it resulted in increased abundance of ARGs in the rhizosphere, bulk soil and even in plant endophytes.[287] The addition of biochar to fields has been shown to reduce the abundance of antibiotics and ARGs in the soil.[285, 288] However, there are no data available on the extent to which this technology is currently being used in practice. Moreover, the effects of biochar are not necessarily beneficial. Soil moisture levels and addition of biochar have been found to have significant effects on ARG retention and on the maintenance of soil bacterial diversity. ARGs dissipate more slowly in dry soils and soils supplemented with biochar because of biochar's microporous structure and adsorption of ARGs on the surface of the carbon within it; it is likely that concentrations may change over time because the adsorbed ARGs may dissipate from the aging biochar as it loses its original potential.[281, 287] More research and improved biochar technologies are needed in order to improve the positive effects of biochar in ARG removal from soils.

As bacteriophages play a major role in the horizontal gene transfer of ARGs in the environment, the control of phages could also help reduce the spread of ARGs in the soil. Bacteriophages occur naturally in huge numbers (10^9 virions/gram) in the soil.[289] Phage therapies probably have potential as environmentally friendly soil management practices: polyvalent phages have no host specificity, and it has been found that they can be applied to combat pathogenic bacteria and the spread of ARGs when combined with biochar amendments.[290] However, some studies have highlighted the possibility that viable phages carrying ARGs could infect new hosts with them, leading to their persistence not only in the soil but also in the edible parts of crops and thus increasing the risk that antibiotic resistant bacterial strains will emerge.[291] More field studies would be needed to evaluate whether widespread agronomical application of phages would be feasible and risk-free or whether the presence of phages favours the persistence of ARGs in the environment.[292]

Genetically modified microorganisms, such as engineered plant-growth promoting bacteria and multicontaminant-removing bacteria,[293] have been developed and no adverse widespread effects on the soil microbiome have been found in studies so far.[294, 295] However, regulatory constraints mean that they are rarely used in agriculture or released into the open environment. Several questions regarding the advantages and disadvantages of genetically modified microorganisms and the possible ecological damage they may cause to native biodiversity have been raised in recent decades by scientists.[296] Genetic engineering could improve microbial traits or multiple beneficial mechanisms and traits could be combined in single microbial strain. There are concerns that genetic engineering could lead to the release of potentially invasive microbes, but such examples have not been reported and should be preventable by strict regulation.

2.3. Global climate change and elevated atmospheric carbon dioxide levels

It is not fully clear how carbon cycling in different regions will be affected by climate change. For instance, research shows that the thawing of arctic permafrost increases the abundance of methanogens and the release of methane into the atmosphere.[297] However, developing technologies for directed bacteriophage infections or the application of methanotrophs, i.e. microbes that utilize exclusively methane as carbon source, could mean that in the future it will possible to influence the speed of this process.[298]

Altitudinal and temperature differences are known to drive the community composition of nitrogen-fixing bacteria in the soil. A study in an alpine environment showed that different diazotrophs colonized different altitudes, that rainfall stimulated the activity of the diazotrophs and that higher temperatures triggered changes in the abundance and diversity of the soil microbial community.[299] These findings suggest that climate change may strongly affect biological nitrogen fixation, as some psychrophilic (cold-loving) diazotrophs are sensitive to temperature changes and microorganisms perform worse during drought.

An experiment on the effects of a simulated 2 °C increase in environmental temperature on fungal communities in tropical grassland soil found that some fungal taxa showed increased abundance, while the relative abundances of others decreased significantly.[300] This highlights the non-uniform effects that climate change has on the microbiome of an ecosystem. Furthermore, the same study found that the abundance of phytopathogenic fungi increased in response to drought because of their adaptedness to reduced water availability. The main conclusion of a long-term multifactorial global change experiment on grasslands was that it is important to adjust soil biodiversity conservation and ecosystem management to account for expected future soil temperatures, altered precipitation and land use.[301] This recommendation was based on the finding that warming has a predominant role in accelerating both the taxonomic and the phylogenetic temporal scaling rates of soil microbial communities, which are measures of changes in community distribution and taxonomical composition over time.[301]

In areas with heavy-metal pollution or persistent organic pollutants (pesticides) in the soil, the concentration of pollutants and the toxicity can worsen when temperatures increase and soil moisture content decreases, [302] which threatens some soil invertebrates, such as earthworms[303] and springtails and other small soil insects.[304] A study of soil nematodes found them to be sensitive to daytime warming and dry soil.[305] A predictive analysis of expected climate and land-use changes between 2015 and 2070 conducted as part of a global soil field survey covering archaea, bacteria, fungi, protists and invertebrates found that species richness and diversity of soil biological and biochemical functionality in soil conservation hotspots will decline, with the effects mainly occurring in the Global North.[14] Global warming is predicted also to lead to elevated crop losses through increased insect pest damage.[306, 307] This could lead to an increase in the number of available pesticides and in pesticide application, which would pose a threat to biodiversity. Atmospheric CO_2 levels have increased since the industrial revolution from a base level of 280 µl/l to 424 µl/l.[308] In a six-year *in situ* experiment on grassland, elevated atmospheric CO_2 levels were found to contribute to the extinction of larger-diameter nematodes by inducing structural changes in the soil.[309]

2.4. Examples of elevated local risks of extinction of soil microorganisms and invertebrates

In 2014, Tsiafouli *et al*.[11] reported that soils sampled from intensive rotations in Greece lacked earthworms and predaceous collembolans and that those sampled from intensive rotations in Sweden lacked fungivorous mites and predaceous collembolans. A study in North-America found that the bacterial phylum Verrucomicrobia, which commonly occurs in soils and potentially contributes to biogeochemical cycling,[310] was dominant in undisturbed prairie soil and that intense agricultural management significantly lowered its abundance.[311] Similarly, a study in Switzerland found that fungi from the order Sebacinales, mycorrhiza-forming organism belonging to the Basidiomycota, were found in organic farms but absent from conventional farms.[312] A European study reported reduced occurrence of fungal species,[313] for example a 45 percent decline of sporocarps of ectomycorrhizal fungi, especially those of the genera *Phellodon, Hydnellum, Suillus, Tricholoma* and *Cortinarius*. This decline is probably connected to a change in land use and increased nitrogen fertilization.[314] Also, in New Zealand, the soil-dwelling earthworm *Aporrectodea longa* disappeared from farm soils with high irrigation.[315] Invasive species drive local extinctions, such as that of the North-American earthworm *Driloleirus americanus*[271] and the European fungus *Hymenoscyphus albidus*, which was replaced by its invasive morphologically identical “cryptic” counterpart *H. pseudoalbidus* .[279]

Chapter 3. State of the sustainable agricultural use and conservation of soil microorganisms and invertebrates used for bioremediation and nutrient cycling

Microorganisms in the soil and in the microbiome of soil invertebrates form complex ecosystems through their interconnected networks. Even where specific soil functions or nutrient-cycling activities can be assigned to specific taxonomic groups or species, unravelling the intricate associations in soil communities is challenging. This complexity of interdependence between individual microorganisms makes it difficult to single out members of a microbial community. Both *in situ* and *ex situ* conservation methods and sustainable agricultural practices need to be highly targeted to support stable and productive locally adapted native microbial ecosystems.[316] Locally adapted, rather than commercially available microorganisms, are preferrable for biostimulation or targeted soil-management practices, as they have far greater effects on plant growth.[317-319]. Agricultural practices that have been found to lead to the loss and disruption of the native soil biota need to be researched in various regions and climatic conditions and replaced by alternative measures whose effects are less negative.

Assessing the need for management interventions requires good-quality ecological data. However, collecting such data, particularly long-term population data, can be time-consuming and costly. Because of inadequate data on the distribution or the ecology of the target species, it is often not possible to transfer conservation models and applications to other areas.[320] Transferring models into concrete ecosystem scenarios to predict ecosystem changes under future environmental conditions and support regional conservation and management of protected areas requires standardization of data collection, laboratory protocols, data analysis and modelling.

Sequeira *et al*.[320] discuss several concepts that could be used in the development of better predictive models and to provide guidelines for ecologists and conservationists on how to improve the transferability of ecological biodiversity prediction models into real life soil biodiversity conservation and management scenarios. A metastudy of publications on the prediction of changes in biodiversity found that most studies related to biodiversity loss focus on a single threat, frequently climate change, and neglect to integrate other factors, such as changes in land use and land cover, and therefore do not provide sufficient information to allow effective planning of biodiversity management interventions.[321] The authors of a recent study on global hotspots for soil biodiversity conservation[14] propose that management strategies and conservation approaches should be updated and adjusted so that they align with the microbial and ecological reality of the respective region and conservation area. They report that global soil biodiversity can be differentiated into (occasionally overlapping) hotspots of community dissimilarity, species richness and ecosystem services and that therefore different regions have different soil conservation needs. For example, it may be appropriate for areas that have a global community-dissimilarity biodiversity hotspot to focus on biodiversity indicators and species-conservation goals, while it may be appropriate for areas with ecosystem-service hotspots to focus on specific indicators related to the supply of ecosystem services. The study defined critical and priority areas for soil biodiversity conservation as regions that support relatively high levels of soil biodiversity or soil ecosystem services and found that only 10 percent of these areas are currently under nature protection. Some parts of the world, for example high altitude areas of Canada and the Russian Federation, the Amazon, southeast Asia and most of the African continent, are particularly lacking in data on the abundance of soil microorganisms. More research, especially on the microorganisms of agricultural lands and on beneficial and surrogate soil organisms, is therefore needed.[322-324]

The lack of knowledge on the state of conservation and sustainable use of soil organisms is highlighted in the results of a 2019 survey, to which 57 countries responded, presented in the FAO-Global Soil Biodiversity Initiative (GSBI) publication *State of Knowledge of Soil Biodiversity*.[6, 217] Twenty-two of the responding countries indicated that they had conducted comprehensive assessments of the status and trends of soil biodiversity; few reported that they have national information systems for soil biodiversity. The report also indicates that some responding countries have direct and indirect references to soil biodiversity in their National Biodiversity Strategies and Action Plans (NBSAPs), although it also notes that direct links to soil biodiversity will need to be reinforced in future NBSAPs.

Scientists have recently drawn attention to the need to combine the objective of promoting higher crop yields worldwide with that of promoting soil's roles in the provision of multiple ecosystem services.[325, 326] Viable strategies for achieving high crop yields while also reducing the use of external inputs rely on enhancing the efficiency of natural nitrogen fixation. Reducing external nitrogen inputs into agricultural fields is increasingly regarded as imperative, as soils in some agricultural regions are already heavily overloaded with nitrogen and phosphate, with potentially adverse consequences for soil biodiversity.

3.1. Agricultural management practices and the sustainable use and conservation of soil microorganisms and invertebrates used for bioremediation and nutrient cycling

It has been argued that the objective of sustainable agriculture is to create a self-regulating production system that meets future food, feed and fibre requirements using local natural resources and without adverse environmental impacts or additional land consumption.[325] Evaluating the effectiveness of sustainable management methods requires qualitative and quantitative background data. To assess soil quality under particular management practices there is a need to measure key soil parameters or to use standardized soil-health indices. One such standard index, known as alteration index three (AI3), is a measure of the balance between three microbially secreted enzymes, β-glucosidase, phosphatase and urease, with lower AI3 values indicating better soil quality. The use of such indices in studies assessing the functionality of the soil microbiome and the impact of treatments on soil enzymatic activity should be encouraged.[327]

Regenerative agriculture aims to promote soil health, quality and biodiversity while allowing profitable production of nutrient-dense food. This involves sustaining or restoring useful soil organisms, including the microorganisms and invertebrates that contribute to the biogeochemical cycling of nutrients and those that can be used in bioremediation. Regenerative agricultural approaches help to increase soil fertility and maximize desirable biological networks between organisms in the soil.[326] Agricultural fields are underestimated as areas for potential conservation and creation of reservoirs of useful and diverse soil organisms.[316] The main practices associated with regenerative farming are avoiding or reducing tillage, avoiding periods when the soil is left bare, using cover crops, practising multiple cropping, reducing pesticide use and integrating livestock and crop production. Regenerative agricultural systems can include a variety of different management practices and approaches, the aim being to offer standardized uncomplicated practices to the farmer while also benefiting soil health and biodiversity under local environmental conditions.[326] Increasing carbon capture is another objective. Practices that can help reverse the loss of soil biodiversity include the following: maintaining soil cover, for example using mulch or cover crops; agroforestry practices, including silvopasture; diversified crop rotations; and reduced pesticide use. A study on the profitability of regenerative maize farming systems in the United States of America found that regenerative fields had 29 percent lower grain production but 78 percent higher profits than conventional fields.[328]

As noted above, one approach that can contribute to regenerative agriculture is to increase plant diversity. A metastudy of 122 studies that examined the effects of crop rotation on soil carbon and nitrogen found that adding a rotation of one or more crops to a monoculture increased the soil carbon and nitrogen content of the microbial biomass by more than 20 percent.[329] There are several ways of increasing plant diversity in agriculture and forestry. Interseeding and planting crop mixtures, cover crops or pasture grasses are promising ways of conserving soil biodiversity and reducing weed prevalence without using pesticides.[330] Intercropping with legumes can increase the resilience of agricultural systems to extreme weather events, such as periods of heavy rainfall, and help regenerate soil microbial and nematode communities.[331]

Agroforestry, or tree-based intercropping, is a rising star among regenerative agriculture practices. It involves combining patches or alleys of trees with non-tree crops or livestock. Several worldwide studies have found that agroforestry can increase soil carbon and nitrogen content[332] and improve the composition of soil-bacterial communities[333] and soil-fungal communities.[334] However, a study in a region with a monsoon climate found that the abundance and diversity of ground arthropods in a rubber-based agroforestry system in which *Ficus macrophylla* was grown within and between the rows of rubber trees was lower than in rubber monocultures.[335] The probable reasons for this is reportedly that *F. macrophylla* was an inappropriate choice as an accompanying species because its strong sprouting ability inhibits the growth of other plants under the rubber trees and because it reduces soil temperature by shadowing of the ground.[335] A study on the conservation of arbuscular mycorrhizal fungi (AMF) in subtropical areas of Ethiopia did not find that multistrata agroforestry was better for the conservation of AMF than monocropping in khat cultivation and highlighted the need to conserve natural forests where AMF richness is high as *in situ* genetic reserves of locally adapted mycorrhizal symbionts.[336]

