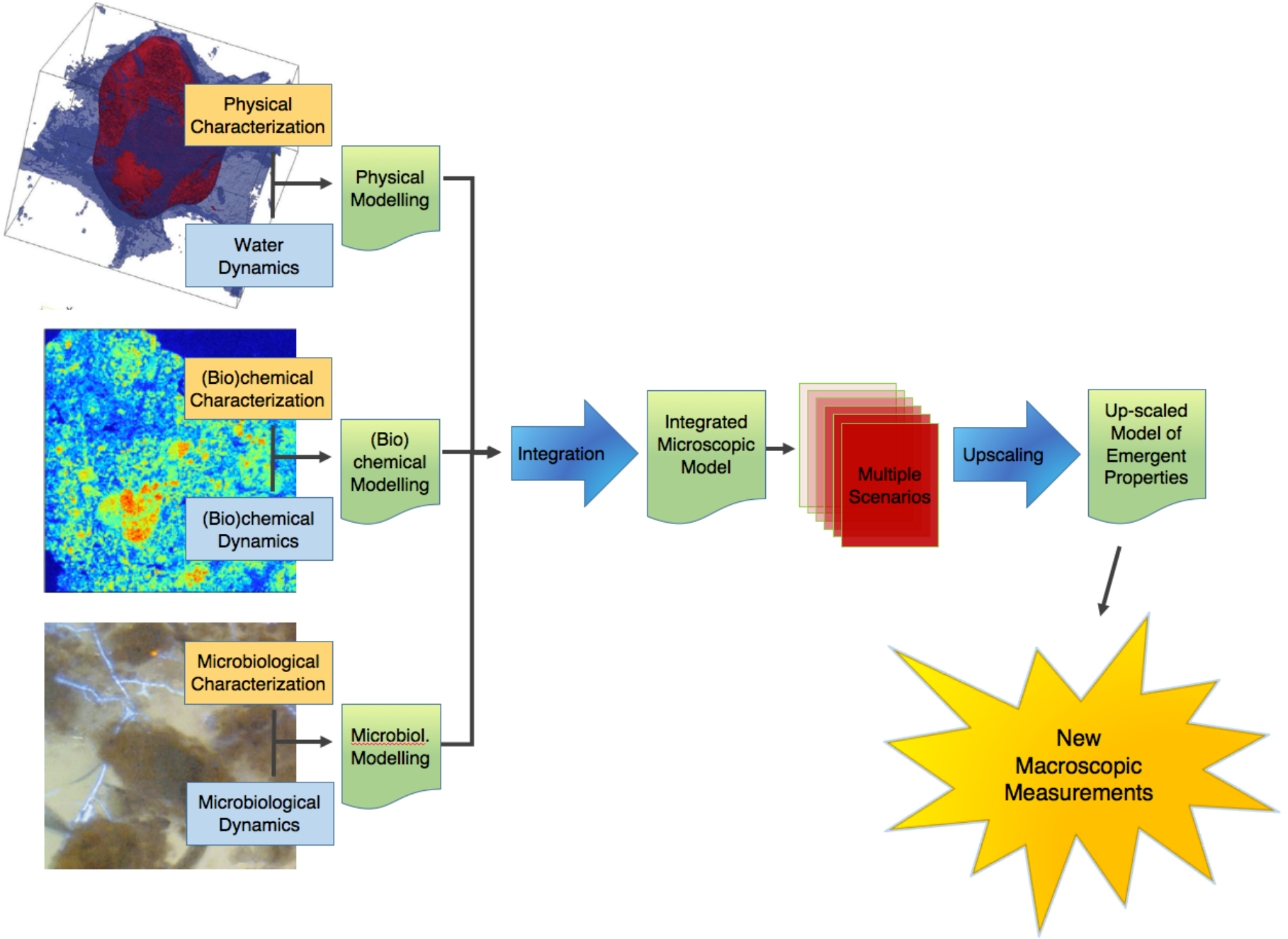
MicroSoil 2018

Workshop on “Elucidating microbial processes in soils and sediments: Microscale measurements and modeling”

Château de Saint Loup sur Thouet
Saint Loup Lamairé, Deux Sèvres, France

June 26-29, 2018.


