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## HIGHLIGHTS ON PROGRESS IN FOREST SOIL BIOLOGY

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### ABSTRACT

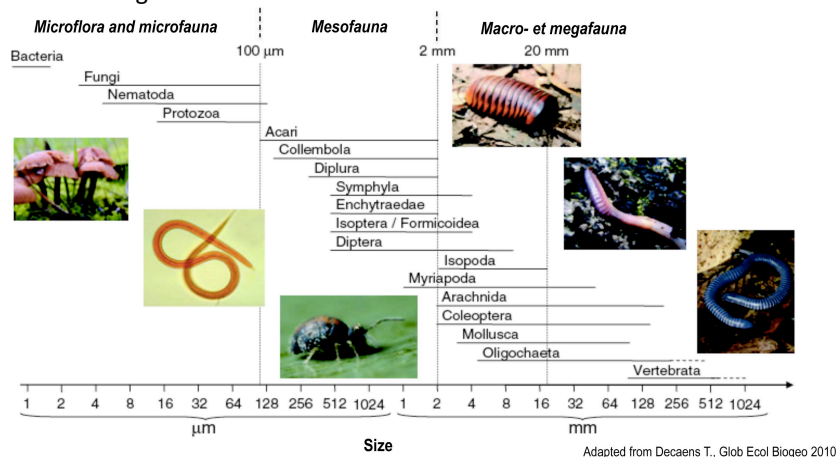
Determining the identity and function of forest soil organisms is essential to understand their relative roles, but also to determine their resilience after environmental perturbations. These characteristics are scientific challenges because of the high biological diversity of forest soil organisms, but also because many of them currently remain unknown. In this context, this review presents a snapshot of the difficulty associated with soil organism characterization, the uniqueness of forest soils and methodological and conceptual developments of the last decade. This review also presents the progress in political consideration of soil biology and highlights recent projects related to soil biology and ecosystem services.

### 1) FOREST SOIL BIOLOGY: DEFINITIONS AND CONSTRAINTS

Besides forests' ecosystem services related to biomass production, water cycling, air quality, as well as their cultural and recreational uses, forests also play a key role in term of biodiversity. However, a full understanding of this forest biodiversity is not so easy, even for the forest managers and hikers. Indeed, most visible biodiversity related to forest cover and its associated fauna is well understood, but the diversity of forest soil organism's remains little known.

Soil biology includes all living organisms inhabiting the soil, as well as their interactions and functions. Understanding the huge diversity of soil organisms is a challenge, and we are far from having a complete inventory of the living organisms that compose it. Consequently, it is particularly important to investigate and preserve soil biodiversity. One challenge is to classify organisms whose sizes range from a few centimetres for the largest invertebrates (snails), millimetres for mites, springtails and fungi, to micrometres for bacteria (Fig. 1). Moreover, these organisms have heterogeneous densities, ranging from few dozen per gram of soil for nematodes to several million per gram of soil for fungi and bacteria. These organisms' characteristics and their heterogeneous development in the soil matrix make them difficult to study and represent true scientific challenges to overcome. Despite these difficulties, several conceptual, technical and collaborative developments have occurred in the last decade in the field of soil biology.

**Figure 1 :** Distribution of soil organisms as a function of their size



## **2) UNIQUE FOREST SOIL CHARACTERISTICS**

In temperate regions, forest soils can be differentiated from those of annual cultures and grasslands by three main factors. The first corresponds to soil quality. For historical reasons, soils with high nutrient availability for plants have been intensively exploited for crops and grasslands. In contrast, nutrient-poor and rocky soils, usually with extreme pHs, have been left as forests. The second concerns the presence of trees, which can modify soil properties in the long term as a function of tree species. These characteristics and the lack of tillage allow forest soils to develop soil horizons, which correspond with the gradient of available nutrients. Under these conditions, trees can accumulate water and nutritive cations in all the soil horizons, even the deepest. These nutritive cations are then recycled during litter decomposition. The last factor corresponds to forestry practices (e.g. planting, thinning, harvesting, soil compaction, fertilisation and amendment), which modify soil characteristics. All of these factors strongly impact the structure of biological communities and consequently soil biological functioning.

## **3) ROLE OF SOIL ORGANISMS**

The activity of soil organisms in nutrient cycling plays an essential role in soil structuring, tree nutrition, and the homeostasis of forest ecosystems.