Another important aspect of sustainable agriculture is the conservation of traditional techniques and fulfilment of the needs of local communities while also preserving soil biodiversity. Indigenous knowledge is culture-based knowledge that is specific to a given community. Such knowledge is severely overlooked in the management of soil biodiversity in some areas.[337] Some successful agricultural management practices used for generations could provide valuable information on how to ensure the long-term sustainability of soil conservation strategies.[338] In areas with strong indigenous movements in agriculture, communities have access to a wider variety of food products, use land-management practices suited to small-scale and local needs, and self-identify culturally through these practices.[339] One element of traditional agriculture is the use of indigenous crops. For example, multipurpose trees are dominant features of traditional agroforestry. These tree species offer fruit, fodder, wood and timber, improve soil fertility, reduce soil erosion and benefit local biodiversity.[340] In another example, it was found that smallholder farmers in Kenya who grew African indigenous vegetables in crop rotations significantly increased the diversity of soil bacteria and fungi and enhanced the enzymatic status of the soil, which was associated with increased soil fertility.[341]

One way of involving local farmers in technology, selection and management of natural regeneration in their fields is farmer-managed natural regeneration (FMNR), which is a low-cost, specific, sustainable regenerative form of agroforestry.[342] Crucial elements of the approach include the use of dormant tree stumps to regenerate land, regular pruning and pollarding to encourage ideal tree growth, collection of local native seeds and involvement of the local community. A study of FMNR in the Sahel found that the most important factor influencing regeneration was human impact, particularly protection of trees from livestock grazing, and that the next most important factor was the natural occurrence, diversity and density of the tree species.[342] The study also found that higher intensity of land use for agriculture inhibited the regeneration of land, and concluded that in the case of tree species whose natural dispersal is limited, FMNR can be complemented with tree planting. When the approach is used correctly, it benefits local biodiversity and provides agricultural benefits that lead to economic growth.[343] FMNR enhances soil quality, reduces soil erosion, increases water retention capacity and therefore indirectly increases soil microbial and invertebrate diversity.[344]

Strip cropping is another approach that can potentially benefit soil biodiversity. Preliminary results from studies in the Netherlands indicated that this approach enhanced biocontrol potential in wheat and potatoes [345] and reduced herbivore pest damage in cabbages.[346] Strip cropping can be implemented using 3-metre-wide strips, which correspond to the dimensions of commonly used agricultural machinery, and does not require complicated reconfiguration of the production system. It could thus offer a fairly straightforward way of diversifying crop fields and thereby supporting soil microbiome functions, enhancing soil fertility and eventually improving soil health.[347]

Buffer zones separating farmland from adjacent fields, or from grasslands or forests, can help stop the spread of diseases and pollution. For example, they may help protect the soil biodiversity in organic fields from the effects of agrochemicals used nearby. However, diffuse pollution is hard to avoid and can come from several sources. Studies have found that pesticides – and associated lower levels of microbial biomass and lower AMF abundance – can be detected even on organically farmed land where conventional farming has been discontinued for several decades.[348, 349] These studies not only suggest the importance of having big buffer zones around organic farms but also highlight the need for more research on pesticide dispersal, diffusion and accumulation in the soil.

Adding organic amendments, such as compost and organic litter of different sorts, to the topsoil can not only substantially improve the physicochemical properties of the soil but also stimulate native microbial activity and even reduce heavy-metal concentrations by changing the pH of the soil and through microbial enzymatic activities.[350, 351]

Composting has been used for centuries to turn waste into fertilizer. Compost is a mixture of various types of decomposing organic litter containing specific saprophytic microorganisms, for example aerobic mesophilic and thermophilic bacteria belonging to the Firmicutes and Actinobacteria,[352] as well as fungi such as yeasts and moulds, protozoans and, in specific cases, earthworms, nematodes and other detritivore invertebrates.[353] Use of compost in agriculture has been shown to provide long-term benefits for soil nutrient content, carbon-sequestration potential and soil biodiversity. However, data on its effects on soil biodiversity are limited.[354] Adding nitrification inhibitors, such as 3,4-dimethylpyrazole phosphate (DMPP),[355] dicyandiamide (DCD) or biochar,[356] as organic amendments can increase the efficiency of nitrogen fertilization by slowing the environmental degradation of nitrate and reducing the relative abundance of ammonia oxidizers.[250, 357, 358] Addition of biochar to the soil also has the potential to increase carbon sequestration and promote beneficial plant–microbe interactions in phytoremediation by enhancing microbial activity in the rhizosphere.[359]

Including pasture as part of the rotation can stimulate the microbial biomass and increase fungal and bacterial species richness,[360] although higher microbial biomass does not imply beneficial changes or that the indigenous microbiome is being conserved. However, grazing may decrease soil microbial diversity and ecosystem multifunctionality.[361] More studies on the effects on soil biodiversity of including pasture in crop rotations are needed.

3.2. Agricultural technologies using cultured microorganisms or reared invertebrates to enhance nutrient availability or for bioremediation of soil contaminants

Biofertilizers are formulated agricultural products that contain cultured and selected microorganisms that can increase the availability of soil nutrients. Beneficial bacteria that have plant growth-promoting traits or nitrogen-fixing abilities and are widely used in biofertilizers include those from the genera *Rhizobium*, *Azotobacter* and *Azospirillum*. There are also numerous products on the market containing AMF. However, the viability and the reliability of many of these inoculants remain questionable. For example, a recent study[362] conducted at three sites on three continents, tested 28 commercial AMF inoculants and observed that under greenhouse conditions none of the inoculants led to enhanced AMF colonization and only one increased plant biomass. The same study found that under field conditions only one inoculant colonized roots and enhanced plant biomass. The main conclusion of the study was that most of the products studied do not contain viable AMF propagules. This finding is similar to that of an earlier study[363] that found that five out of eight products tested did not produce mycorrhiza and concluded that there was a need to require better preliminary trials prior to the commercialization of products. A meta-analysis based on 97 peer-reviewed publications on microbial inoculation to enhance crop productivity reported positive effects on crop yield and plant size; however, the authors also noted that most of the studies were conducted under greenhouse conditions and that the field efficacy of microbial inoculants remains inconsistent.[274] Furthermore, if microbial inoculants have competitive advantages over resident organisms, they may have a negative effect on the indigenous soil microbial community [276, 364, 365] and reducing the abundance of some taxonomic groups.[366] A meta-analysis of 180 studies found that 86 percent of rhizosphere inoculation campaigns modified local microbial communities in agricultural systems in the short or long term.[367] However, most studies report that soil microbial communities remain undisturbed after biofertilizer application, which implies that their use is ecologically safe.[368] A systematic review on the safety of bioinoculants for resident microbial communities found that bacterial communities were more likely to change than fungal communities after inoculation experiments;[369] however, it is unclear whether changes to biodiversity are transient or last for a longer period.

Apart from the issue of their potential effects on soil biodiversity, the efficacy of microbial inoculants has also been debated. A literature synthesis of 27 inoculation studies reported that native soil-microbiome restoration increased plant biomass production by 64 percent on average.[316] Co-introduction of native AMF strains with native plant seeds from a protected location increased the success of restoration efforts in former mining areas in Estonia.[370] Similar results were obtained in another restoration study that involved native soil microbial communities on post-agricultural land.[371] Results of studies on the efficacy of bioinoculants have varied with the plants, microorganisms, invertebrates and soils/regions involved, and more systematic research is needed to assess their potential.

Biopesticides – products containing microorganisms or invertebrates specifically selected to counteract plant pathogens or herbivores – are potential alternatives to chemical pesticides, which are known to severely affect microbial (and other) biodiversity. Biopesticides are beyond scope of this paper. However, it is worth noting that their impact on native soil microbial communities, as well as their efficacy, also needs to be investigated.

Bioremediation technologies rely almost exclusively on cultured microorganisms and reared invertebrates because of the highly specific metabolic characteristics needed for the removal of toxic substances. Two main types of soil bioremediation intervention can be distinguished: *in situ* methods, i.e. those carried out on the site directly in the contaminated soil; and *ex situ* methods, i.e. those that involve moving contaminated soil to bioreactors or other external sites.[113] Both *in situ* and *ex situ* bioremediation techniques can be used to degrade organic contaminants such as petroleum hydrocarbons, solvents and pesticides, and to change the form of trace elements and reduce their bioavailability. Microbial strains used for bioremediation often do not have the same degradation capabilities in field conditions as they have in laboratory conditions.[372, 373] Options for mitigating this problem include stimulating the indigenous soil microbiome by adding nutrients and electron acceptors or taking steps to ensure that the added microbial population remains stable.[374] Some biostimulation practices are already used in the management of soil nutrients, for example the addition of wood dust and nitrogen to the soil to enhance saprotrophic fungal growth.[375, 376]

Vermifiltration, treatment of suspended soils or sewage sludge using earthworm- and microorganism-inoculated biofilters, can be used to stabilize and remove heavy-metal contamination while also enhancing soil-nutrient content.[377] A study on the use of the earthworm *Eisenia fetida* to treat sewage sludge used as fertilizer found that it significantly decreased toxic copper and cadmium levels and increased crop biomass.[378] This earthworm is a common species originally from Europe that has spread to other continents. Another study found that earthworms can remove trace elements, pesticides and lipophilic organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), from the soil.[379] Amendment of animal manure with additional organic matter and earthworms can mitigate the risk of heavy-metal contamination that frequently accompanies the use of untreated manure.[380] A study on the use of microorganism-rich liquid vermicompost extract (LVE) and subsequent planting of berseem clover, lentils and sunflower concluded that it increased AMF root colonization.[381] Berseem clover and sunflower provided an increase of more than 30 percent in shoot biomass and grain yield, which could be explained by the increased AMF root colonization and the LVE's high content of plant growth-promoting bacteria.[381] Earthworms and LVEs are commonly available for purchase for composting and vermicomposting.

3.3. Agricultural technologies involving the use of microbiomes and soil transplants

The use of whole microbiomes (or microbial consortia) rather than single species or species mixes as biostimulants, biofertilizers and biopesticides in agriculture is emerging as a novel approach. The 2022 FAO publication *The soil microbiome: a game changer for food and agriculture*[382] provides an overview of the agricultural practices affecting the soil microbiome and recommends that resources should be channelled into research on the question of what constitutes a healthy soil microbiome and the connections between the microbiome, the environment and overall ecosystem functioning. It also highlights the need to unify or standardize research protocols for the study of the soil microbiome and to improve interdisciplinary links between microbiome research communities (human, environmental, plant and animal).

A metastudy covering about 2000 AMF-inoculation experiments found that the response to inoculation can be highly specific to the plant host.[383] It also found that simultaneous inoculation with multiple fungal species resulted in better plant growth responses than single-species inoculation and noted that this might be explained by the complementarity of fungal species with respect to the benefits provided to the plant. A 2022 study on grasslands in the United States of America improved the rate of native plant restoration by reintroducing native AMF communities and whole soil microbiomes.[384] Because of their complex nature, plant-associated microbiome applications involve a number of challenges, including those related to regulatory approval, which currently requires strain-identification in microbial products, something that is not possible for a microbiome product that contains hundreds of thousands of microorganisms.[385] It has been proposed that the rhizosphere microbiome could be manipulated or engineered to create a “microbiome-mediated smart agriculture system” (MiMSAS) in which complex but synthetic microbiomes would be used to improve the field-application success rates of biofertilizers.[386] The use of native microorganisms sourced from local “healthy” soils can be advantageous, as microbiomes not only show high plant-host specificity[387, 388] but are also highly adapted to particular local biotic and abiotic conditions.[389]

Transplanting soil with its whole microbiome, which has the advantage that it does not require microorganisms to be isolated and maintains the whole microbial diversity of the donor soil, has been successfully used in the restoration of terrestrial ecosystems.[390] A 20-year study found that the composition of the soil-nematode community changed significantly after continuous soil-inoculation (soil transplant) treatment and reported that this seemed to be a persistent long-term change.[391] The potential disadvantages of soil transplantation and the criteria for select donor soils have not been sufficiently studied.

All the technological and research advances discussed above highlight the importance of conserving soils and soil biodiversity, especially in the centres of origin of important crops.

3.4. Conservation planning and biodiversity surrogates in agriculturally relevant areas

Applying conservation biology concepts to agricultural landscapes and to specific groups such as organisms involved in soil nutrient cycling and bioremediation is challenging. If conservation and profitable agriculture are to be successfully integrated there is a need to acknowledge the diverse goals involved and aim for mutually beneficial outcomes.[392] One of the most well-known basis for individual species conservation and recognition of threatened species is the well-curated International Union for Conservation of Nature (IUCN) Red List of Threatened Species. However the list is based on a species definition that in its present form is not applicable for microorganisms. Hence, it has been suggested that the IUCN Red List should be expanded and adapted to cover a wider range of species, including threatened microbial species or consortia.[316]

Planning the conservation of an area or of a specific group of organisms requires high-resolution, high-coverage, long-term abundance data. However, such data are hard to obtain and some conservationists therefore rely on proxies.[393] These may consist of data on surrogate species that serve as indicators of the desired conservation objective.[394] Indicators of ecosystem or soil health such as SOC content and water retention can also serve as surrogates. A study that attempted to find surrogates for the diversity of predatory arthropods found that ground beetles could serve as surrogates for other ground-dwelling predators, including in agricultural contexts.[395]

Developing statistical ecological models that can optimize multiple conservation- and productivity-related objectives is challenging. Surrogate-based optimization approaches can provide management frameworks with acceptable prediction accuracies that are highly adaptable to different parameters and types of spatial and temporal data. Using an artificial neural network, a biogeochemical metamodel of this kind has recently been developed for optimizing agricultural landscapes in the United States of America with respect to SOC, GHG, soil nitrogen, irrigation-water use, farm profits and crop yield.[396] Use of this metamodel increased farm profits, SOC and grain yield and reduced GHG emissions. Guerra *et al*.[397] suggest a set of soil-ecological indicators based on essential biodiversity variables for use in a global monitoring framework for soil biodiversity and ecosystem function. The proposed variables are intraspecific genetic diversity, population abundance, community traits of roots, taxonomic diversity, functional diversity, soil biomass, litter decomposition, soil respiration, enzymatic activity, soil aggregation, nutrient cycling and habitat extent.