Physical Characterization
Water Dynamics
Physical Modelling
(Bio)chemical Characterization
(Bio)chemical Dynamics
(Bio) chemical Modelling
Microbiological Characterization
Microbiological Dynamics
Microbiol. Modelling
Integration
Integrated Microscopic Model
Multiple Scenarios
Upscaling
Up-scaled Model of Emergent Properties
New Macroscopic Measurements

Programme

Tuesday June 26, 2018

Wednesday June 26, 2018

8:00-9:00: Breakfast in the medieval tower of the château of Saint Loup sur Thouet.

9:00-9:30: Welcome and organizational aspects of workshop

9:30-10:30: Invited talk: "**Emergent properties of microbial activity in heterogeneous soil microenvironments: An overview",** Wilfred Otten [40 minutes + 20 minutes for discussion]

10:30-10:45: Coffee break (first chance to see posters of Poster session 1)

Session I: Physical description and modelling. Chair: Paul Hallett

10:45-11:20: "**Recent progress in the treatment and segmentation of X-ray CT images of soils**", Steffen Schlüter [20 +15]

11:20-11:55: "**Quantitative analysis of X-ray CT images of soils**", Fernando San José Martinez [20 +15]

11:55-12:30: "**Sharing soil CT images and image analysis software: How do we do it, and should we come up with soil standards?**" open discussion centered around 2 short presentations by Marine Lacoste and John Koestel [35]

12:30-14:00: Lunch (cold buffet in the Orangerie, on the grounds of the château)

14:00-14:45: Invited talk: "**Soil microaggregates: How useful are they in the research on microscale processes?**", Kai Uwe Totsche [30 +15]

14:45-15:20: "**Where do we stand on modelling the microscale physics of soils, and what aspect of the physics of soils should we emphasize in the future?**", Philippe Baveye [20 +15]

Session II: (Bio)chemical characterization and modelling.
Chair: Hans-Jörg Vogel

15:20-16:05: Invited talk: "**Using X-ray CT to identify SOM distribution patterns in soils**", Sasha Kravchenko [30 +15]

16:05-16:35: Coffee break (+ posters)

16:35-17:10: "**How life shapes the liquid phase in soils**", Pascal Benard, Mohsen Zarebanadkouki, Andrea Carminati [20 +15]

17:10-17:45: "**Role of sugars in the contribution of root exudates to soil structural stability**", Mathilde Brax, Chrsitain Buchmann, Kilian Kenngott, Gabriele Ellen Schaumann, Dörte Diehl [20+15]

17:45-18:20: "**Submicron-scale imaging of soil organic matter dynamics using NanoSIMS**", Carsten Müller [20 +15]

18:25–18:45: Open discussion (including discussion of posters)

Evening: Dinner in the restaurant of the Hôtel Relais du Chapeau Rouge, in the town of Saint Loup

Thursday June 26, 2018

8:00-9:00: Breakfast in the medieval tower of the château of Saint Loup sur Thouet.

Session II (continued): (Bio)chemical characterization and modelling.

Chair: Hans-Jörg Vogel

9:00-9:50: Invited talk: "**Using statistical techniques to extrapolate 2D chemical maps to the 3rd dimension**", Simona Hapca [30 +15]

9:45-10:00: "**Where do we stand on modelling the microscale (bio)chemistry of soils? Brief overview of a black hole**", Philippe Baveye [10+5]

10:00-10:30: "**Areas we should focus on in the (bio)chemical characterization/description of soils**", Open discussion [30]

10:30-10:45: Coffee break (first chance to see posters of Poster session 2)

Session III: Microbial characterization and modelling.

Chair: Matthias Kästner

10:45-11:20: "**Microbial hotspots and hot moments: Conceptual background**", Evgenia Blagodatskaya [20 +15]

11:20-11:55: "**Soil-microorganisms interactions revealed through transmission electron microscopy**", Françoise Watteau [20 +15]

11:55-12:30: Poster session

12:30-14:00: Lunch (cold buffet in the Orangerie, on the grounds of the château)

14:00-14:35: "**FISH-related techniques to assess the spatial distribution of soil-microorganisms in thin sections**", Hannes Schmidt [20 +15]

14:35-15:00: "**Microbial dynamics at the microscale: How can we move to 4D and are micromodels part of the answer?**", Philippe C. Baveye [15 +10]

15:00-15:25: "**Round-table discussion: Why are there so few measurements of the microscale spatial distribution and activity of microorganisms, and how can we get more?**" Discussion led by Naoise Nunan and Wilfred Otten [35]

15:25-16:00: "**Computer simulation of the activity of micoorganisms in soils: Where are we?**", Valérie Pot [20+15]

16:00-16:30: Coffee break (+ posters)

16:30-17:05: "**Individual-based modeling of C and N turnover in soils**", Christina Kaiser [20+15]

17:05-17:35: "**Areas we should focus on in the microbial characterization/description of soils**", Open discussion [30]

Session IV: Integration and upscaling

Chair: Wilfred Otten

17:35-18:10: "**Example of integration of disciplinary perspectives: Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere**", Alix Vidal. [20 +15]

18:10-18:45: Poster session

Evening: Barbecue in the Orangerie, on the grounds of the château

Friday June 26, 2018

8:00-9:00: Breakfast in the medieval tower of the château of Saint Loup sur Thouet.