### ***3.1 Soil ventilation and structuring***

Soil ventilation, permeability and water-holding capacity strongly depend on soil structure, porosity and biological activity. Indeed, soil microorganisms influence soil structural stability by producing polysaccharides, which cement soil particles. This stabilisation is reinforced by the action of the hyphal network developed by soil fungi. In addition, earthworms play a key role when soil conditions allow them to develop. From the litter surface to several dozen centimetres below the litter, earthworms looking for organic matter (OM) or favourable moisture conditions create galleries along which water infiltrates, gas circulates and soil roots grow. Mixing hundreds of tons of soil per hectare per year (theoretically, 240 tons of faeces produced per hectare), earthworms ingest the equivalent of 25 cm of topsoil over 10-20 years. Their faeces, with more OM and nutrients than the surrounding soil, allow proliferation of microfauna, fungi and bacteria, which impact soil porosity as a feedback effect.

### ***3.2. Involvement in nutrient cycles and tree nutrition***

#### **3.2.1- ORGANIC MATTER DEGRADATION**

Forest soil OM is composed of biological products degraded and enriched seasonally to differing degrees by falling leaves and dead wood. This environment is colonized by active and complex groups of organisms that help degrade and turn over OM.

##### **a) Role of fauna**

Microarthropods (springtails, mites), millipedes, nematodes, woodlice, earthworms and some gastropods are among the first to degrade OM. Their activity combines mechanical degradation, biochemical modifications, and mixing of OM with minerals with an increase in microbial activity and the rupture and dispersal of fungal hyphae. Through multiple activities, OM is modified i) in a few hours during digestion, ii) in few days through an increase in microbial activity, iii) in few weeks to months with some of the OM beginning to be sequestered in resistant soil components and iv) in a few years to decades, during which OM turnover is modified due to dispersal of OM particles in the soil profile.

#### b) Fungal activity

In association with soil invertebrates, fungi participate in OM degradation, specifically impacting the most resistant compounds. Saprophytic fungi have long been considered the only actors in this degradation, but recent findings highlight that ectomycorrhizal fungi share this degradation ability (Buée *et al.*, 2009). The spatial distribution of these two fungal communities in the first few centimetres of the soil suggests potential functional complementarity or at least functional redundancy between them. Indeed, saprophytes are located mainly in the surface leaf litter, where cellulolytic activity is the most intense. In contrast, mycorrhizal fungi dominate in the deeper part of the organic horizon, where they feed off organic nitrogen (N) from residual lignin, humic compounds and chitin residues from dead insects and fungi. Temporal distribution of these functional groups of fungi is also observed during wood decay.

### 3.2.2- MINERAL WEATHERING AND THE NITROGEN CYCLE

Because most of the forest ecosystems are developed on nutrient-poor soils, one may wonder how trees collect the nutrients they need to grow and stay healthy in such conditions, and from where come the nutrients necessary for long-lasting functioning of forest ecosystems.

#### a) Mineral weathering

Besides nutrients recycled from OM and from atmospheric deposits, the main source of nutritive cations and phosphorous is soil minerals. However, these nutritive elements are trapped in the crystal structure of the minerals and require weathering, involving both abiotic and biotic processes, to make them available to soil biota. For decades, biological mineral weathering in forests was mainly attributed to symbiotic mycorrhizal fungi, which are known to participate in tree nutrition and mobilization of essential nutrients directly from soil minerals or OM. These nutrients are then transferred through fungal hyphae to tree hosts. Besides the implication of fungi in nutrient access, soil bacterial communities have also been demonstrated as important actors in mineral weathering and OM degradation. Recent findings suggest that mycorrhizal fungi and bacteria complement one another to feed trees with the essential nutritive cations they require for growth (Uroz *et al.*, 2011). In this context, trees would select in their root vicinity nutrient microbial helpers particularly effective at mobilising these nutrients.

#### b) Nitrogen cycle

The N present in forest ecosystems essentially comes from the atmosphere: atmospheric deposits, free-living N fixers in the soil (bacteria) or symbiotic fixation by species such as *Alnus* sp. or legumes. However, the quantity of available N remains low in forest ecosystems, increasing the importance of recycling through litter mineralisation. Development of analytical methods based on stable isotopes (<sup>15</sup>N) have helped to understand the N cycle of these soils, identify the sources of available N and describe the microorganisms involved in the N cycle.