3.5. Microbial culture collections and biological reference collections

Microbial culture collections serve as hubs of soil microorganism identification and preservation, and as sources of microorganisms for agricultural research and use. According to the 1977 Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, the deposit of microorganisms is recognized by all the treaty's parties as a part of the patent procedure, irrespective of where the depository authority is located. Culture collections are often complex bioresource centres that conserve fungi, bacteria, diverse eukaryotes, viruses, fungal spores and bacterial plasmids. The resources held are only available for research or technological development purposes, and the handling, growth and bioformulation of individual organisms requires trained personnel.

The most comprehensive catalogue of culture collections and database of recognized microorganisms is available via the webpage of the World Federation of Culture Collections (WFCC), which contains 768 culture collections from 76 countries. The World Data Centre for Microorganisms (WDCM) database is a directory of worldwide collections of 3 370 507 microorganisms and cell lines across 831 culture collections from 78 countries. Of these 831 collections, 351 are university based, 311 are governmental, 35 are semi-governmental, 60 are private and 25 are industry based. Most of the collections are in Asia (306) and Europe (263). The catalogues contain 61 578 soil isolates of microorganisms and 5 909 species of nitrogen-fixing Rhizobium in worldwide collections. [4] Various public and private institutions provide accessibility to these organisms and related services. Some collections are at risk of being lost because of a lack of funding, including for staff, or because of natural disasters, and action is needed to ensure that they are preserved for the future.[398]

A number of different conservation technologies can be employed, depending on the objectives. Long-term conservation methods include cryopreservation, underwater storage and lyophilization. In the case of some organisms, conservation in the most viable form requires soil- and substrate-based maintenance, occasionally together with the organism's symbiotic partner, for example in the case of AMF.[399] Although some require high-energy equipment, such as -80 °C freezers or -180 °C containers, long-term conservation techniques have many advantages and are used in most culture collections.

3.6. Invertebrate breeding and mass rearing

The rearing of earthworms used for vermicomposting is called vermiculture. Earthworms can usually be bought locally for composting, use as fishing bait or animal/pet feed or for other purposes. These earthworms are mainly *Eisenia* spp., *Dendrobaena* spp. or *Lumbricus* spp. Various cocoons or live earthworms can be ordered from online shops, and because of a lack of regulation even non-native species are easily accessible for most customers outside Australia, Canada, Malta, New Zealand, the United Kingdom and the United States of America. Nematode products for soil applications can be found on the biological pest-control market in the form of capsules or dried cultures. Species of *Heterorhabditis* and *Steinernema* entomopathogenic nematodes are commonly used in agricultural pest management and are mass produced via incubation in bioreactors with their crucial symbiotic bacteria in the culturing media.[400]

Selective breeding of soil invertebrates is quite uncommon. Promising results have been obtained at research level for characteristics such as biomass, maturation time, cocoon production rate and hatching success in the earthworm *Eisenia fetida*.[401] Attempts to selectively breed soil nematodes for improved attraction to a root signal,[402] desiccation tolerance[403] and selective host-finding[404] have shown that manipulating key traits can be effective if the heritability of the selected trait is high enough or if beneficial traits are stabilized in inbred lines.[405] A few soil invertebrate species, including some earthworms, millipedes, centipedes, are listed as threatened on the IUCN Red List. However, there are no species-specific active conservation efforts involving *ex situ* breeding and recovery, and the

[4] https://wfcc.info

protection of such species is limited to "wildlife protection" efforts in some countries where collecting and possessing them is a criminal offence.

3.7. Threats to the use and conservation of soil microorganisms and invertebrates used for nutrient cycling in sustainable agriculture and for bioremediation

As discussed above, studies have indicated that terrestrial microbial biodiversity is being affected by climate change, agricultural land-use changes and other anthropogenic activities.[406, 407] The organisms of interest to the present study (soil microorganisms and invertebrates useful in nutrient cycling and bioremediation) exist as components of complex ecosystems. Their ability to survive and function adequately depends on the presence of favourable abiotic conditions and on interactions with other organisms. Conserving them *in situ* thus requires sustainable agricultural practices that improve soil health and reduce soil disturbance. As also discussed above, microbial culture collections are vital resources for *ex situ* conservation. Volunteer taxonomists and museum collections of invertebrates need to be recognized as crucial components of soil organism conservation and monitoring and require appropriate support. Indigenous ecological knowledge and traditional management techniques are severely threatened. Many such practices could disappear before their efficiency can be evaluated. Appropriate education programmes and strategies for communication with holders of local and indigenous knowledge are required.

Chapter 4. State of policies and legislation

4.1. International and national instruments

The *State of Knowledge of Soil Biodiversity* publication[6] presents a comprehensive compilation of worldwide policies, programmes, regulations and environmental frameworks related to soil biodiversity. The following paragraphs provide an overview of the instruments most relevant to the sustainable use and conservation of soil microorganisms and invertebrates.

The Convention on Biological Diversity (CBD) is the key international legal framework for the conservation of biodiversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising from the utilization of genetic resources. In 2002 the sixth meeting of the Conference of the Parties to the CBD decided to establish the International Initiative for the Conservation and Sustainable Use of Soil Biodiversity. FAO and other relevant organizations were invited to facilitate and coordinate this initiative, [408]and the Conference of the Parties adopted the Framework for Action for the Initiative in 2006.[409] According to the results of a survey of Parties to the CBD conducted for a 2020 review of the initiative,[410] soil biodiversity-related practices are poorly implemented. Initiatives and research programmes supporting the development and implementation of soil management practices are in place. However, they do not specifically target the sustainable use and conservation of soil biodiversity. Only a few national assessments directly or indirectly linked to soil biota were reported. Moreover, national soil biodiversity monitoring schemes and arrangements to ensure the inclusion of conservation and sustainable use of biodiversity in national planning and policy development are rare. The review emphasized the need for the following actions, identified by governments and stakeholders, to improve the conservation of soil biodiversity and increase awareness of its importance:

“(a) Description of soil biota in conditions of natural and agricultural ecosystems to assess degrees of vulnerability and initiating a new round of research on soil microorganisms using molecular methods;

(b) Development of methods and technologies for ensuring the recovery of soil biota;

(c) Development of soil biodiversity information systems to establish a national standard for soil quality;

(d) Modernization of soil biology educational institutions, including modern equipment and technical facilities;

(e) Organization of training programmes for soil microbiology and zoology professionals;

(f) Creation and publication of training and information materials on soil biodiversity;

(g) Increasing the social significance of soil biodiversity and ecosystem services through workshops and round tables with farmers and local communities.”

In 2022, the 15th meeting of the Conference of the Parties to the CBD urged Parties to the Convention, as well as other governments and organizations, to mainstream soil biodiversity across sectors and provide financial support to promote research, technology transfer and monitoring of soil biodiversity. More importantly, the meeting endorsed an updated plan of action for the initiative, covering the period 2020 to 2030, [411] which includes the following objectives:

“(a) Implementing coherent and comprehensive policies for the conservation, restoration and sustainable use of soil biodiversity at the local, subnational, national, regional and global levels, considering the different economic, environmental, cultural and social factors of all relevant productive sectors and their soil management practices, and mainstreaming their integration into relevant sectoral and cross-sectoral plans, programmes and strategies;

(b) Encouraging the use of sustainable soil management practices and existing tools, sustainable traditional practices, guidance and frameworks to maintain and restore soil biodiversity and to encourage the transfer of knowledge and enable women, particularly rural women, indigenous peoples and local communities and all stakeholders to harness the benefits of soil biodiversity for their livelihoods, taking into account national circumstances;

(c) Promoting education, awareness-raising and developing capacities in the public and private sectors on the multiple benefits and application of soil biodiversity, sharing knowledge and improving the tools for decision-making, fostering engagement through collaboration, intergenerational transmission of traditional knowledge of indigenous peoples and local communities and partnerships, and providing practical and feasible actions to avoid, reduce or reverse soil biodiversity loss;

(d) Developing voluntary standard protocols to assess the status and trends of soil biodiversity, as well as monitor activities, in accordance with national legislation, to address gaps in knowledge and foster relevant research, and to enable compilation of large data sets to support research and monitoring activities;

(e) Recognizing and supporting the role, and land and resource rights of indigenous peoples and local communities, in accordance with national legislation and international instruments, as well as the role of women, smallholders and small-scale food producers, particularly family farmers, in maintaining biodiversity through sustainable agricultural practices."

The Framework for Action on Biodiversity for Food and Agriculture,[412] which was negotiated by the Commission on Genetic Resources for Food and Agriculture as a policy response to the report on *The State of the World's Biodiversity for Food and Agriculture,* [413] was endorsed by the 168th Session of the FAO Council in December 2021. It features 57 individual actions grouped into three strategic priority areas: characterization, assessment and monitoring; management (sustainable use and conservation); and institutional frameworks. In each of the priority areas, specific references are made to soil biodiversity and soil health. For instance, recommended actions include improving capacity for research, in particular research on soil biodiversity and other associated biodiversity, through the formation of multi-, inter- and transdisciplinary research teams and by strengthening mechanisms for cooperation and exchange of information between scientists, producers and other stakeholders.

At the level of individual countries, NBSAPs have been put in place as instruments to promote the implementation of the CBD.[5] The 2020 CBD *Review of the International Initiative for the Conservation and Sustainable Use of Soil Biodiversity*[410] reports that 120 out of 170 NBSAPs reviewed featured some action or initiative targeting the improvement of soil quality in general. However, only 23 NBSAPs recognized the importance of soil biodiversity conservation and included actions targeting soil organisms, and only ten aimed to enhance the conservation of soil biodiversity by promoting sustainable agricultural practices. In their 2020 national reports to the CBD, 76 parties out of 83 countries mentioned the implementation of at least one action related to improving soil quality or biodiversity, while 33 mentioned that they prioritized soil conservation and 24 that they prioritized increasing soil fertility. In their national reports, countries referred to difficulties in identifying and understanding soil microfauna and macrofauna and stated that there was a lack of expertise and tools in this field. A recent publication on soil biodiversity conservation[397] emphasized that there is great uncertainty about the impact that national and international nature conservation policies have on soil systems. It concluded that the data needed to track the implementation of policy targets are currently lacking, especially at global scale.

A systematic analysis of national and regional policy and legal frameworks is beyond the scope of the present study. However, the 2015 report on *The status of the world´s soil resources*[414] summarized the state of soil-related policy and governance at the time and noted that only a few countries had put in place effective policies on soil conservation and land-use change, and that – apart from Australia and New Zealand – these were mainly in Europe and North America. Overall, policy and legal frameworks related to soil biodiversity vary considerably across the different geographical regions of the world. The following paragraphs provide some examples.

The EU has adopted a number of relevant instruments, including "A Soil Deal for Europe", which is as one of five "missions" launched in 2021 within the Horizon Europe research and innovation programme. A Soil Deal for Europe explicitly aims to establish 100 living labs (collaborative initiatives between multiple partners and diverse actors, such as researchers, farmers, foresters, spatial planners, land managers and other citizens, who come together to co-create innovation aimed at meeting jointly agreed objectives) and lighthouses (farms where scientifically proven good practices and solutions are

[5] https://www.cbd.int/nbsap

demonstrated) and has eight key mission objectives, among which number 6 is to improve soil structure to enhance soil biodiversity.[415] Relevant EU legislation includes Directive 2009/128/EC, which requires EU member states to adopt national action plans aimed at reducing the undesired effects of pesticides on the environment, including on biodiversity. In 2014, a Soil Framework Directive for combating soil degradation was withdrawn because of insufficient support from EU member states.[416] However, the EU's 7th Environment Action Programme, [417, 418] which covered the period 2014 to 2020, addressed soil protection and soil bioremediation. Key commitments set out in the European Biodiversity Strategy for 2030, which was launched in 2021,[419] include making significant progress in remediating contaminated soil sites and placing at least 25 percent of agricultural land under organic farm management. The Biodiversity Strategy also emphasizes soil ecosystem restoration, protecting soil fertility, reducing soil degradation and increasing soil organic matter. A key action to be taken by the European Commission under the Biodiversity Strategy is to revise the Thematic Strategy for Soil Protection.