Session IV (continued): Integration and upscaling

Chair: Wilfred Otten

9:00-9:35: "**Example of integration of disciplinary perspectives: In situ X-ray tomography imaging of soil water and cyanobacteria from biological soil crusts undergoing desiccation**", Estelle Couradeau and Vincent Felde. [20 +15]

9:35-9:55: "**Types of integration we should focus on in the near future**", Open discussion [20]

9:55-10:30: "**Emergent properties: Scaling up soil carbon dynamics from microbial cells to ecosystems**", Anke Hermann [20+15]

10:30-10:45: Coffee break

10:45-11:05: **"Continued open discussion on scaling-up and new macroscopic measurements"**, Open discussion [20]

11:05-12:00: **"Where do we go from here? Outlining a possible COST proposal to create European-scale network + other funding opportunities"** discussion led by Philippe C. Baveye, Patricia Garnier and Naoise Nunan [55]

Session V. Wrapping up:

12:00-12:30: **Concluding comments and award for best poster and runner-up (Sponsored by *Frontiers*)** Philippe C. Baveye [30]

12:30-14:00: Lunch (hot buffet in the Orangerie, on the grounds of the château)

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Title:
Facing pore or solid matrix? Location of organic matter in macroaggregates from two volcanic soils of contrasting organic matter level examined by synchrotron-based X-ray CT

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Abstract:

Andisols, developed from volcanic parent materials, have unique features for soil organic matter (OM) and microbes: high contents of nano-sized mineral particles, organic matter and microbial biomass and physically-stable aggregate structure. Using physical fractionation coupled with nano- to micro-scale imaging techniques (STXM, SEM/TEM/EDX), we previously characterized micro-aggregates and their subunits in detail and developed a conceptual model of aggregate hierarchy for Andisols.

Here we used synchrotron-based X-ray CT in combination with osmium-based carbon staining technique to characterize the macro-aggregates as a whole and to explore how physical aggregate structure control OM dynamics. Specifically we tested the hypothesis that the OM exposed to pore is preferentially decomposed by comparing the Andisol under different long-term management (“Till” conventional tillage vs. “NT” no-till plus compost addition). Using beamline 20XU at SPring-8 (Japan), we obtained CT images at 0.5 micron resolution and identified the locations of OM within 100-micron-sized macro-aggregates. Pore-size distribution was similar between Till and NT aggregates. While image analysis is in progress, our initial analyses using aggregate section images showed that greater proportions of identified OM were exposed to pores in NT whereas the OM in Till was more fragmented and in association with soil solid matrix. We hope to discuss these results in relation to “microbial accessibility to OM” and "aggregate hierarchy concept".

Direct measurement of cell-mineral interactions of natural soil materials using atomic force microscopy – Method development

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Until now it is unclear to which extent soil bacterial cells and their residues contribute to the occurrence and persistence of water repellency in soil. Thus, interaction mechanisms between mineral particles and bacterial cells, surface properties of bacterial cells, minerals and their associations will be characterized by an innovative approach combining contact angle measurements and surface free energy calculations with information on surface chemical structure and nanomechanical properties obtained by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

In order to characterize bacteria-mineral interactions by AFM, a method is needed by which forces between mineral surfaces and bacterial surfaces can be directly obtained quantitatively. However, direct AFM measurements between two materials require a sample of the one and a tip of the other material. Thus a method for the preparation of mineral coated tips is needed. In addition, quantitative force measurements strongly depend on the contact area between tip and sample. Therefore, the mineral functionalized tips need to be characterized for their morphology such that the real contact area between tip and sample in dependence of height and deformation of the sample is known.

