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From the micro-scale to the habitat: Assessment of soil bacterial community structure as shown by soil structure directed sampling

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ABSTRACT

Natural structural units of a luvisol under maize crop were studied to assess if soil structure directed sampling could improve the understanding of arrangements of bacteria in spatially constraint location. Three habitats were defined: (i) soil around fine lateral roots (rhizo-aggregates), (ii) soil close to basal roots (core clods) and (iii) unplanted soil between rows (bare soil clods). These habitats were also investigated with maize plants resulting from *Azospirillum lipoferum* CRT1 inoculated seeds as a model of enhanced fine root system. Rhizo-aggregates were clearly separated from each other (disconnected habitat) in contrast to micro-samples (fragments) from clods, which belong to cohesive macro-structures. Genetic fingerprints on metagenomic extracts were used to characterize the structure of bacterial communities on 95 micro-samples from the three habitats. For eubacteria, automated RISA (Ribosomal Intergenic Spacer Analysis) of ITS (Internal Transcribed Spacer) profiles were performed. PCR–RFLP on *nifH* gene were used to describe the N-fixer guilds. Exploratory multivariate analyses (PCA and MDS) revealed bacterial community patterns in the sampled habitats. On the basis of ITS profiles, rhizo-aggregates harboured closely related communities, distant from those of the unplanted soil, and each sampled rhizo-aggregate could therefore be considered as a sub-unit of the whole macro-habitat, comprising all the fine roots. The observed low dissimilarity of disconnected rhizo-aggregates is likely to result from the direct influence of maize root tips on the recruitment of rhizosphere bacteria. Molecular fingerprints of *nifH* from basal root clods (core) were more similar to bare soil than to rhizo-aggregates, indicating similar ecological conditions without, or with, at least, poor maize exudating root influence. Although our study was performed on a limited number of situations, the distribution of bacteria was revealed to be patterned by soil structure units, which is a first step to improve the modelling of microbial ecology in soils.

1. Introduction

Established ecological theory may help to frame the description of microbial habitats (Head and Prosser, 2007). Spatial patterns at different scales have been proposed by theoretical ecologists to describe the interrelationships between organisms and their environment and to explicit the heterogeneity of soil organisms (Ettema and Wardle, 2002). Besides the immediate environments of the individual organisms, the effects of roots, particles (organic and mineral) and soil layer structure can be described as nested in plot scale effects which are in turn nested in a macrosystem (soil profile, topography and bioclimatic conditions) (Ettema and Wardle, 2002). From the micrometer size of biofilms and micropores to macroscopic environments, influenced by other environmental factors (topography and vegetation system) it is increasingly obvious that the spatial assessment of soil bacteria ecology is scale dependent (Wimpenny et al., 1984; Franklin and Mills, 2003; Izquierdo and Nüsslein, 2006).

Most studies on microbial environments ignore the natural organization of soil components, i.e. the various patterns of soil structure. The specific organization of soil texture components in a define structural arrangement is a fundamental property of soil type, as it results from the soil layer genesis and functioning through the grouping of individual soil particles into secondary units of aggregates and peds (Ettema and Wardle, 2002). Assemblage of particles may range from low to high level of secondary

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units. If soil does not contain secondary units, the structure is particular, with high porosity in sands. In contrast, the absence of secondary units in loam soils results in massive structure, with no macropores and lack of oxygen (USDA, 1975). Morphology of assemblages of soil components in secondary units shows four arrangements depending on soil processes: aggregates (crumbs and granular units), blocks (large clods), platelets (resulting from sealing process) and prismatic structure (in sodic soils). In the plough layer of cultivated soils, the internal structure of centimeter size clods have been defined (Curmi et al., 1996): δ clods resulting from recent or older compaction due to wheel traffic under moist conditions and Γ clods resulting from the cohesive stress of soil components involving colloids and mainly due to desiccation. Γ clods which are dominant, except following ploughing, exhibit irregular fractures with several macropores (Curmi et al., 1996). The most beneficial secondary units of soil structure are aggregates and crumbs. Aggregate size ranges from less than 50 μm to 4 mm (Jocteur Monrozier et al., 1991), with cohesive strength and durability (Cosentino et al., 2006) depending on size and biological activities. Root growth and soil fauna also contribute to soil structuring (Oades, 1993) depending on soil texture and management.