In Asia, China has significantly improved national funding for ecological studies on agriculturally relevant soils.[420] It has established biodiversity monitoring networks, increased the capacity of seed banks, botanic gardens and protected areas, and launched initiatives such as the Grain for Green Program and the Returning Grazing Land to Grassland (RGLGP) and Returning Agricultural Land to Forest Projects.[420-422] The scientific basis for supposing that returning grazing lands to grassland under the RGLGP can be linked to a positive future impact on soil biodiversity is supported by a study conducted on the Tibetan plateau that showed that animal excretion altered the structure of soil microbial community and negatively affected the balance of harmful and beneficial bacteria.[252] A study on the impact of these initiatives in the Weihe River Basin described increased ecosystem services, increased soil carbon storage and improved soil conservation over the period between 2000 and 2018.[423] According to some reports, the initiatives slowed the local decline of biodiversity thanks to investments and targeted initiatives by increasing protected undisturbed areas, increasing forest and grassland coverage and reducing the area exposed to the strain caused by constant high manure load. However, there is limited evidence regarding actual impacts on biodiversity, particularly on the soil biodiversity.[420, 422, 423]

In Africa, soil biodiversity-related policy and legal frameworks are relatively underdeveloped. Egypt has a Biodiversity Strategy and Action plan for the period 2015 to 2030. It envisions soil conservation and reduction of biodiversity loss by 2030. It also aims to ensure that pressures on biodiversity are reduced, biological resources are sustainably used, benefits arising from the utilization of genetic resources are shared in a fair and equitable manner, biodiversity issues and values are mainstreamed into relevant policies and that such policies are implemented effectively and in a participatory way.[424] Support is, or has been, provided via initiatives such as the Global Environment Facility's Food-IAP: Fostering Sustainability and Resilience for Food Security in Sub-Saharan Africa an Integrated Approach (IAP-PROGRAM)[6] and the Alliance for a Green Revolution in Africa's (AGRA) Soil Health Program, which aims to increase income and food security by promoting the wide adoption of integrated soil fertility management on sub-Saharan smallholder farms and includes the implementation of practices such as the use of legumes in crop rotations and appropriate use of manure and fertilizers. The key objective of AGRA is to promote regenerative agricultural practices, reduce soil erosion and increase crop biodiversity across 800 projects in sub-Saharan Africa. However, the current goals do not include specific targets related to soil biodiversity or soil biodiversity conservation, and no recent data are available on the impact the regenerative practices implemented have had on soil biodiversity.[425] In North Africa and the Near East, countries have established programmes to fight desertification, although the enforcement of environmental regulations in these countries has proven to be challenging. [6, 426]

In North-America, federal agencies in the United States of America (U.S. Code 7 (2010), § 136r-1.) and in Canada (Environmental Management Act, SBC 2003, Chapter 58) are required by legislation to promote integrated pest management in their regulations, procurement and other activities [427, 428], which indirectly benefits soil biodiversity through reduced pesticide use.[429] The United States Conservation Reserve Program[7] is based on a so-called payments for ecosystem services (PES) mechanism and is a

[6] https://www.thegef.org/projects-operations/projects/9070
[7] https://www.fsa.usda.gov/programs-and-services/conservation-programs/conservation-reserve-program

successful voluntary land-conservation programme run by the Farm Service Agency, under which landowners are paid for removing land from agricultural intensification. As agricultural intensification is directly linked to soil biodiversity reduction[11] and habitat loss is a direct threat to soil biota,[430] the conversion of crop lands to natural areas can be a good way to restore land and protect soil biodiversity. However, more research on the effects these measures have on soil biodiversity and the restoration of soil microbiome is needed.[431]

In Latin America and the Caribbean, concerns about the rapid decline of soil biodiversity and increased soil erosion associated with the exploitation of natural sources have led some countries to implement soil protection policies. For example, Uruguay has implemented a sustainable intensification model under which each farm is required to have a soil-management plan and implement crop rotation,[432] and local scientists have called for the establishment of policies on biodiversity and natural-resource conservation in agricultural environments.[433]. The region's biggest agricultural producer and exporter, Brazil,[434] also promotes sustainable agricultural practices, soil conservation programmes and PES initiatives. [435]. However, some of these have not provided sufficient protection for threatened areas, and the implementation of related legislation is affected by a number of constraints, including persistent conservative values with regard to farming practices, financial struggles, large socioeconomic inequalities between regions and groups and fears that introducing more sustainable agricultural practices might decrease food security.[6, 434, 436]

4.2. Genetic resource sharing protocols and legislation, including soil movement restrictions

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity[437] is a supplementary agreement to the CBD in force since 2014. It ensures that the country of origin of sampled biological material receives benefits from any commercialization of this material.

It is not possible to comprehensively restrict or prohibit movement of specific microorganisms and invertebrates. This is mainly because there are limited data available on native microbial and invertebrate communities that would allow non-native and invasive species to be differentiated.[316] However, the import and movement of soil and other biological material is strictly regulated in several countries. For example, import of soil into the EU and the United Kingdom is prohibited unless it is for research or testing purposes.[438] However, it has been reported that the tracking of soil movements across borders within the EU is difficult.[439] The United States of America requires a permit from the Animal Plant and Health Inspection Service for the import of soil samples, which have to undergo sterilization and meet quarantine requirements.[440] Member states of the Community of Latin American States (CELAC) have put in place an agreement on the exchange of soil samples.[441] However, samples are always treated as phytosanitary material and samples shipped to Brazil have to be collected from areas that are free of *Globodera* spp. (plant-pathogenic cyst nematodes) (information from SIMPLE GLOSOLAN). The SIMPLE (Soil IMPort LEgislation) database,[8] maintained by the Global Soil Laboratory Network (GLOSOLAN), provides information on the rules for the import of soil samples to countries worldwide.

The import of living organisms is usually subject to quarantine regulations. For example, the following rules for the import of earthworms to the United States of America have been in place since October 2022: [442]

- "Earthworms must be reared on a diet free of soil or bedding containing pathogens. The diet may contain paper pulp, sawdust, or pasteurized vegetables (vegetables that have been held at a temperature of 180°F (83ºC) for a minimum of 30 minutes).
- At least 15 days prior to shipment, all imported earthworms must be placed on a cleansing diet that is free of any materials that may contain plant or animal pathogens.

[8] https://www.fao.org/global-soil-partnership/glosolan-old/simple-soil-import-legislation/custom-control-procedure-database/en

- At no time during the rearing or packaging process are earthworms to be fed soil, uncooked or partially cooked vegetables.
- At all times during the rearing operation, worms must be kept separated from the ground by a heavy layer of plastic, fiberglass, metal, or other material that is not biodegradable."

These actions aim to protect soils and native biodiversity and to prevent the spread of soil-dwelling pathogens of plants and animals.[443] A similar regulation on terrestrial earthworms has been in place in Canada since 2020.[444] In the United Kingdom, the import of invertebrates is prohibited if the organism is listed as a plant pest (listed in Annex 2A of the Plant Health Regulations 2020). It is also subject to the rules set out in the Balai Directive (Article 4 of Council Directive 92/65/EEC); however, the directive does not list any invertebrates as prohibited. The import of invertebrates into the EU is regulated in the case of honeybees and plant pests (listed in Annex II of Article 36 of Regulation (EU) 2016/2031 and Annex IIA and IIB in Regulation (EU) 2019/2072 of 28 November 2019), while other invertebrates are only regulated by the EU Invasive Alien Species Regulation (No 1143/2014). The only invertebrate soil organism listed under the EU Invasive Species Regulation is the New Zealand flatworm (*Arthurdendyus triangulatus*).[446] The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international convention that requires its parties to implement national wildlife trade laws to stop trade in endangered organisms. At the moment no soil invertebrates are listed, although this could be updated based on new research findings.[445]

Chapter 5. State of organizations and networks

5.1. International and national organizations, initiatives and networks, research institutions, initiatives and citizen science programmes

This section provides short descriptions of organizations and networks that make important contributions to the sustainable use and conservation of soil microorganisms and invertebrates.[9]

The Global Soil Partnership (GSP)[10] is a partnership established by FAO in 2012[447] that aims to improve soil governance to guarantee productive soils that support food security and climate change adaptation and mitigation in the context of sustainable development. In August 2022, GSP announced the development of a global map of soil nutrients and associated soil properties. GSP's five pillars of action are: (1) promote sustainable management of soil resources for soil protection, conservation and sustainable productivity; (2) encourage investment, technical cooperation, policy, education awareness and extension in soil; (3) promote targeted soil research and development focusing on identified gaps and priorities; (4) enhance the quantity and quality of soil data and information: data collection, analysis, validation, reporting, monitoring and integration with other disciplines and on synergies with related productive, environmental and social development actions; and (5) harmonize methods, measurements and indicators for the sustainable management and protection of soil resources.

Global Soil Laboratory Network (GLOSOLAN)[11] is a network established by the GSP that brings together soil analysis laboratories to harmonize soil analytical data, share information and develop standards (standard operating procedures) and training materials. GLOSOLAN launched the SIMPLE (Soil IMPort LEgislation)[12] database, which contains information on countries' soil-import procedures to facilitate research and exchange.

International Network on Soil Biodiversity (NETSOB)[13] is a network established by the GSP in 2021 to promote the sustainable use and conservation of soil biodiversity. It addresses the need to expand and improve knowledge of soil biodiversity and soil biodiversity loss. It is an open network, and all scientists, organizations, institutions and other stakeholders can become members and engage in its work.

International Network on Soil Pollution (INSOP)[14] is a network established by GSP in April 2022 that focuses on stopping soil pollution and achieving the global goal of zero pollution. INSOP's mission is to support and facilitate joint efforts to reduce the risks of soil pollution and effectively remediate already-polluted areas using nature-based biological remediation techniques. It is an open network that brings together governments, academia, the private sector, NGOs and other stakeholders from around the world who share the vision of a world with zero pollution and healthy soils.

The Intergovernmental Technical Panel on Soils (ITPS)[15] consist of 27 soil experts representing all regions of the world and provides scientific and technical advice and guidance on global soil issues to the GSP. The ITPS regularly releases policy letters and reports on topics related to soil health.

The World Federation of Culture Collections (WCCF)[16] is a multidisciplinary commission of the International Union of Biological Sciences (IUBS) that harmonizes the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells. Its webpage provides access to a range of guidelines and information, for example on the preservation of microorganisms. WCCF serves as an international information network linking culture collections and users. It organizes

[9] The information presented is based mainly on material available on the websites of the networks and organizations described.
[10] https://www.fao.org/global-soil-partnership/en
[11] https://www.fao.org/global-soil-partnership/glosolan/en
[12] https://www.fao.org/global-soil-partnership/glosolan-old/simple-soil-import-legislation/custom-control-procedure-database/en/
[13] https://www.fao.org/global-soil-partnership/netsob/en
[14] https://www.fao.org/global-soil-partnership/insop/en
[15] https://www.fao.org/global-soil-partnership/itps/en
[16] https://wfcc.info/home_view

conferences and workshops and is active in scientific publishing. It collaborates with the World Data Centre for Microorganisms (WDCM), which hosts an online global catalogue of microorganisms.

Microbial Resource Research Infrastructure (MIRRI)[17] is a pan-European distributed research infrastructure whose goals are the preservation, systematic investigation, provision and valorization of microbial resources and biodiversity.

CEEweb for Biodiversity[18] is a central and eastern European network that strives to conserve the natural heritage of the region. It aims to integrate the concept of sustainability into agricultural policies and practices in the EU and in the countries of central and eastern Europe.

The Soil Ecology Society (SES)[19] is an international organization dedicated to raising awareness of soil ecology and its relevance to human and environmental well-being and to science. It organizes an annual symposium and various public-outreach events.

The Soil and Water Conservation Society (SWCS)[20] is an international and intersectoral organization of professionals working on the conservation of natural resources. Sustainable land and water management are at the core of its work. It organizes annual conferences and chapter meetings and creates online content on conservation practices for the general public.

The International Network of Soil Information Institutions (INSII)[21] is a network of institutions with the ability to develop and share selected national soil information and data. It provides information to a number of international collaborations and global soil-mapping initiatives.

The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)[22] is an independent intergovernmental body that aims to strengthen the science-policy interface for biodiversity and ecosystem services. The members are representatives of states. The global need to slow land degradation and promote the restoration of degraded soils was the main topic of its 2018 assessment report.[448]

The International 4 per 1000 Initiative,[23] which was launched in 2015 at the 21st meeting of Conference of the Parties to the United Nations Framework Convention on Climate Change, highlights the need to increase soil carbon content globally by 0.4 percent annually.

The European Soil Data Centre (ESDAC)[24] is a thematic centre for soil-related data in Europe that provides access to datasets, maps, documents and information on relevant events. Its Land Use and Coverage Area frame Survey (LUCAS) gathered data on topsoil properties in 23 EU member states.[449]

The Consultative Group on International Agricultural Research (CGIAR)[25] is the world's largest global agricultural innovation network. One of CGIAR's impact areas is environmental health and biodiversity. Its research centres include the Center for Tropical Agriculture (CIAT), the International Rice Research Institute (IRRI) and the International Maize and Wheat Improvement Center (CIMMYT).

The Centre for Agriculture and Bioscience International (CABI)[26] is an international, intergovernmental non-profit organizations that provides information and scientific expertise that helps solve agricultural and environmental problems. It currently has 49 member countries. CABI maintains a Crop Protection Compendium that contains information on several biological control agents and an Invasive Species Compendium that provides accessible datasheets on the invasive species present in different territories.

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[17] https://www.mirri.org
[18] https://www.ceeweb.org/index.php
[19] https://www.soilecologysociety.com
[20] https://www.swcs.org/
[21] https://www.fao.org/global-soil-partnership/insii/en
[22] https://ipbes.net
[23] https://4p1000.org/?lang=en
[24] https://esdac.jrc.ec.europa.eu
[25] https://www.cgiar.org
[26] https://www.cabi.org

Society for the Protection of Underground Networks (SPUN)[27] is a research organization whose mission is to protect and harness mycorrhizal networks, map these networks and advocate for the protection of underground ecosystems.

The Global Environment Facility (GEF)[28] serves as a global financial mechanism for several environmental conventions. It supports the work of developing countries on issues such as biodiversity loss, chemicals and waste, climate change, food security, land degradation, sustainable forests and cities. It launched the programme Fostering Sustainability and Resilience for Food Security in Sub-Saharan Africa (Food-IAP), also known as the Resilient Food Systems (RFS)[29] programme.

The Global Biodiversity Information Facility (GBIF)[30] is an international network and data infrastructure that aims to provide open-access data on various organisms. Soil organisms are poorly represented. However, the data hub is well established and could be extended.

The Global Soil Biodiversity Initiative (GSBI)[31] is a global collaboration of scientists that aims to inform the public, promote the integration of research information into environmental policy and create a platform for the current and future sustainability of soils. It has a diverse scientific advisory committee and hosts online informal webinars and global meetings on soil biodiversity. As a joint initiative with the European Commission Joint Research Centre, GSBI published the freely available and highly detailed *Global Soil Diversity Atlas*.[3]

The Soil Biodiversity Observation Network (SoilBON)[32] is a global partnership launched by GSBI that involves several global and regional partners and makes available soil biological and ecosystem observations that contribute to the sustainable use and conservation of soil resources. It focuses on expanding existing essential biodiversity variables (EBVs) on soil ecological features. EBVs are defined as "the measurements required to study, report and manage biodiversity change" and can be used for monitoring and decision-making.

The Earth Microbiome Project[33] was a collaborative research effort that aimed to characterize the microbiomes of all natural environments on Earth. It required researchers to use the protocols and standards provided on its webpage. It resulted in 60 peer-reviewed publications on different environments, with open-source data made available in online databases and codes in a GitHub repository.[450]

Edaphobase[34] is an online information system dealing with the distribution and ecological preferences of soil animals. It is a joint research project involving several German research institutions and museums and contains data on various soil invertebrates and metadata on their environments.