We present a method by which a fresh sharp silicon nitride (SNL) tip with a defined shape (obtained by measuring the roughness of a standard titanium sample, and post blind tip reconstruction analysis, BTR) was extracted from its body and fixed rigidly on a surface, serving as a ”well-defined’’ characterizer. The detailed morphology of a kaolinite functionalized probe was determined by imaging the SNL characterizer with the kaolinite probe. Finally, we present a first application of a kaolinite functionalized tip for AFM imaging of bacterial cells of *Rhodococcus erythropolis*. Results indicate the potential of this method to characterize interactions of minerals with single bacterial cells.

EVALUATION OF ENZYME ACTIVITY IN SOILS BASED ON 3-D MODELING

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Quantitative interpretation of the membrane-based soil zymography requires an accurate assessment of rates and distances at which substrates and products can diffuse in and out of, as well as within, an substrate-saturated zymography membrane. The goals of this study were: (i) to collect experimental data on diffusion rates and diffusion distances of the enzyme catalysis substrates and products; (ii) to model the diffusion and substrate catalysis in the zymography membrane/soil surface system; and (iii) to assess the spatial distribution and activity of the enzymes based on the results of experimentation and modeling. The studied enzyme was β-glucosidase. Dynamics of 4-methylumbelliferone (MUF) production and redistribution in the membranes were measured in a set of membrane incubation experiments. The areas of contact between the membrane and the soil surface, as well as the distances from the membrane to the soil surface, were measured using X-ray computed micro-tomography and laser scanning. The diffusion of the substrate and the product was modeled using the HP2 program of HYDRUS-2D/3D software. The Michaelis-Menten equation was introduced into the HP2 program to model MUF production by β-glucosidase. Results of diffusion experiments demonstrated that β-glucosidase itself did not diffuse from the soil into the membrane; only diffusion of the substrate and the product took place during zymography incubations. The catalysis of MUF-β-D-glucopyranoside by the enzyme occurred on the membrane surface and in the portions of the soil surface, which were in the hydraulic contact with the membrane.

The results also indicated that a large portion of the fluorescing areas on zymography membranes, which are commonly attributed to presence of enzyme active zones, actually result from diffusion of MUF from soil to the membrane and its diffusion inside the membrane. The incubation time affected the spread of MUF in the membrane and the size of “enzyme active zone”. Thus, fluorescent brightness patterns on the zymography membranes could be associated both with high enzyme concentration and with short distance between the soil and the membrane. Therefore, first, assessment of distances between the soil surface and the membrane and, second, modeling diffusion and catalysis processes are the prerequisites for accurate quantitative measurements of the enzyme activities from zymography membranes.

Abiotic regulation of the biodegradation of organic matter in the soil structure at the level of the microbial habitat

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Directors: Claire Chenu & Laure Vieublé-Gonod

Soil organic matters (SOMs) are among the key factors that underline the importance of role of soil to regulate climate or manage the sustainability of agriculture. Among their many features, these SOM are, in part, at the origin of soil quality but also compose the largest carbon stock in the continental biosphere. Hence, it is fundamental to apprehend and predict the dynamics of carbon in soil, particularly through microscale mechanistic approach. Soil carbon mineralization dynamics modulated by microorganisms-substrate accessibility is controlled by transport processes in soils, themselves modulated by abiotic factors of the soil. The thesis subject focus on these abiotic factors and poses the following problematic: What are the abiotic descriptors that account for the accessibility of microorganisms-substrate in soils and what is their hierarchy?
Thus, the objectives are: (i) To evaluate the impact of abiotic factors (porosity, water content, localization of microorganisms and substrates) on the microscale carbon mineralization and (ii) to make a monitoring of the spatio-temporal evolution of microorganisms and their activity at the scale of the microbial habitat.
The experimental set-up is based on incubated soil cosmes with addition of localized complex organic substrates (a central layer of ^{13}C labelled maize residues) in which water content varies. The monitoring the ^{13}C and total carbon mineralization was done and, on additional cosmes, selected at 4 different dates of the incubation, cuts from the residues layer to the bulk soil had been done in order to analyze the microbial community structure and its activity by processing PLFA and SIP-PLFA.
We observed that the addition of organic substrate enhances the respiration from the degradation of residues and soil organic matter with a more pronounced effect following the increasing of water content. We also observed a priming effect which seems to be also accentuated with the increasing of the water content. The analysis of microbial communities shows a hotspot phenomenon with a gradient of activity from the residues to the bulk soil.

