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Abstract summary

Despite its importance for human activities, soil biodiversity remains largely unknown and threatened. Aware of the need for large-scale monitoring, we studied the feasibility to add a soil biodiversity survey (RMBS-Biodiversity) to the already existing French soil quality monitoring network (RMQS). We worked with soil biodiversity experts to couple RMBS-Biodiversity with RMQS i.e. to choose soil taxons and functions, the methodologies and the sampling design. According to the experts, the sampling design of RMQS fits with a soil biodiversity survey. We propose sampling protocols for *i)* bacteria and fungi, *ii)* protists, *iii)* nematodes, *iv)* mesofauna (particularly springtails), *v)* below-ground macrofauna (earthworms), *vi)* surface macrofauna (beetles and spiders), and considering functions, *vii)* soil porosity, *viii)* enzymatic activity and *ix)* organic matter degradation. The RMBS-Biodiversity data would be used to assess the distribution and monitor the changes in soil biodiversity at the scale of the French territory. In addition, coupling data providing from RMBS with data from RMQS-Biodiversity, allows deciphering links between biodiversity,

physical-chemical characteristics of the soil and human activities (practices, heavy metal loading, and pesticide residues for instance).

Keywords: Large-scale survey, soil taxons, soil functions, soil physical-chemical characteristics, practices, biodiversity distribution

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Introduction, scope and main objectives

Soil biodiversity is essential for human activities and particularly for agriculture, by governing soil structure, organic matter degradation, nutrient and water cycles (Wagg *et al.*, 2014). Despite of its importance, soil biodiversity remains largely unknown and threatened by human activities (Decaëns, 2010; Orgiazzi *et al.*, 2016).

From the early 1990's, research programs are launched in order to know better soil biodiversity. In France, several surveys exist or have existed, varying according to sampling design, spatio-temporal breadth and studied taxon and functions: ENVASSO (Bispo *et al.*, 2009), EcoFINDERS (CORDIS. 2015), LUCAS (Toth, Jones & Montanarella, 2013), Bioindicator programme (Bispo, Grand et Galsomies, 2009) and Landmark (LANDMARK, 2015). It remains the need to a global surveillance network of soil biodiversity based on standardized methodologies, covering the whole French territory (metropolitan and ultra-marine areas), including almost the main soil biodiversity components as it is done elsewhere (Dragicevic, 2008; McKenzie, Van Leeuwen et Pinder, 2009; Rutgers, 2011; Schmidt *et al.*, 2011; CENBAM, 2012).

Building a large-scale survey with several methodologies needs a strong organization and substantial resources to cover large spatiotemporal scales. It could be advantageous to lean on an existing survey, already operational. Beyond the organization benefit, this coupling allows to study the links between different data with a high statistical power. Besides, linking different biodiversity surveys is one of the main goals of the French Biodiversity Office (OFB) (Touroult *et al.*, 2017). However, it is necessary to assess if the sampling design of the existing survey could fit with the new one. For example, the grid pixel size and the number of studied size can impact the results (Nielsen *et al.*, 2009; Soberón *et al.*, 2007).

Aware of the need for large-scale monitoring and of the opportunity of this coupling for research issues, we studied the feasibility to add a soil biodiversity survey to the already existing French soil quality monitoring network (RMQS). The objectives of the present work were: 1) Constitute an expert group involving in the feasibility evaluation of a long-term soil biodiversity survey, 2) Choose protocols and assess human, financial and technical needs and 3) Propose a sampling design accommodating all the biodiversity methodologies and the pedologic measurements.

Methodology

In order to study the feasibility to add a soil biodiversity survey (RMBS) to the already existing RMQS, we first installed a group of

experts and discussed the way we could adapt the existing sampling design to biodiversity measurements.

RMQS

The first campaign of the RMQS took place from 2000 to 2015 in continental France and overseas territories (French Guiana, West Indies, Reunion and Mayotte islands) and the second campaign began in 2016. The RMQS is based on the monitoring of 2,240 sites spread over a 16*16 km grid on French territory. These sites can be agricultural plots, meadows, urban gardens, wastelands, forests, vineyards and orchards and other types of natural environments.

For each site, a sampling area of 20m*20m, divided into 2m*2m plots, was selected. Monitoring is carried out on 25 of these plots (those with plot n°2 for the second RMQS campaign, Figure 1). A pedological profile is carried out near the sampling area.