The Australian Microbiome Initiative[35] is a continental scale, collaborative research project aspiring to characterize the diversity and ecosystem-service provision of microorganisms in Australia. It aims to create a public resource containing microbial genomic datasets and site-specific comprehensive metadata from a range of environments, including soils.

Other continents and countries have launched similar projects, for example the African Soil Microbiome Initiative,[451] the soil and plant biogeochemistry sampling campaign National Ecological Observatory Network[36] in the United States of America,[452] and the China Soil Microbiome Initiative.[453] To increase awareness of soils and below-ground biodiversity the United Nations launched the International Year of Soils in 2015.

[27] https://www.spun.earth
[28] https://www.thegef.org
[29] https://www.resilientfoodsystems.co
[30] https://www.gbif.org
[31] https://www.globalsoilbiodiversity.org
[32] https://www.globalsoilbiodiversity.org/soilbon
[33] https://earthmicrobiome.org
[34] https://portal.edaphobase.org
[35] https://www.australianmicrobiome.com/initiative
[36] https://www.neonscience.org

The GlobalFungi database[37] is a global online database of information on fungal occurrences obtained from high-throughput-sequencing metabarcoding studies.[324] It contains publicly available mapped and validated data on the composition of soil fungal communities in terrestrial environments, including soil and plant-associated fungi. It accepts findings from relevant studies from all around the world.

Citizen-science programmes can make important contributions to the collection of scientific data in several fields, including on species distributions, with the help of volunteer data collectors. For example, the Earthworm Society of Britain[38] had a successful campaign called Earthworm Watch[39] that allowed it to collect information on earthworm diversity and distribution in different environments and soils with the help of volunteer citizen scientists. Restor,[40] a global platform launched by ETH Zürich, Switzerland, allows people to share and monitor their nature conservation and restoration projects. Participants can upload photos and data files and find a forum for collaboration. iNaturalist[41] is a popular application via which people upload species observations from various environments to a database and participate in local missions. Since 2015, the Netherlands has been holding successful Soil Animal Days,[42] a national citizen science project in which volunteers are urged to explore their direct environment and provide observations on soil biodiversity in a soil animal chart.[454]

5.2. Strategic areas of collaboration

The numerous networks and initiatives involved in work on soil invertebrates and microorganisms often have similar functions. However, they generally operate independently with the exception of those operating under the auspices of FAO (e.g. GSP). More umbrella organizations or networks could help to synchronize activities and organize the scientific, economic and social outcomes of projects and initiatives.

Closer involvement of stakeholders and education of scientists on policymaking processes and on the work of relevant governmental and intergovernmental organizations would facilitate transparency and the efficient planning of scientific projects. Collaboration and intersectoral partnerships between academic partners, policymakers, NGOs and other stakeholders should be encouraged and better funded.

The Organisation for Economic Co-operation and Development's (OECD's) Co-operative Research Program in Sustainable Agriculture and Food Systems[43] is an example of an initiative that facilitates international cooperation among scientists and institutions by providing funding for international researcher mobility, conferences, workshops and similar activities to promote coordination among stakeholders and support policymaking. These OECD Fellowships are available in several relevant topics, such as invasive species, agricultural soil emissions, ecological rhizosphere management for enhanced nutrient efficiency, stress resilience and biodiversity in sustainable agro(ecosystems).

[37] https://globalfungi.com
[38] https://www.earthwormsoc.org.uk
[39] https://earthwormwatch.org
[40] https://restor.eco
[41] https://www.inaturalist.org
[42] https://bodemdierendagen.nl/soil-animal-days
[43] https://www.oecd.org/agriculture/crp

Chapter 6. Education, human resources and training

6.1. Higher education and training – taxonomic impediment

Properly cataloguing, measuring and conserving soil biodiversity requires an enormous amount of interdisciplinary and intersectoral collaboration. Several challenges need to be recognized and addressed. There is a growing need for up-to-date taxonomic knowledge and to account for organisms' roles and functions in the environment. Existing knowledge at the habitat level may be lost or become inaccessible if research focuses on a range of species that is too narrow. It is well recognized that over the last three decades a shortage of trained taxonomists and curators has created a "taxonomic impediment", i.e. a lack of capacity to update information on some taxa and misidentified species and to deal with the vast amount of taxonomic data constantly being added to databases.[455, 456] Questions related to the current biodiversity crisis cannot be properly answered while phylogenetic understanding remains outdated, museum cabinets are full of unidentified specimens and capacity to cultivate microorganisms in the laboratory remains limited. The low number of taxonomists is presumably a consequence of a lack of interest in the subject and the perception that taxonomy-focused publications have weak citation power, although this has not been found to be reflected in actual citation metrics, and journals could benefit from taxonomy-focused papers.[457]

Funding for environmental and agricultural research has increased in several parts of the world, including for example the United States of America, the EU and Australia, because of the need to feed a growing population or to modernize and increase the sustainability of the sector.[458-461] There has been an exponential boom in the number of research papers, reviews, books, emerging journals, special issues, conferences and scientific networks addressing relevant topics. As of August 2022, there were 40 704 hits on the PubMed[44] search engine for the keyword "sustainable agriculture" and 16 136 for "soil biodiversity", of which 6 202 and 1 041, respectively, were reviews.

6.2. Stakeholder education and public outreach

The key to fostering visibility and awareness of scientific findings is public outreach and provision of educational materials for farmers and landowners. Online information materials with multimedia content on soil organisms, for example the webpage "It's Alive!",[45] can make soil ecology more comprehensible to the public. The farmer field school approach[46] is an example of direct stakeholder education that allows farmers to observe and experiment with new technologies. A 2004 review of studies of the impact of IPM farmer field schools[462] found that measuring impact was complex and lacked an agreed conceptual framework but that several studies had reported measurable reductions in pesticide use, higher crop yields and that continued learning had been stimulated. The 2020 review of the International Initiative for the Conservation and Sustainable Use of Soil Biodiversity [410, 411] mentions that 15 NBSAPs include plans to educate farmers and stakeholders on soil management practices and that 23 include plans to support multidisciplinary research networks targeting soil biodiversity conservation and improved understanding of soil organisms and the soil-related benefits of agroforestry.

[44] https://pubmed.ncbi.nlm.nih.gov
[45] https://biology.soilweb.ca
[46] https://www.fao.org/farmer-field-schools/en

Chapter 7. Knowledge gaps and future needs

This chapter identifies gaps in our understanding of how best to improve the sustainable use and conservation of soil microorganisms and invertebrates. It identifies research priorities and policy interventions that can help overcome hurdles to improvements in this field. A major general conclusion is that soil and soil organisms should be subject to protective measures similar to those already in place for groundwater and surface waters, including measures for investigating contaminants.

7.1. Soil organisms in nutrient cycling

Soil-nutrient cycling is immensely complex, as it involves multiple biogeochemical transformations that are not yet fully understood. Specifically, there are major gaps in our understanding of the microorganisms and invertebrates involved in the various soil-nutrient cycles. Because of this, it has not yet been possible to successfully forecast changes in SOC content and the associated soil biodiversity in agricultural settings.[27, 463] Our knowledge of how soil biodiversity and SOC content are affected by agricultural management practices such as the use of organic nitrogen amendments remains incomplete, mainly because it is primarily based on small-scale studies.[464] Likewise, we are insufficiently aware of the factors involved in SOM cycling and specifically of how SOC types pass from one fraction into another (living, decomposing and stable). It is well established that SOM drives soil food webs, the decomposition of external organic material in the soil and the mineralization of several essential nutrients.[223, 463] From this it follows that more research effort is needed on the links between SOM content and soil biodiversity, SOM-derived nutrients and nutrient cycling. Monitoring soil nutrients would also improve our understanding of how agricultural management practices affect SOM quality and the soil food web.

Given that it has become increasingly apparent that methanogenic archaea are major contributors to soil nitrogen fixation in some areas,[465] more research is needed on the relationship between biological methane production and atmospheric nitrogen fixation. Similarly, the use of polyphosphate-accumulating organisms as green manure is a promising alternative to conventional phosphorus fertilization. However, the cellular biochemical mechanisms underlying polyphosphate accumulation have remained largely unknown and under-researched.[466] In addition to the urgent need for research on specific organisms with potential uses in improving soil fertility, improving nutrient management in agriculture also requires better knowledge of interannual variability and the effects of climate change.

There is strong consensus that when dealing with soil food webs there is a need to focus on functional groups rather than individual taxa. This implies the need for a more mechanistic understanding that allows microbial groups to be linked to soil functions. Despite the huge progress made in sequencing and data analysis capacities in recent times, it is still not possible to effectively link taxonomic diversity to functions in nutrient cycling. This emphasizes the need for improved microbial gene databases and novel methods for predicting and quantifying microbial functions.[467, 468]

Crop diversification will be important for soil biodiversity protection. However, the importance of crop diversity is context dependent, as plant and microbial diversity are not necessarily coupled.[19, 216] It also seems that some taxonomic groups of microorganisms and soil invertebrates are significantly under-researched. In particular, the roles of protozoa and of bacteriophages (viruses that target bacteria and archaea) and other viruses in soil ecosystems are not well understood.[6, 51]

Petabytes of microbiome data from greenhouse and field experiments as well as from studies of natural habitats are available online and offer opportunities for data mining and analysis that could help answer existing research questions without the need for new experiments. However, many of these data have been obtained using slightly different approaches, i.e. without following unified standards, and this makes comparative analysis more challenging.

7.2. Soil organisms in bioremediation

Bioremediation is increasingly important in agricultural areas for restoring and ameliorating cultivable land needed for food production.[120] Holistic approaches that consider the interaction of bacteria, fungi and invertebrates would help improve understanding of the processes underlying bioremediation. In this context, the use of invertebrates to enhance the bioremediation of heavy metals and pesticides should be at the forefront of research interest. The biodiversity of contaminated soils is often depleted, but if specific functionalities needed for contaminant removal are still present, bioremediation can still be effective. Studies focusing on *in situ* bioremediation are needed, as it is economically feasible and because it is easier to implement methods that do not require excavation and removal of the native soil.[206, 469] Multicontamination sites in agriculture are common,[125, 207] but most studies and projects only focus on the removal of single contaminants. Developing effective methods for the bioremediation of contaminant mixtures would therefore be very valuable in the agricultural context. Bioindicator organisms such as earthworms and soil microarthropods should be key components of approaches to assessing contamination levels, contaminant degradation potential and the nutrient-cycling functionalities of sites. The use of microorganisms and invertebrates as bioindicators in agricultural settings could be further explored, for example the use of the lichen *Ramalina farinacea*, a proposed bioindicator of fertilizer toxicity,[470] the use of nematodes as indicators of soil heavy-metal pollution[471] or the use of bacteria as indicators of various aspects of soil health.[472]

Given the current interest in urban farming, potential contamination of plant produce with heavy metals[473] or other harmful substances needs to be considered. This also implies that contaminant levels in urban areas and potential effects on soil biodiversity functions need to be assessed.

7.3. Soil organisms in agricultural management

7.3.1. Microbial products, invertebrate products and biodiversity

The transfer of research findings to the field is a crucial step in the development of microbial products. Microbial strains applied as biofertilizers often do not have the same effects under field conditions as they have in the greenhouse or under *in vitro* experimental conditions. Microbial products also have problems with viability,[362] and strains may fail to colonize root tissues in competition with already-existing soil microbiomes. Consequently, there is a need to address the competitive ability and plant compatibility of inoculant strains, as well as their tolerance of environmental stresses, in order to allow the development of formulations and application technologies that enable better establishment of the applied microorganisms. Furthermore, there is a need to determine the environmental conditions in which microorganisms are able to efficiently degrade or transform pollutants and to improve plant growth.

Determining whether the use of a single highly competitive microbial strain or the use of microbial consortia is the more effective biocontrol strategy in given contexts is another priority. If a single strain is relied on, the treatment may only be effective in conditions that suit that strain. The use of microbial consortia may allow the treatment to be effective in a wider range of conditions, for example in different seasons or different weather conditions, because the different taxa used may be metabolically active at different times.

As microbial inoculants may have non-target effects on native biodiversity and soil functions, any undesired effects on soil biodiversity need to be carefully considered. Another concern is that applying single, highly competitive strains may disrupt the native microbial ecosystem. More studies are needed on how soil inocula become established and how they affect the existing soil food web and soil functions.[474] Strains that have an engineered “turn off” or “suicidal gene”[475] function could be used. Other challenges include a lack of information on the potential for horizontal gene transfer between microbial inoculants and the environment and the fact that different countries take contrasting approaches to the regulation of the use of engineered strains.[476] Furthermore, non-native invertebrates used in bioremediation or for enhancing nutrient cycling and composting could also pose a threat to the natural biodiversity of an area and could potentially be the source of invasive species in the soil.

In practical terms, microbial products containing consortia of multiple strains need to be produced using multiple production lines and later combined. This is costly and creates a major bottleneck in the production of multimicrobe products. Limits to the storability of microbial products are another issue, as in some cases the products applied in the field do not contain enough viable bacterial or fungal cells. This raises the need for more control and testing in the biologicals industry. Furthermore, the nature of long-term plant–soil feedbacks can differ depending on whether bacteria or fungi are applied to the soil, and this needs to be accounted for in the timing and mode of inoculant applications.

7.3.2. Agricultural soil management practices

In general, it is hard to track the negative or positive effects that an agricultural practice may have on the soil if there is no consensus as to what constitutes a "healthy" soil or what soil biota components are required. According to a definition provided by ITPS, "soil health" means "the ability of the soil to sustain the productivity, diversity and environmental services of terrestrial ecosystems."[477] Above all, there is a need to establish standard operation procedures for sampling and for the measurement and evaluation of soil health.

There are no reference sites available at national/international or regional levels for use in biodiversity assessments relating the abundance or diversity of different soil taxa. However, studies usually include a no-treatment field or a local non-disturbed agricultural or forest area as a reference site. If the aim is to reduce the use of agricultural practices that may disturb the biodiversity of beneficial soil organisms, there is a need to highlight the harmful effects proven to occur under various conditions and offer viable and affordable alternatives. Organic soil additions that can inhibit soil denitrification are promising fertilizer alternatives that could help reduce the need to constantly add nitrogen fertilizer to fields.