These arrangements (crumbs, aggregates and clods) deserve consideration as they are determinant factors for the spatialization of soil microbiota but are rarely used to define the spatial distribution of microbial habitats. Usually, the structure of soil bacterial communities is investigated on either random aliquots from macro-samples (~100 g) or on several micro-samples which are further averaged. Since such sampling methods ignore the soil structure, they could miss the specific habitat of the various communities. A more relevant sampling strategy might be based on the natural structure units of the soil matrix which could embody the natural habitats of telluric bacteria. In this context, those in disconnected aggregates might exhibit more variability than bacterial communities living in connected structure like clods. Usually the most stable aggregates are encountered in the rhizosphere of plants, specifically graminaceous species, where plant exudates and microbial products provide the agents for aggregation (Kandeler and Murer, 1993). Desiccation due to high water withdrawal by plants reinforces the stability of rhizo-aggregates in soil under graminaceous plants. To test the hypothesis of a possible relationship between these structural units and the bacterial habitat, we sampled soil under a graminaceous crop, *Zea mays*, which has a strong potential for water withdrawal.

Maize model provides several other advantages to address the relationships between soil structure units and bacteria communities. First, adventitious and seminal roots are formed, differing in their role in exchange of nutrient and water with soil (Walker et al., 2003). Second, the number of active fine seminal roots may be increased using seed inoculation with strains of *Azospirillum* genus. This treatment, which improves root proliferation through phytohormones released by the inoculum (Okon and Labandera-Gonzales, 1994; Dobbelaere et al., 1999), enhances the level of soil aggregation in the vicinity of active roots. Soil microstructure under inoculated and non-inoculated maize was described by Watteau et al. (2006), who showed that active root (tips) causes aggregate formation in the rhizosphere. Rhizospheres of inoculated and non-inoculated maize seeds were investigated, corresponding respectively, to larger or lower extension of the rhizo-aggregate system.

From the same plots, Γ clods (Curmi et al., 1996) from the crown of non-exudating mature roots of maize and from bare soil (between rows) were fragmented following internal zones of fracture and bacterial community structure was compared to that of disconnected rhizo-aggregates. To link individual natural soil units and microbial community at both the micro-scale and the habitat level, we studied metagenomic DNA extracted from rhizo-aggregates and from fragmented clods from the core of the nodal rhizosphere and from the bare soil. Bacterial community structure was evaluated in each micro-sample by A-RISA, and N-fixers community structure was investigated by *nifH* RFLP, because of their abundance in the vicinity of maize active roots.

2. Materials and methods

2.1. Sampling

Samples from a field experiment continuously planted with maize for more than 15 years near La Côte Saint André (LCSA, Isère, France) were investigated. LCSA soil is a silty loam Luvisol with an organic matter content of 2% and a Cation Exchange Capacity (CEC) of 64 meq/kg in the top layer (Ap). A field trial was established in early 2001 to assess the consequence of maize seed inoculation with *Azospirillum lipoferum* CRT1, a free nitrogen fixer bacteria, on soil biota and maize biomass (El Zemrany et al., 2006). Field trials consisted of plots, planted with maize, inoculated, or not, every year during three successive crops. Samples were collected at mid-summer (90-day-old plants), the third year of the experiment. Two replicates of whole maize root system and adhering soil were taken from inoculated plot and from non-inoculated control plot, respectively. Samples from the first layer (5–15 cm) of the bare soil between plant rows were also collected from the same plots (non-rhizospheric clods). Samples were placed in plastic bags and brought to the laboratory where sub-sampling was done on the same day.

Sub-sampling was performed with sterile tools to collect various micro-samples from three soil habitats (Fig. 1):

- 39 natural rhizo-aggregates, clearly separated from each other, formed around fine lateral roots,
- 20 micro-samples from clods in the core of the nodal rhizosphere (central upper part of root system),
- 36 micro-samples from approximately 6 cm size non-rhizospheric clods (bare soil).

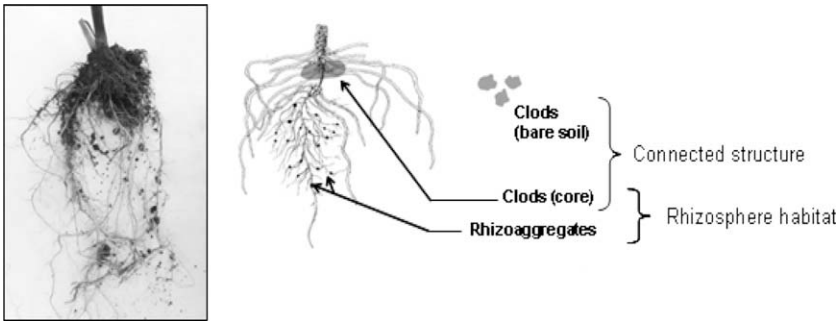

Fig. 1. Sample location in maize plant environment. Clods are both representative of connected structure, with (core) or without (bare soil) plant influence; rhizosphere samples are both under plant influence with (core) or without (rhizo-aggregates) connection.