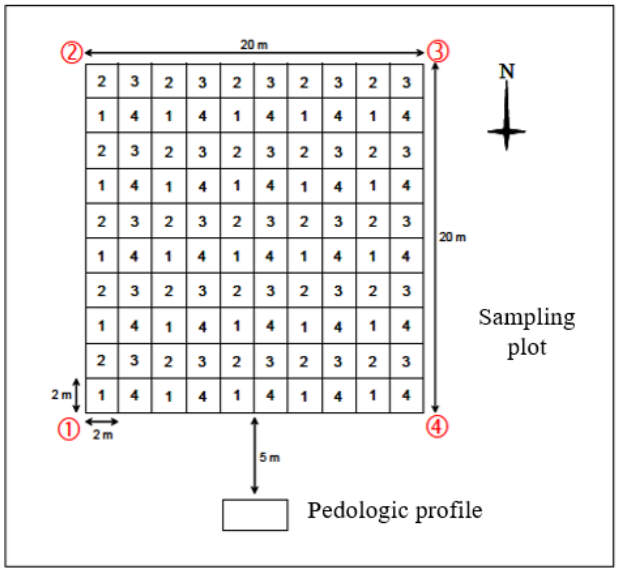

Figure 1: Sampling design of the RMQS

The data collected correspond to the physico-chemical characteristics of the soil, including contaminants, organic carbon stocks and human activities (agricultural or soil management practices). Some biodiversity monitoring has already been done on RMQS plots in three projects. Flora and forestry characteristics are measured since 2000 in plots of the ICP forest network. In the ECOMIC-RMQS project, microorganism community was determined for all RMQS plots. Similarly, fungi analysis is in progress. In the RMQS-Biodiv, several taxa (microorganisms, mites, springtails, nematodes, earthworms) and functions (organic matter degradation and enzymatic activity) were sampled in 107 Brittany sites. More recently, the enzymatic activity is measured on RMQS soil samples collected since 2016 (5 enzymes in 2016 and 2017, 10 enzymes from 2018).

Setting up the soil biodiversity survey

The OFB expressed the need to build a survey of soil biodiversity, which will be part of the larger survey of terrestrial biodiversity. The first step is to demonstrate the feasibility of such surveillance, taking into account scientific and practical aspects. Thus, we contacted around 30 soil biodiversity experts to install a working group where we built a survey. The questionnaire included 59 questions divided into 4 main categories: sampling (16 questions), laboratory and data analysis (23 questions), costs (5 questions) and interpretation of the results (15 questions). The survey was used to gather the opinions and knowledge of the experts (e.g. usefulness of the taxon, budget, way of implementation, interpretation limits) and collect the details of both field and lab methods for each taxon and function. We circulated the survey within the group. We performed face-to-face interviews to discuss more practical aspects (such as field and analysis time, workload, coordination with other teams, analysis difficulties) and collect suggestions for the most efficient way of working. Finally, during the 4 plenary meetings we all together i) discuss the results of the questionnaire, ii) study the feasibility of coupling RMBS with RMQS, iii) choose soil taxa, functions and associated methods, iv) figure out the sampling design and v) list practical needs (labour supply, costs, material, time). Such meetings were also used to explain the aims of the project, present the monitoring network, the advancement of the work, make a synthesis of the different information and reach agreements.

Results

During the last two years, 21 completed answers and 2 uncompleted answers to the questionnaire were received, together with 14 interviews. We discussed the results and shared our views during 4 plenary meetings with all the experts.

The working groups composed by the contacted experts, is representative of almost all the soil biodiversity taxonomic groups, and some functions. According to experts, the sampling design of RMQS seems be appropriate to perform the soil biodiversity survey. However, some aspects of the RMQS protocols were discussed to be adapted to the RMBS-Biodiversity. We decided that the frequency (10-15 years) and season of sampling, and the sampling grid (16*16 Km) of RMQS fits to the objectives of RMBS-Biodiversity.

We collectively propose a survey of *i)* bacteria and fungi, *ii)* protists, *iii)* nematodes, *iv)* below-ground mesofauna (springtails mainly), *v)* below-ground macrofauna, *vi)* surface macrofauna (beetles and spiders) and mesofauna, and considering functions, *vii)* soil porosity, *viii)* enzymatic activity and *ix)* organic matter degradation (Tab. 1). Flora and sporocarp fungi should also be part of the RMBS

but discussions about sampling protocols are still in progress. The expert group still need to be set up for French ultramarine areas.

The development of RMBS-Biodiversity has also allowed federating a multidisciplinary network around soil biodiversity, composed of researchers (pedologists, agronomists and ecologists), botanical conservatories, nature managers, naturalist associations, the administration and private research offices.

Table 1: Taxons, functions and methodologies selected

Taxons-Functions	Data collection	Lab analysis
Bacteria and fungi	Composite sample	Mass sequencing
Protists	Composite sample	Sequencing
Nematodes	Composite sample	Taxonomic identification
Mesofauna	Soil corers	Taxonomic identification
Belowground macrofauna	Spade test and mustard application	Taxonomic identification
Surface macrofauna and mesofauna	Pitfall traps	Taxonomic identification
Soil porosity	Soil column	Soil column scan
Enzymatic Activity	Composite sample	Absorbance measure
Organic matter degradation	Cotton strips	Tension test

Discussion

After 2 years of discussion, meetings and literature review, we considered RMQS sampling design adapted to monitor soil biodiversity at large scale. As there are no studies at different scales of soil biodiversity, so it is difficult to define the most suitable sampling design density and therefore the most appropriate grid cell. Coupling a soil biodiversity survey with a project such as the RMQS, will certainly provide some answers to this question. Indeed, a soil biodiversity survey (RMBS-Biodiversity) using the RMQS tool represents a real opportunity to conduct a study on a national scale. Even if the study grid is quite large in relation to existing work (e.g., plot scale), it will provide a global vision at a national scale of soil biodiversity and thus provide a better understanding of the biogeography of soil organisms. Due to the lack of information about the wide scale distribution of soil organisms, the adaptation of RMQS protocols to soil organism's study should constitute a starting point to understand the main drivers influencing soil biodiversity at large scale.

The RMBS-Biodiversity will start with a testing phase in 2020. A soil expert group is engaged in this project and methodologies were chosen.

The next steps are to identify methodologies to survey Flora and fungi sporocarps and to define sampling designs in ultramarine territories.

Conclusions

Adding a soil biodiversity survey (RMBS-Biodiversity) to the RMQS seems feasible and will be tested. It is a promising project that will contribute to a better knowledge of soil biodiversity on the French territory and to understand links between different kind of soil biodiversity, physical-chemical characteristics of the soil and human activities.

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