The effects of tillage on soil biodiversity are still not clear,[478] and in this context there is a need for guidelines on standardized soil sampling and the choice of parameters, as well as for common measurement protocols.[225, 479] Similarly, the effects of long-term monocultures on the components of soil biodiversity need more research – and eventually regulation to promote appropriate crop diversification. More research is needed on shifts in the composition and functional properties of regenerating microbial and invertebrate communities in farmer-managed natural regeneration and on the functions of the targeted ecosystems and the benefits provided by restoration.[342] There is a need to develop a better knowledge base on how different agricultural management practices affect soil biodiversity and functions in order to predict which practices should be used under which conditions.

Furthermore, the presence of pesticide residues in native soils and organic fields even decades after pesticide use has ended is concerning and needs to be assessed. Our understanding of the long-term impact of new pesticides on the soil food web is incomplete. However, as pesticides create complex problems, their environmental effects need to be discussed and measured by interdisciplinary teams that include environmental chemists, biologists, agronomists and other scientists.

It is questionable whether knowledge acquired on one farm about how a particular agricultural management regime affects soil biodiversity and soil-quality conditions can be transferred to other farms. This is particularly the case where the transfer of findings from smaller plots and smaller farms to industrialized commercial agriculture is concerned. The importance of smallholder farming relative to industrialized farming varies greatly by region, and therefore more information on the effect of farming practises in different settings, under different environmental conditions and in different geographical areas is required. A study on soil biodiversity and indigenous practices in Africa identified cultural and language barriers to consent, along with inaccessible locations, as big constraints to the selection of fields for sampling,[341] and these factors probably contribute to researchers' lack of interest in working with smallholder farmers. This problem could be solved by providing local help for researchers by selected soil "ambassadors" or representatives whose job it is to ensure good communication between researchers and farmers.

7.4. Roles of soil organisms in mitigating the effects of a changing climate, invasive species and antibiotic resistance genes

Given the inevitable impacts of climate change on agriculture, there is a need for more investment in research on how it affects soil biodiversity and how such effects can be mitigated. Extreme weather events, such as floods, droughts and heavy, long-lasting rainfall, may give rise to then need for interventions to restore soil and soil biodiversity. Many organisms are involved in the mineralization of atmospheric CO_2 through the oxalate-carbonate sink. However, they do not receive sufficient research attention in spite of their potential for use in carbon sequestration.[31] Soil aggregation and bioturbation by soil organisms and the role of these functions in carbon sequestration require more research, especially given the controversy surrounding the results of studies that suggested that earthworms may increase GHG emissions from the soil.[29] Although oxalate-carbonate pathways are important contributors to carbon and calcium cycles, the diversity and taxonomy of the organisms involved remain neglected in the scientific literature, even if some papers have called for them to be explored and utilized in agricultural management.[32]

Besides CO_2, some volatile organic atmospheric carbon components need to be considered, for example methanol, which is known to be present in higher concentration in the air in rural areas than other areas because of the higher plant coverage.[28] According to some experts, the diversity of aerobic methanol oxidizers in the soil should receive more research attention, especially in agricultural settings with more methanol turnover measurements in the soil.[30] The natural cycling of methanol, and all the terrestrial factors involved in its production, are still poorly understood and there is uncertainty about its global sources and sinks and its effects on tropospheric photochemistry.[41, 43, 44]

There are no efficient strategies available for preventing the spread of invasive earthworms introduced into soil.[480] More information is needed on the effects of invasive earthworms and other invertebrates on plant biodiversity and soil quality.[220, 270] Therefore, efforts to prevent future introduction and human-mediated dispersal, even in areas that have already been invaded, are crucial, even if restoring the original diversity is unlikely to be possible.[480]

More research is needed on the effects of antibiotic resistance genes (ARGs) on the environment and the technologies that can be used attenuate these effects. In particular, the use of bacteriophages could be a promising way of reducing the spread of ARGs.[481]

7.5. Conservation and restoration

Averill *et al.* [316] identified three key principles of ecosystem conservation and monitoring surveys: (1) the spatial and geographic coverage of datasets should be expanded, particularly in less-disturbed regions that can be regarded as "baseline" soils for comparison; (2) long-term and frequent surveys of biodiversity are needed, especially in threatened areas; and (3) information sharing should be made more efficient, and all relevant studies should be transparent and shared via open-access platforms. As specific actions, these authors recommend the following: (1) extending the IUCN Red List of Threatened Species; (2) incorporating microbial biodiversity into conservation planning; (3) incentivizing agricultural management practices that are beneficial for soil microbial diversity; (4) properly documenting and sharing key metadata (climate, date and location).

7.5.1. **In si***tu and* **ex situ** *conservation*

Most biodiversity protection guidelines and incentives concentrate on macrofauna and above-ground biodiversity and neglect microorganisms and below-ground meso- and microfauna.[13, 218, 316]. Important spatial and quantitative data on the loss of soil biodiversity from natural areas and areas used for agriculture are unavailable. The categories and criteria used for the IUCN Red List are not appropriate for microorganisms or for most eukaryotic single-celled organisms, and microorganisms are simply excluded. The Red List categories and criteria were last updated in 2001,[482] and the guideline document states that "there is sufficient range among the different criteria to enable the appropriate listing of taxa from the complete taxonomic spectrum, with the exception of micro-organisms." Frequently, policymakers do not adequately consider the significance of microorganisms as components of ecosystems. The European Environmental Agencys EUNIS habitat classification defines habitat types (synonymously used with the term "ecosystem") as "plant and animal communities as the characterising elements of the biotic environment, together with abiotic factors operating together at a particular scale." A more inclusive definition can be found in the 1992 EU Habitats Directive 92/43/EEC, which considers natural habitats to be "terrestrial or aquatic areas distinguished by geographic, abiotic and biotic features, whether entirely natural or semi-natural." The directive provided the basis for the creation of the Natura 2000 ecological network and is still in use as a definition. There is a need to consider taxa and species that are not included in the Red List but are threatened.

In 2018, IUCN published a document entitled *Soil Biodiversity and Soil Organic Carbon: keeping drylands alive*, which presents a good set of policy options for a soil-biodiversity conservation but barely considers microorganisms even though the main focus of the document is on SOC.[484] Soil organisms lack media and public visibility because of their "hidden" nature and a lack of appreciation of their ecological contributions.

Ecosystem conservation cannot be separated from the conservation of soil biodiversity. There is a huge gap in knowledge of the connectedness between below-ground habitats and soil biodiversity hotspots and between above-ground and below-ground biodiversity. Also, because biodiversity hotspots of terrestrial microorganisms do not correspond to those of above-ground biodiversity,[14] there is a need for appropriate conservation approaches for them. Most importantly, a better understanding of the functions of soil organisms in the soil-food web at ecosystem level is required. Some taxa are still better studied than others, and taxa such as protozoa and bacteriophages need more research.

The scientific literature and the expert opinions canvassed for this study clearly indicate that there is a need for long-term studies of soil biodiversity to be conducted at diverse geographical locations on disturbed and undisturbed sites and for seasonal variation to be taken into consideration. Only long-term studies can account for environmental and climatic variations and for natural seasonal variations in biodiversity; moreover, some organisms may grow or be active only under particular conditions or at a particular times of year.[485] Existing biodiversity monitoring programmes could be employed and specific elements of soil-biodiversity monitoring integrated into them. Conservation programmes for indigenous crops and their indigenous microbiota and invertebrates are needed.

For *ex situ* conservation, but also to improve understanding of microbial functions, there is a need to develop protocols and high-throughput technologies that can bring "uncultivable" groups and whole microbiomes into cultivation. There is also a need to centralize the deposition of microbial strains. Shortages of funding and trained personnel are currently big constraints to *ex situ* conservation. Establishing collections that specialize in the cultivation of overlooked soil organisms or organisms that are hard to breed or cultivate under laboratory conditions is crucial.

7.5.2. Soil restoration

Heavily disturbed areas, for example those where soils have been degraded by agricultural activities, are typical targets for restoration. Contaminant removal through bioremediation should be followed by restoration activities for soil biodiversity. Restoration ideally requires information on the important organisms and functions associated with the targeted soil. Lost soil organisms could be obtained from *ex situ* collections and reintroduced. There is a need to develop approaches that can promote or stimulate indigenous soil microbes and soil fauna for restoration purposes. Microbiomes rather than single organisms or limited groups of organisms need to be targeted, as many microorganisms and microbial interactions are only fully functional in complex communities. There is a huge gap in knowledge on soil invertebrates and their associated native microbiota. Increasing the efficiency of soil nutrient cycling, restoration and bioremediation will require holistic understanding of the interrelationships between plants, invertebrates, protozoa, bacteria, fungi, viruses and connected soil functions.

Soil transplantation is a promising cultivation-independent soil restoration method. However, baseline information on which soils to use as donors is lacking, and there is also a lack of guidelines and official recommendations on soil transplantation. Large-scale campaigns are also prohibitively costly.

7.6. Accessibility, databases, linking networks and organizations

7.6.1. Accessibility of scientific results and databases

Legacy maps based on data collected by various field surveys using various methods exist, for example those available from the FAO Soil Maps and Databases web page,[47] including those developed by the GSP.[48] Selected soil parameters from various regions recorded in maps and databases provide an overview of the state of soil resources. These maps and databases could be updated with additional parameters by using new technologies such as remote sensing, drones and robots. Compiled data on soil biodiversity parameters, such as areas where invasive soil organisms are known to be present, where the abundance of core taxa has declined or where indicator taxa for specific environmental factors are present or absent, could be useful in the identification of threatened areas and targets for biodiversity restoration.[486]

A comprehensive review or metastudy mapping the contamination levels of various heavy metals and pesticides would provide a better overview of the severity of the contamination of arable soils globally and would highlight critically polluted areas. Such a study would allow better prioritization of goals and allocation of research and economic resources.

7.6.2. Regulatory, dissemination and outreach issues

There is a need to improve some regulations relevant to the management of soil biodiversity. For instance carbon-offset schemes leave too much scope for exploitation.[487-489] Another issue is that the requirement for strain-level registration potentially hinders the introduction of products containing multiple microbes into agricultural use.[385] Stricter control of the import of invertebrates could also be considered. Other requirements include improving the quality control of the viability of microbial products and closely involving scientists and curators of culture collections in policymaking.

The huge number of publications and reports on soil conservation and sustainability topics is hard to follow at times, and more-effective platforms for communicating research findings are needed. There is a need to better communicate research results, such as those related to the benefits of conservation agriculture and soil biodiversity, to farmers and the wider public and to better involve them in research, dissemination and development activities. This will create trust and improve understanding of the importance of conservation and restoration. Soils, soil functions and soil biodiversity merit more public awareness and protection, as the quality and sustainability of food production depends to a significant degree on below-ground biodiversity.

7.7. Strategic areas for collaboration

Areas requiring strategic, multidisciplinary, international collaboration include the following:

1. development of strategies for better public and stakeholder outreach and communication, including information materials on soil organisms and their use;
2. facilitation of interdisciplinary and international research and partnerships on topics related to soil biodiversity;
3. transfer of knowledge between the agricultural, academic, industrial and policymaking sectors to improve products, relevant legislation and funding schemes for research;
4. coordination of research, and development of protocols defining the concept of a "healthy" soil microbiome and for commonly used laboratory and analysis techniques; and
5. harmonization of soil biodiversity-relevant monitoring programmes, networks, initiatives and databases.

[47] https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/en
[48] https://www.fao.org/global-soil-partnership/resources/publications-new/data-products/en

7.8. Opportunities for the Commission and its members

The Commission and its members could potentially contribute to addressing gaps and weaknesses in the sustainable use and conservation of soil microorganisms and invertebrates used for bioremediation and nutrient cycling in the following ways.

1. Provide standards and commonly agreed definitions. Research and technological development on the use of soil microorganisms and invertebrates in sustainable agriculture and soil biodiversity conservation would greatly benefit from improved standardization and more consensus on research priorities. For instance, implementation guidelines or standard operation procedures for the measurement of "healthy soil" for different geographical regions would facilitate international collaboration and the compilation and sharing of knowledge. Guidelines could include sampling protocols, key soil parameters for biodiversity assessments and the most important soil organisms for quantification. Information on a baseline of "healthy soil" conditions for a given region and season would be valuable. It should be increasingly recognized that soil quality and fertility and soil ecosystem functions need to be included in environmental studies, bioremediation campaigns and land-restoration initiatives.

2. Foster the development of consensus on (a) what are the most important soil functions, (b) parameters for use in assessments of the effects that new agricultural methods have on soils, (c) key soil biodiversity parameters for use in assessments of the impact of soil contamination, (d) sampling and laboratory practices and gene-sequencing and bioinformatics procedures for use in soil-biodiversity studies.

3. Substantiate what are the best practices in agricultural soil management. To address gaps in knowledge derived from long-term observations, a metastudy on the impact of farming practices using uniform methodologies at various geographical and regional scales and including reference sites with native, undisturbed soils could be initiated. This could be the basis for substantiating best practices for management interventions in terms of soil biodiversity conservation under particular soil conditions.

4. Support the uptake of promising agricultural practices that are beneficial to soil biodiversity conservation. This would involve:
   (a) supporting evaluation of the applicability of the practice (i.e. whether it is affordable, easily understandable and does not require new machinery or radically new local agricultural practices);
   (b) supporting evaluation of potential negative effects;
   (c) supporting uncomplicated implementation of products and tools.
5. Support the merging of relevant databases on soil biodiversity. Several existing databases (e.g. GLOSOB and Soil BON) could be combined to provide better access to more data. This could include an easy-to-use map of the state of agricultural soils around the world containing parameters such as nutrient content, heavy-metal contamination, pesticide contamination and major risks (FAO GSP announced the creation of such a map[49] for soil-nutrient budgets in August 2022). A novel database of reference or indicator taxa for healthy soils for various geographical and climatic conditions could be created and be used in the evaluation of agricultural practices.

6. Foster the establishment of interdisciplinary research initiatives. Current societal challenges relate to complex ecological and environmental problems that require comprehensive and inclusive approaches. Multidisciplinary teams need to address the full range of the potential impacts of human activities on soil biodiversity on a global scale. Incentivizing such research efforts could be done through an intergovernmental interdisciplinary platform.