Analysis of microbial function in the N cycle has revealed that each step of the cycle (i.e. mineralisation, nitrification, denitrification) is performed by specialised microorganisms and via well-described genes. Among these steps, nitrification (oxidation of ammonium to nitrate) is usually considered a key step. Development of functional markers to follow functional communities has highlighted the link between soil physico-chemical properties (pH, N) and the diversity of nitrifying bacteria. However, our knowledge remains limited, especially for acidic soils, where the known functional groups are uncommon or even absent, suggesting the involvement of other actors. A potential role of archaeal communities in ammonium oxidation has been suggested, but it remains to be demonstrated for forest soil environments. Finally, various fungi and heterotrophic bacteria could also contribute to nitrification, but knowledge about their roles and relative contributions is limited.

#### 4) GROWING INTEREST IN SOIL BIOLOGY

At the international level, the Convention on Biological Diversity (1993) aimed to preserve biological diversity and develop its sustainable use. In 2006, an initiative was introduced to gather knowledge about the role of soil biota and to better integrate soil biota and its preservation into agriculture and land-management practices.

At the European level, strategies for preserving biodiversity were initially based mainly on delimitating protected areas (nature reserves). More recently, the 1992 Habitats Directive and the establishment of Natura 2000 areas have enlarged the size of protected ecosystems. In 2002, soil degradation was reported in a communication of the European Commission [(COM(2002)179)], accepting *de facto* the need to preserve soils (especially soil biodiversity) and asking for establishment of a Thematic Strategy for Soil Preservation<sup>1</sup> [COM(2006)231]. Notably, this strategy emphasises that soil is an essential and irreplaceable natural resource that plays a fundamental role in ecosystem functioning and needs to be preserved. To do so, the strategy suggests i) integrating soil protection in national and European policies, ii) reinforcing soil surveys for future policy resolutions, and iii) alerting the public about the importance of protecting soils.

In practice, however, no action has been proposed to preserve soil biodiversity, due to the tremendous knowledge gaps in this field. Moreover, the apparent functional redundancy among soil organisms has not allowed “keystone” species to be identified and has made it impossible to establish an action plan to preserve soil biodiversity.

Nevertheless, understanding functions of soil biodiversity and transferring this knowledge to the public have been identified as European priorities. For example, more funding has been allocated to European Commission Framework Programmes (see section 6), and a European Atlas of Soil Biodiversity was recently published (Jeffery *et al.*, 2010).

#### 5) EVOLUTION OF ANALYTICAL TECHNIQUES

The accessibility of the organisms in the soil matrix and their identification remain important stranglehold in soil ecology. In the past decade, several methods have been developed to bypass such difficulties. Separation of organisms from the soil is often the first problem. Depending on the organism, separation may involve manual extraction (for all fauna), cultivation-dependent approaches on Petri dishes (for bacteria and fungi), specific techniques based on selective extraction procedures through density gradient. For some organisms (bacteria), a complete physical separation can be impossible and a direct identification based on cell constituents is required. Among cell constituents, the fatty acids, the proteins and especially the nucleic acids have given the higher technical developments. Identification of marker genes (e.g. 16S rDNA for the bacteria) led to development of monogenic approaches, which has been applied with success to cultivated organisms but also on environmental DNA directly extracted from the soil. Notably, most of these targeted approaches depend on selective amplification (PCR) of marker genes, their sequencing and comparison of their bases with international databases, which allows taxonomic identification of organisms. These targeted approaches have led to quantification methods (quantitative PCR) and high-throughput sequencing methods (e.g. pyrosequencing, Illumina, Ion Torrent) that quantify and decipher the diversity of organisms in a soil sample. High-throughput sequencing is probably the most important technological progress in the past few years, giving unprecedented access to soil biodiversity (Fig. 2). Along with an increase in the number of sequences produced per run (from 10-100 at first to several million in the past decade), the cost of sequencing has greatly decreased (from 10€ per sequence at first to 0.006€ in the past decade). The development of shotgun metagenomics has also increased in the past few years. Based on direct sequencing (without PCR) of environmental soil DNA, this approach gives access to genetic information about the biodiversity of all soil biota in a sample. Such metagenomic analyses should increase understanding about the functional potential of soil communities. This DNA-based method will be supplemented by metatranscriptomic and