7. Promote improved coordination between existing research networks related to the sustainable use and conservation of soil microorganisms and invertebrates.

[49] https://www.fao.org/global-soil-partnership/resources/highlights/detail/en/c/1601502

8. Foster public outreach and awareness building via stakeholder education campaigns and initiatives promoting sustainable agricultural practices and protection of soil organisms. Promoting citizen science may foster public engagement and raise public interest in soil organisms and their benefits to society and the environment.

9. Facilitate better coordination of currently scattered *ex situ* conservation initiatives and related research. This could include initiating joint research programmes using advanced technologies for the cultivation of entire microbiomes and overlooked groups of soil organisms.

10. Identify short-term and long-term goals in the *in situ* and *ex situ* conservation and protection of soil organisms and invertebrates used for bioremediation and nutrient cycling, and set priorities among them.

Annex I. Most important functional marker genes used in assessing the microbial biodiversity of nutrient-cycling microorganisms

Gene	Function/enzyme activity	Ecosystem role
mcrA	Methyl coenzyme M reductase	Methanogenesis. Archaea
pmoA	Particulate methane monooxygenase	Methanotrophs. Bacteria
mmoX	Soluble methane monooxygenase	
mxaF	Methanol dehydrogenase large subunit	Methylotrophy. Proteobacteria
fae	Tetrahydromethanopterin hydrolase	Methylotrophy. Methylobacterium
mtdB	NAD(P)-dependent methylenetetrahydromethanopterin dehydrogenase	
mch	Methenyltetrahydromethanopterin cyclohydrolase	Methylotrophy. Methylothermaceae
fhcD	Formylmethanofuran--tetrahydromethanopterin formyltransferase	Methylotrophy. Proteobacteria
cmuA	Chloromethane methyl transferase	Methylotrophy, chloromethane oxidation. Bacteria
xoxF	Lanthanide-dependent methanol/methanethiol dehydrogensase	Methanol oxidation. Bacteria
mox1	Methanol oxidase 1	Methanol oxidizing. Yeasts
fdh1	Formate dehydrogenase	
das	Dihydroxyacetone synthase	
frc	Formyl-coenzyme A (CoA) transferase	Oxalotrophy. Bacteria
oxc	Oxalyl-CoA decarboxylase	
ureA,B,C	Urease	Microbially induced calcite precipitation (MICP). Bacteria
nifH	Reductase subunit of nitrogenase	Nitrogen fixation. Bacteria, diazotrophs
amoA	α-subunit of ammonia monooxygenase	Nitrification. Bacteria, archaea
hao	Hydroxylamine oxidoreductase	
narG	Nitrate reductase α-subunit	Denitrification. Bacteria, archaea
napA	Periplasmic nitrate reductase	
nirB	Nitrite reductase large subunit	

nirK	Copper-containing nitrite reductase	
nirS	Nitrite reductase	
norB	Nitric oxide reductase	
nosZ	Nitrous oxide reductase	
nirA	Nitrogen assimilation transcription factor (*niaD* and *niiA* genes)	Nitrate assimilation
niaD	Nitrate reductase	Nitrite oxidation
nxrA		
niiA	Nitrite reductase	
nasA	Catalytic subunit of assimilatory nitrate reductase	Assimilatory N reduction to ammonium
nrfA	c-type cytochrome nitrite reductase	Dissimilatory N reduction to ammonium (DNRA). Bacteria
gdh	Glutamate dehydrogenase	Nitrogen mineralization (ammonification)
ureC	Urease	
acpA	A nonspecific acid phosphatase	Organic phosphate solubilization
appA	Acid phosphatase/phytase. Bi-functional	
appA2		
napA	A nonspecific acid phosphatase class B	
napD	Nonspecific periplasmic acid phosphatase	
napE		
phoC	A nonspecific acid phosphatase class A	
phyA	Neutral phytase	
gabY	Promotes pyrroloquinoline-quinone and glutamate dehydrogenase combination. Gluconic acid production	Inorganic phosphate solubilization. Mineral phosphate solubilization (MPS)
mps	Pyrroloquinoline-quinone synthase	
pcc	Phosphoenol pyruvate carboxylase	
ppa	Inorganic pyrophosphatase	
ppk	Polyphosphatase kinase	Polyphosphate accumulation

ppx	Exopolyphosphatase	
phoA	Alkaline phosphatase	

Annex II. Detailed taxonomic list of soil microorganisms and invertebrates involved in nutrient cycling

Carbon fixation	Kingdom/clade	Organism(s)	Mode and/or location of action
Photoautotrophs (oxygenic)	Bacteria	Phylum Cyanobacteriota	Use of atmospheric CO_2 as C source and light as energy source. Only in the top few mm of the soil
	Stramenopile (Protist Yellow-Brown Algae)	Phylum Ochrophyta (Class Bacillocariophyceae, Eustigmatophyceae, Xanthophyceae)	
		Genus Ralstonia	
		Genus Rhodopseudomonas	
		Genus Thermodesulfobium	
Heterotroph decomposers, saprotrophs and detrivores	Animalia (Microfauna)	Phylum Nematoda (Nematodes)	Use of organic materials as both C and energy sources. Results in CO_2 release and C biomineralization
		Phylum Rotifera (Rotifers)	
		Phylum Tardigrada (Tardigrades)	
	Animalia (Mesofauna)	Subclass Acari (Mites)	
		Phylum Annelida (Segmented worms)	
		Class Collembola (Springtails)	
		Order Diplura (Bristletails)	
		Family Enchytraeidae	
		Order Protura (Proturans)	
		Order Pseudoscorpiones (False Scorpions)	
		Class Thermoplasmata	
	Bacteria	Various	
	Fungi	Various	
	Protists	Various	
Oxalate producers	Fungi	Class Agaricomycetes Genera *Ganoderma*, *Hebeloma*, *Paxillus*, *Plurotus*, *Polyporus*, *Pycnoporus*, *Rhizopogon*, *Suillus*, *Trametes*	Oxalate production and accumulation
	Protists	Phylum Amoebozoa Families Cribrariaceae, Dianemataceae, Trichiaceae	Calcium oxalate production and accumulation
Oxalotrophs	Bacteria	Class Actinobacteria	

		Genera *Arthrobacter*, *Intrasporangium* (f. *Humihabitans*), Kribbella, *Streptomyces*	Use of ubiquitous oxalate as carbon and energy source. Oxalate–carbonate pathway (OCP). Mainly in rhizosphere but also in plant tissues. Some associated with mycorrhizae (*Streptomyces, Burkholderia*) and lichen (*Herminiimonas*)
		Class Alphaproteobacteria Genera *Afipia*, *Azospirillum*, *Bradyrhizobium*, *Ensifer*, *Methylobacterium*, *Rhizobium*, *Starkeya*, *Xanthobacter*	
		Class Bacilli Genera *Bacillus*, *Paenibacillus*, *Psychrobacillus*	
		Class Betaproteobacteria Genera *Achromobacter*, *Burkholderia*, *Cupriavidus*, *Herminiimonas*, *Janthinobacterium*, *Oxalicibacterium*, *Polaromonas*, *Variovorax*	
		Class Gammaproteobacteria Genera *Lysobacter, Pseudoxanthomonas, Stenotrophomonas, Xanthomonas*	
	Fungi	Class Agaricomycetes Genera *Agaricus*, *Pleurotus*, *Polyporus*	Likely in association with oxalotrophic bacteria
$CaCO_3$ precipitation	Bacteria	Genus *Bacillus*	Biomineralization of CO_2. Microbially induced calcite precipitation (MICP). Urease activity
		Genus *Halomonas*	
		Genus *Sporosarcina*	

C1 cycling	**Kingdom/clade**	**Organism(s)**	**Mode and/or location of action**
Methanogens	Archaea	Superphylum Euryarchaeota Orders Methanobacteriales, Methanocellales, Cand. Methanofastidiosales (WSA2), Methanomassiliicoccales?, Methanomicrobiales, Methanopyrales, Methanosarcinales	Conversion of CO_2 with H_2 into CH_4. Used in biogas production and wastewater treatment

		Superphylum TACK Phyla Bathyarchaeota, Verstraetearchaeota	
		Superphylum DPANN?	
		Superphylum Asgard?	
Methylotrophs-Methanotrophs	Archaea	ANME 1, 2, 3	Anaerobic methanotrophy. Reverse methanogenesis. Sulfate, nitrate or metal oxides as electron acceptors
		Genus *Methanosarcina*	Electrogenic anaerobic CH_4 oxidation
		Candidatus Family Methanoperedenaceae (*Methanoperedens nitroreducens*)	Denitrifying methanotrophy under anaerobic conditions
	Bacteria	Class Alphaproteobacteria Genera *Methylocapsa*, *Methylocella*, *Methylocystis*, Methyloferula, *Methylosinus*	Aerobic methanotrophy, Type II
		Class Gammaproteobacteria Genera *Methylobacter*, *Methylocaldum*, *Methylococcus*, *Methylomicrobium*, *Methylomonas*	Aerobic methanotrophy, Type I
		Cand. Phylum NC10 Cand. Genus *Methylomirabilis* (*Methylomirabilis oxyfera*)	Denitrifying methanotrophy under anaerobic conditions. Produces N_2 and O_2
		Phylum Verrucomicrobia Genus *Methylacidiphilum*, *Methylokorus*	Aerobic methanotrophy
Methylotrophs – Non-methanotrophic Methanol oxidizers	Bacteria	Phylum Actinobacteria Genera *Amycolatopsis*, *Arthrobacter*, *Mycobacterium*	Facultative. *Mycobacterium* can also utilize CO
		Class Alphaproteobacteria Genera *Acidomonas*, *Afipia*, *Hyphomicrobium*, *Methylosulfomonas*, *Paracoccus*, *Rhodoblastus*	Methanol plus other C1 (methanosulfonic acid)

		Family Beijerinckiaceae (Class Alphaproteobacteria)	Lanthanide-dependent PQQ-MDH
		Genus *Methylobacterium* (Class Alphaproteobacteria)	Connected to nitrification. In soil and in the phyllosphere of plants
		Genus *Bacillus*	Facultative methylotroph
		Class Betaproteobacteria Genera *Methylobacillus*, *Methylovorus*	Obligate methylotrophy. In soil and in plant roots
	Fungi	Order Saccharomycetales Genera *Candida (*former *Torulopsis)*, *Komagataella* (*K. phaffii* former *Pichia pastoris*), *Ogataea* (*O. polymorpha* former *Hansenula and* f. *Pichia methanolica*)	Conversion of CH_4O into CO_2
		Genus *Trichosporon* (Basidiomycota)	
Acetogens	Archaea	ANME-2a	Simultaneous methanotrophy or methanogenesis
		Genera *Methanotrix* (former *Methanosaeta*), *Methanosarcina*	
	Bacteria	Genus *Acetobacterium*	Mainly known as acetogenic but can also utilize methanol
		Genus *Clostridium*	
Nitrogen fixation	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
Primarily symbiotic	Bacteria	Genus *Azorhizobium*	Endosymbionts with plants of genus *Sesbania*, also rice and wheat
		Family Rhizobiaceae Genera *Rhizobium, Allorhizobium, Bradyrhizobium*, *Pararhizobium, Mesorhizobium, Neorhizobium,* Sinorhizobium/Ensifer	Endosymbionts in nodules of legumes
		Genus *Frankia*	Endosymbionts in actinorhizal plants
		Genus *Methylobacterium*	Legume root-nodulation. Methylotrophic
Free-living	Archaea	Phylum Euryarchaeota	Methanogenic Euryarchaeota in soils. Presumably

		Genera *Methanobacterium*, *Methanococcus*, *Methanosarcina*, *Methanosphaerula*, *Methanospirillum*, *Methanoregula*	important in wetlands, rice fields, rainforest areas. Produces methane
	Bacteria Class Alphaproteobacteria	Family Acetobacteraceae Genera *Asaia, Gluconacetobacter, Swaminathania*	Plant endophytes. Associative in rhizosphere. Free living in soil
		Genus *Azospirillum*	Associative in rhizosphere (*A. brasilense*). Some species exclusively endophytes. Can also denitrify
		Order Hyphomicrobiales Genera *Rhodopseudomonas, Xanthobacte*r	In rhizosphere
		Genus *Rhodobacter*	In soil. Phototrophic
	Bacteria Class Betaproteobacteria	Genus *Azoarcus*	Endophytes of *Poaceae* (rice). Also free living in soil
		Genus *Burkholderia*	Endophytes of plants and AMF. Some free-living in rhizosphere and soil
		Genus *Herbaspirillum*	Endophytes in Monocots (*Poaceae*, *Musaceae*) and in some Eucots (soybean). Colonize all plant tissues
		Genus *Paraburkholderia*	Associative nitrogen fixers. Endophytes
	Bacteria Class Gammaproteobacteria	Order Enterobacterales Genera *Enterobacter*, *Klebsiella, Pantoea*	Endophytes in various plants. In soil. In rhizosphere
		Order Pseudomonadales Genera *Azotobacter, Pseudomonas*	Endophytes in various plants. Free living in soil. Associative in rhizosphere
	Bacteria	Phylum Cyanobacteria	Endophytes in Cycad coralloid roots. Symbionts in the rhizome of *Gunnera* species. As part of lichen. Free living in soil and rhizosphere
	Bacteria Phylum Firmicutes	Order Bacillales Genera *Bacillus, Paenibacillus*	Associative in rhizosphere. Free-living in soils. Endophytes

		Order Eubacteriales Genera *Clostridium, Heliobacterium*	Some *Clostridium* species free-living in soils *Heliobacterium* spp. free living in soils, particularly in tropics
Nitrification	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
Ammonia Oxidation	Archaea (AOA)	Phylum Thaumarchaeota Genera *Nitrosocosmicus*, *Nitrosarchaeum*, Cand. *Nitrosotalea*, *Nitrososphaera*	Free-living in soils. Anaerobic and aerobic reaction
Ammonia Oxidation	Bacteria (AOB)	Phylum Planctomycetes Genus *Kuenenia*	Anaerobic ammonium oxidation (Anammox) into N_2
Ammonia Oxidation	Bacteria (AOB)	Class Betaproteobacteria Genera *Nitrosococcus*, *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus*	Free living in the soil
Nitrite Oxidation	Bacteria	Genus *Nitrobacter*	Free living chemolithoautotrophs in the soil
Nitrite Oxidation	Bacteria	Genus *Nitrococcus*	Free living chemolithoautotrophs in the soil
Nitrite Oxidation	Bacteria	Genus *Nitrolancea*	Free living chemolithoautotrophs in the soil
Nitrite Oxidation	Bacteria	Genus *Nitrospira*	Free living chemolithoautotrophs in the soil
Comammox	Bacteria	Genus *Nitrospira*	Complete ammonia and nitrite oxidation to nitrate, some even to N_2
Denitrification	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
	Archaea	Order Methanosarcinales	Denitrifying anaerobic methane oxidation (DAMO)
	Bacteria	Phylum Proteobacteria Genera *Burkholderia*, *Paracoccus*, *Pseudomonas*, *Ralstonia* (former *Alcaligenes*), *Xanthomonas*	Heterotrophic. Facultative aerobic. In the soil
	Bacteria	Genus *Bacillus*	Heterotrophic. Facultative aerobic. In the soil
	Bacteria	Genus *Streptomyces*	Heterotrophic. Facultative aerobic. In the soil
	Bacteria	Genus *Corynebacterium*	Only to N_2O
	Bacteria	Genus *Methylomirabilis*	Nitrite dependent anaerobic methane-oxidation (n-DAMO)

	Fungi	Phylum Ascomycota Genera *Cylindrocarpon*, *Fusarium*,*Gibberella*, *Trichosporon*	Only to N_2O. Missing final enzymatic step. In the soil
		Genus *Trichosporon*	
Indirect effects on N cycling	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
Interacting partners in the soil-food web	Animalia	Acariformes (Mites)	Microbivorious mites increase N availability
		Collembola (Springtails)	Below-ground macroaggregation formation. Increased nitrification in casts and burrows. Stimulation of microbial activity
		Chilopoda (Centipedes)	
		Diplopoda (Millipedes)	
		Lumbricidae (Earthworms)	
		Phylum Nematoda (Nematodes)	Presence increases net N availability by retaining higher amounts of N and releasing it as ammonia and by grazing on microbes
	Sar, Eukaryotic protists	Amoeba, ciliates, flagellates	Releasing nitrogen in the soil through microbial predation
	Virus	Bacteriophages	Controlling bacterial and fungal communities. Nutrient release. Process changes, etc.
Phosphorus solubilization	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
Organic phosphate solubilizers	Bacteria	Class Alphaproteobacteria Genera *Rhizobium*, *Sphingomonas*	Extracellular phosphatase/phytase production. Organic acid secretion
		Phylum Actinobacteria Genera *Micromonospora*, *Sinomonas*	
		Order Bacillales Genera *Bacillus*, *Geobacillus*, *Paenibacillus*	
		Class Betaproteobacteria Genera *Achromobacter*, *Advenella* (former *Tetrathiobacter*), *Burkholderia*	
		Class Gammaproteobacteria Genera *Acinetobacter*, *Azotobacter*, *Enterobacter*, *Pantoea*,	

		Providencia, *Pseudomonas*	
	Fungi	Class Sordariomycetes Genus *Myceliophthora*	
		Family Aspergillaceae Genera *Aspergillus* (includes former *Emericella*), *Penicillium*	
		Class Agaricomycetes Genera *Hebeloma* , *Lactarius*, *Tomentella*, *Xerocomus*	Po hydrolization through acid phosphatase secretion. ECM fungus
		Class Sordariomycetes Genera *Chaetomium*, *Marquandomyces* (former *Paecilomyces)*	
Inorganic phosphate solubilizers	Bacteria	Phylum Actinobacteria Genera *Arthrobacter*, *Micromonospora*, *Rhodococcus*, *Streptomyces*	Solubilizing tricalcium phosphate by producing organic acids or organic acid anions
		Genus *Aerococcus*	
		Order Bacillales Genus *Bacillus*, *Listeria*, *Lysinibacillus*, *Paenibacillus*, *Sporosarcina*	
		Genus *Chryseobacterium*	
		Phylum Cyanobacteria	
		Class Alphaproteobacteria Genera *Gluconacetobacter*, *Rhizobium*, *Phyllobacterium*, *Xanthobacter*	
		Class Betaproteobacteria Genera *Collimonas*, *Delftia*	
		Class Gammaproteobacteria Genera *Alteromonas*, *Citrobacte*r, *Enterobacte*r, *Klebsiella*, *Kluyvera*, *Kushneria*, *Pantoea*, *Proteus,* *Pseudomonas*, *Serratia*, *Vibrio*, *Xanthomonas*	

	Fungi	Class Agaricomycetes Genera *Hebeloma*, *Laccaria*, *Paxillus*, *Pisolithus*, *Rhizoctonia*, *Rhizopogon*, *Suillus*	Oxalate-producing ectomycorrhizal fungi. Mobilizing insoluble bound Pi in minerals and on soil particles
		Genus *Arthrobotrys*	
		Family Aspergillaceae Genera *Aspergillus* (includes former *Emericella*), *Penicillium*	Pi solubilization through acidification. NH_4^+-driven proton release
		Genus *Cenococcum*	Alkaline phosphomonoesterase activity
		Genus *Glomus*	
		Genus *Trichoderma*	Tricalcium-phosphate solubilization by organic-acid production
		Genus *Yarrowia*	
Phosphorus storage	**Domain/kingdom**	**Organism(s)**	**Mode and/or place of action**
Polyphosphate accumulators	Archaea	*Methanosarcina mazei*	Anaerobic PolyP formation. Alkaline phosphatase.
	Bacteria	Phylum Actinobacteria Genera *Arthrobacter*, *Corynebacterium*, *Friedmanniella*, *Microlunatus*, Cand. *Microthrix*, *Streptomyces*, *Tessaracoccus*, *Tetrasphaera*	Polyphosphate kinase activity
		Genus *Bacillus*	Polyphosphate kinase, exopolyphosphatase, polyphosphatase AMP phosphotransferase activity
		Class Betaproteobacteria Genera. *Accumulibacter*, *Dechloromonas*, *Quadricoccus*, *Malikia*, *Lampropedia*, *Ralstonia*	
		Phylum Cyanobacteria	
		Class Gammaproteobacteria Genera *Acinetobacter*, *Enterobacter*, *Pseudomonas*	Polyphosphate kinase and exopolyphosphatase activity
		Genus *Gemmatimonas*	Enzymatic activity not confirmed yet
	Fungi	Phylum Mucoromycota	

		Blastocladicella emersonii	Polyphosphate polymerase activity
		Saccharomyces cerevisiae	
		Genus *Trichoderma*	
	Protists	Algae Bacillariophyta (Diatoms) Chlorophyta (green), Cryptophyta, Glaucophyta, Haptophyta, Ochrophyta, Rhodophyta (red)	
		Amoebozoa *Dictyostelium discoideum*	Polyphosphate kinase activity
Teichoic acid accumulators	Bacteria	Gram-positive Bacteria	Wall teichoic acids (WTAs), anionic glycopolymers in the peptidoglycan layer
Indirect effects on P cycling	**Domain/kingdom**	**Organism(s)**	**Mode and/or place of action**
	Animalia	Phylum Annelida, Earthworms	Unknown. Likely in the burrows of the earthworms
		Phylum Nematoda	Grazing on microbes. Reduction of P leaching
	Protists	Amoebae, Flagelletes, Ciliates	Grazing of P-solubilizing or storing microbes. Reduction of phosphate leaching
Potassium solubilizers	**Kingdom/clade**	**Organism(s)**	**Mode and/or place of action**
	Bacteria	Phylum Firmicutes Genera *Bacillus*	H^+ or organic-acid excretion into the soil leads to acidification and more available K^+. Weathering of soil and rocks
		Phylum Proteobacteria Genera *Pseudomonas, Klebsiella*	
	Fungi	Phylum Ascomycota Genera *Aspergillus, Torulaspora*	

Annex III. Most important functional marker genes used in assessing the microbial functional diversity of bioremediating microorganisms

Gene	Function /Enzyme activity	Ecosystem role
arsR1/R2	Metalloregulatory protein	Bacteria. As bioremediation
acr3-1/2	Arsenite permeases	
arsC1/C2	Arsenate reductases	
aox	Arsenite oxidation	
msh/mrx-1	Mycoredoxin	A redox system protecting cells against various stresses, such as metals, Reactive Oxygen Species, antibiotics. Present in most Actinobacteria
copA/copB	Copper-exporting ATPase	Bacteria. Cu bioremediation
merA	Mercuric reductase	Archaea, Bacteria. Hg bioremediation
merB	Organomercury lyase	Archaea, Bacteria. Hg bioremediation
pytH/pytZ/pytY	Pyrethroid hydrolase	Bacteria. Pyrethroid biodegradation
estP	Pyrethroid hydrolase	Bacteria. Pyrethroid biodegradation
pye3	Pyrethroid hydrolase	Bacteria. Higher activity and broader substrate specificity

Annex IV. Detailed taxonomic list of soil microorganisms and invertebrates involved in bioremediation

Substances	Kingdom/clade	Organism(s)	Mode and/or place of action
Various substances	Animalia	*Eisenia andrei, Eisenia foetida, Eudrilus eugeniae, Lumbricus terrestris*	Vermicomposting. Vermifiltration. Natively in the soil. Remediation of several heavy metals, hydrocarbons, BTEX (benzene-toluene-xylenes). Also increases soil fertility
Arsenic	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
	Bacteria	Phylum Actinobacteria Genera *Corynebacterium, Kocuria, Micrococcus*	As tolerance through bioaccumulation, absorption, enzymatic oxidation or reduction
		Phylum Bacteroidetes Genera *Flavobacterium*,	
		Phylum Firmicutes Genera *Bacillus*, *Staphylococcus*	
		Phylum Proteobacteria Genera *Acinetobacter*, *Agrobacterium*, *Comamonas*, *Pseudomonas, Sinorhizobium, Sphingomonas*	
	Fungi	Phylum Mucoromycota Genera *Glomus, Rhizoglomus*	Arbuscular mycorrhizal fungi, in symbiosis with the plant
		Genus *Trichoderma*	Decreases As accumulation in crops when inoculated in the soil
	Algae	Phylum Chlorophyta	
		Stramenopile Algae Genus *Nannochloropsis*	
Cadmium	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
Microremediation	Bacteria	Phylum Actinobacteria Genera *Arthrobacter*, *Bifidobacterium*, *Micrococcus*, *Rhodococcus*, *Streptomyces*	Can be biosurfactants. Many genera have PGPR activities in plants. Can often do both bioaccumulation and biosorption of Cd. More details in the review by Kumar *et al*. 2021
		Phylum Bacteroidetes Genus *Flavobacterium*	

		Pylum Cyanobacteria Genera *Microcystis*, *Spirulina*	
		Phylum Firmicutes Genus *Bacillus*	
		Phylum Proteobacteria Genera *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Citrobacter*, *Delftia*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Pseudomonas*, *Rhodobacter*, *Salmonella*	
Mycoremediation	Fungi	Phylum Ascomycota, Genera *Aspergillus*, *Cladosporium*, *Corollospora*, *Fomitopsis*, *Microsporum*, *Monodictys*, *Paecilomyces*, *Penicillium*, *Trichoderma*	Higher cell-to-surface ratio. Intra-/extracellular precipitation. Valence transformation. Active uptake mechanism
		Phylum Mucoromycota Genus *Mucor*	
Cd bioremediation of other organisms	Protists	Algae, Stramenopiles Genera *Ascophyllum*, *Chaetoceros*, *Fucus*, *Planothidium*, *Sargassum*,	Easy application. Low maintenance. Low nutritional requirement. Low operational cost
		Phylum Rhodophyta Genus *Kappaphycus*	Even dry algal biomass effective
Copper	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
Bioaccumulation	Bacteria	Phylum Actinobacteria Genera *Amycolatopsis*	In-cell accumulation with low-molecular weight, cysteine-rich proteins
Biomineralization	Bacteria	MICP Bacteria	Creates localized supersaturated conditions. Metal precipitates from the solution. With Ca^{2+}.
Bioaugmentation	Bacteria	Several plant growth promoting Bacteria	Stimulating the growth and metabolic activity of the plant in phytoremediation
	Fungi	Genus *Rhizoglomus*	Mycorrhizal
Mercury	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**

	Bacteria	*Pseudomonas putida*	Removal of methylmercury, thimerosal, phenylmercuric acetate, mercuric chloride
Lead	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
	Bacteria	Genera *Cupriavidus, Staphylococcus, Enterobacter*	Immobilization through biosorption and siderophore activity in the soil
	Fungi	Phylum Ascomycota Genera *Aspergillus, Penicillium, Saccharomyces, Neurospora*	Biosorption
Nickel	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
	Bacteria	Phylum Actinobacteria Genera *Microbacterium, Micrococcus*	Biosorption, bioaccumulation in soil and water
		Phylum Firmicutes Genera *Bacillus, Streptococcus*	
		Phylum Proteobacteria Genera *Cupriavidus, Desulfovibrio, Enterobacter, Escherichia, Klebsiella, Pseudomonas, Sphingobium, Stenotrophomonas*	
	Fungi	Phylum Ascomycota Genera *Alternaria, Aspergillus, Penicillium*	
Pesticides	Kingdom/clade	**Organism(s)**	**Mode and/or place of action**
Pyrethroids	Bacteria	Phylum Actinobacteria Genera *Brevibacterium, Micrococcus, Streptomyces*	Catabolic and co-metabolic degradation. Usually only one or two pyrethroid compounds, not all of them. Commonly works in soils. Combinations of bacterial strains are highly effective
		Phylum Proteobacteria Genera *Achromobacter, Acidomonas, Catellibacterium, Ochrobactrum, Pseudomonas, Serratia, Sphingobium*	
		Phylum Firmicutes Genera *Bacillus, Clostridium, Lysinibacillus*	

	Fungi	Phylum Ascomycota Genera *Aspergillus, Candida, Cladosporium, Trichoderma*	Catabolic and co-metabolic degradation

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