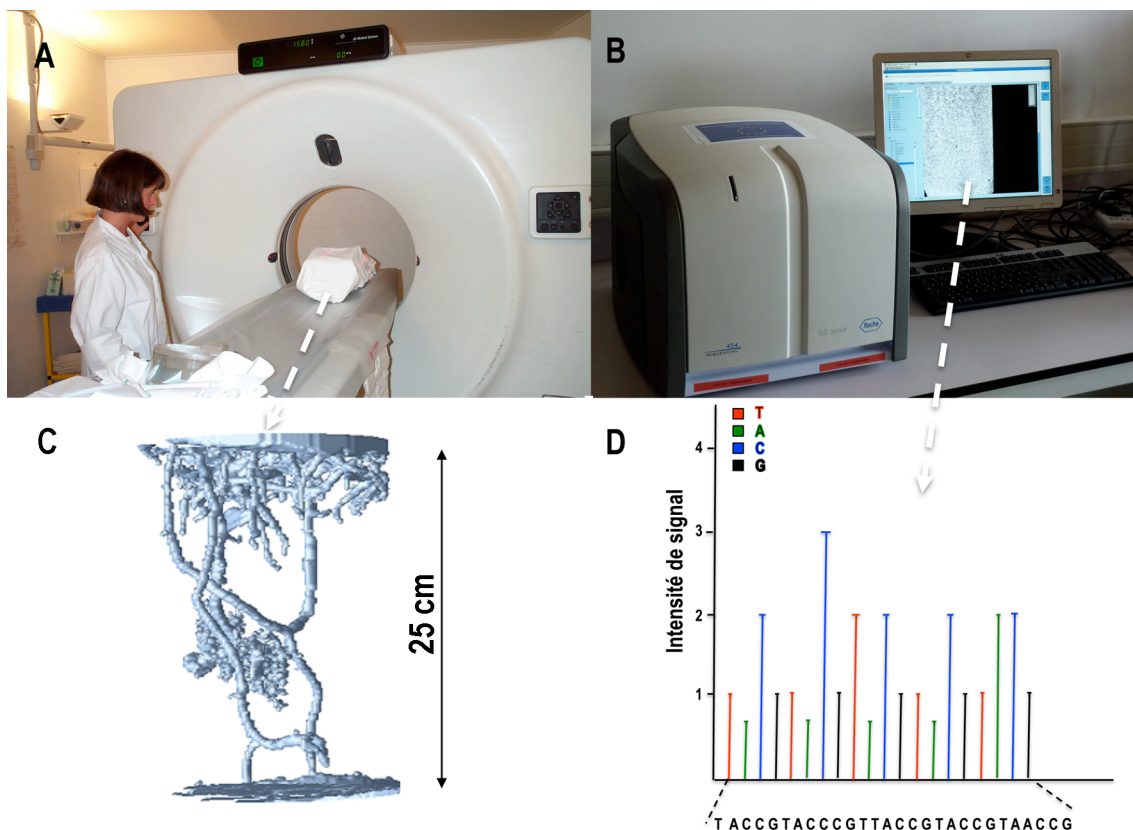
metaproteomic approaches, as well as by developments in bioinformatics. Technologies that can visualise soil structure have also evolved in the past decade. In this direction, several software programs permitting to characterize the 2-D soil porosity have been developed. In addition, X-ray computed tomography can show networks of earthworm galleries in 3-D (Fig. 2).

These new tools have provided the opportunity to develop models that represent soil structure and relations between it and the activity of soil organisms (e.g. the WORM model).

**Figure 2 :** Examples of analytical techniques

**(A)** Use of an X-ray tomograph at Pontchaillou Hospital, Rennes, France (Péres 2003) to obtain **(C)** 3-D images of networks of earthworm galleries. The images allow measurement of parameters related to network structure (e.g. length, diameter, number of branches) and give information about its ability to transfer water.

**(B)** A Roche GS Junior 454 pyrosequencing machine (Roche Diagnostics, Meylan, France), which produces a signal on a computer (Ecogenomic platform, UMR 1136 IAM). Analysis of the signal identifies **(D)** the sequence of bases (A, T, G, C) in a sample, and the machine can generate up to 100,000 sequences per day. It can be used to analyse diversity of bacterial and fungal communities by sequencing marker genes such as 16S and 18S ribosomal genes.



## 6) UNIFYING RESEARCH PROGRAMS

In the past decade, several calls for proposals focusing specifically on ecosystem services and soil biodiversity have been launched (Table S1). One of the first national calls for proposals in France (GESSOL Programme) was initiated in 1998 by the French Ministry of Ecology. This call was orientated toward soil environmental functions (e.g. regulation of nutrient cycles, carbon (C) sequestration, support of biodiversity and the landscape) as well as the main soil-degradation processes (e.g. erosion, compaction, contamination).

Related to the European Union (EU) Thematic Strategy for Soil Preservation (presented in chapter 4), ADEME (Agency of the Environment and the Control of Energy) called for proposals in 2004 related to development and validation of soil-quality bioindicators. This call for proposals was developed in

collaboration with the GESSOL Programme and in relation with the Ecoger and EcoDyn themes of the INSU (National Institute for Earth Sciences and Astronomy). In the framework of the French Soil Quality Monitoring Network (RMQS) of the Soil Scientific Interest Group (“GIS Sol”), several microbial and invertebrate bioindicators have been tested in France (RMQS-ECOMIC) and specifically in Brittany (BioRMQS-Bretagne). After publication of the Thematic Strategy for Soil Preservation, several projects of the EU Seventh Framework Programme focused on soil biodiversity (e.g. ENVASSO, EcoFINDER). In France, creation of the Foundation for Research on Biodiversity (FRB) in 2008, in association with the European network BiodivERsa, provided new input to biodiversity research. The French National Research Agency (ANR) also continues to support this theme through the call for proposals entitled “Genomics, Biodiversity, Agrobiosphere”.

## **7) TOWARDS BETTER UNDERSTANDING OF INTERACTIONS BETWEEN ENVIRONMENTAL PERTURBATIONS AND SOIL ORGANISMS**

### ***7.1. Impact of soil organic matter removal and soil compaction: input of environmental genomics***

Intensification of forest exploitation and use of heavy vehicles can have strong impacts on availability of inorganic nutrients, soil porosity and biological functioning of the soil. Development of DNA-based methods has greatly improved understanding about impacts of these management practices on soil biota. For example, Frey et al. (2011) highlighted short-term impacts of compaction on biological functioning of soil samples collected below beech and spruce stands. Based on molecular techniques and measurements of methane emissions, they demonstrated that soil compaction increased methane production, which appeared to be correlated with an increase in methanogen bacterial communities. Notably, one year after soil compaction, densities of these bacterial communities remained stable, suggesting a persistent effect. However, knowledge about long-term effects of forestry practices remains limited. In this context, Hartmann et al. (2012) analysed effects of intensifying biomass removal practices (i.e. trunk alone, the entire tree, the entire tree and all litter) and increasing compaction (no, moderate or high compaction) on the structure and diversity of soil microbial communities. Their analysis of six long-term observatories revealed that the most intense biomass removal decreased total soil C by 51-84% compared to the control. They also demonstrated that soil compaction increased soil bulk density by 4-20%. Using high-throughput techniques, they revealed that bacterial and, especially, fungal communities remained perturbed for ten years, regardless of the intensification level.

### ***7.2. Impacts of wildfire frequency***

Impacts of wildfire of soil biota are both direct, through immediate destruction of living organisms, and indirect, through environmental modifications (e.g. of soil structure and land cover). In the very short term, wildfires increase soil fertility due to the increase in available OM and nutrients. However, frequent repetition of wildfires can have detrimental effects on soil biota and fertility. Focusing on forests of the Maures mountains (south-eastern France), Vennetier et al (2008) showed that with 100 years between wildfires, the forest ecosystem was able to rebuild the OM stock in the topsoil layer (5 cm). When wildfire frequency was 25 years, the forest ecosystem still appeared resilient but had low fertility and low biological activity that was barely sufficient to regrow the previous forest cover. At higher frequency, however, all soil physico-chemical and biological parameters were degraded, strongly perturbing biological functions related to C and N cycles.

## **8) CONCLUSIONS AND PERSPECTIVES**

Overall, these results highlight the essential role of soil organisms in the functioning of forest soils, as well as the need to protect this resource and maintain efforts to characterize it. In this direction, development of long-term observatories (LTOs) and combination of ecological engineering with new technologies for describing soil biology are essential initiatives. However, the number of LTOs in which forestry practices can be tested at national and international scales remains small and does not sufficiently represent the variety of forestry trends. Nevertheless, new LTOs are essential to

address questions about impacts of forestry practices on the structure of communities of organisms, their functions and their relative roles in ecosystem functioning. For example, LTOs should help determine how soil biodiversity is affected by forest management, how it changes and how long it takes to recover. Consequently, these methods provide new perspectives in the frame of forestry practices and should help to understand, the diversity, structure and functions of soil organisms.

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