The role of self-assembling of root mucilage for the formation of spatiotemporal wettability patterns in the rhizosphere – a planned project

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Root mucilage, a polysaccharide hydrogel, may render soil hydrophobic after drying and buffers extreme hydraulic conditions in the rhizosphere. Until now, little is known about how this buffering behavior depends on chemical and chemico-physical properties of mucilage and how these properties respond to different environmental conditions in the rhizosphere.
We thus aim at understanding which role chemical hydrogel properties play for the physical properties of root mucilage of different plants and, consequently, how these physical properties influence the drying behavior and the resulting spatial patterns of mucilage. Finally, we want to close the circle by unraveling, which consequences these spatial patterns have for hydraulic properties of the rhizosphere during drying and rewetting.
By a combination of dialysis and swelling experiments with mucilage of different plants we plan to study the effect of environmental conditions (pH, cations, surface active substances) on physical hydrogel properties and link it to the results of chemical analysis (monosaccharide, linkage analysis, total contents). Macroscopic rheology, ^{1}H-NMR (for water mobility) and differential scanning calorimetry (DSC, for non-freezing water), will be employed together with microscale methods, such as atomic force microscopy (AFM), for the determination of surface tension, viscosity and elasticity. These AFM measurements will be included as input parameters into a simulation of liquid bridges of mucilage during drying. Therefore, methods from continuum mechanics (describing the network of polysaccharids) are coupled to Lattice Boltzmann methods (describing water flow within the pore space). Furthermore, we will experimentally and numerically quantify the relation between microscopical drying patterns of mucilage to its macroscopic wettability (contact angle) and to macroscopic hydraulic properties such as rhizosphere water retention and its hysteresis. Finally, our results will be tested by insitu ^{1}H-NMR measurements in mini pot experiments.
By coupling molecular-chemical properties and nanoscale-spatial arrangement of mucilage with macroscale-hydraulic processes we intend to significantly improve our understanding of the rhizosphere as a dynamic self-organized system.

An integrated X-ray fluorescence spectroscopy and imaging approach to assess As bioavailability in soils

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Arsenic (As) is a metalloid element, often naturally associated with gold, sulphur, iron and heavy metals. It can be found in soils around former mines and industrial sites treating As-bearing minerals. The assessment of the bioavailability of As in these soils is very important in order to protect human and ecosystems health. The present work aims at evaluating the bioavailability of As in polluted soils sampled in the industrial area of Scarlino (Tuscany, Italy) and in the gold mining site of the Anzasca Valley (Piedmont, Italy). In these soils, As contamination ranges from 20 to 13300 mg/kg and it is associated to different soil phases.
An integrated approach using different X-ray based techniques was employed to investigate As distribution in soil and in *Eisenia andrei,* an earthworm often used as sentinel organism in environmental studies.
After 14 days of *Eisenia andrei* exposure to contaminated soils, no earthworm died, even if an oxidative stress was measured. However, a chronic toxicity (estimated by the OECD reproduction test) was observed after 28 days of exposure, with a reduction of the reproductive rate with the increase in As soil concentration.
µXRF and XRF tomography analysis on earthworm thin sections and whole organisms, respectively, showed that As accumulates mainly in the coelomic cavity. Arsenic quantification in the coelomic fluids was performed by total x-ray fluorescence spectroscopy (TXRF) and compared with the total As concentration in the whole earthworm body. A good correlation was found between the two set of data, which suggests the possibility to assess the As bioavailability using only coelomic fluid extracts. Arsenic concentration in coelomic fluids was also directly related to As soil concentration. However, a saturation level was observed at high As soil concentrations
These results showed the usefulness of laboratory X-ray based techniques for bioavailability studies and could be used as a background information for the development of new procedures for the assessment of As bioavailability in contaminated soils.

Correlations between soil pore network characteristics derived from X-ray CT and soil fungal and bacterial communities at field scale

Marcos Paradelo, Rumakanta Sapkota, Sheela Katuwal, Mogens Nicholaisen, Mogens H. Greve, Per Moldrup and Lis W. de Jonge.

The spatial variation of soil physical properties influences microbial colonization and the soil microbial community structure. In this exercise, we report the correlations between soil physical characteristics derived from X-ray CT scans and the bacterial and fungal communities' composition. Samples in a 15 x 15 m grid were collected from an agricultural field in Greenland (sandy soil). Soil samples for 454-pyrosequencing were extracted by pushing 50-mL sterilized plastic vials into the soil. Intact soil columns 100 cm^3 were collected and scanned with an industrial X-ray CT scanner (129 µm resolution). Bacterial communities were correlated with textural (clay) and X-ray CT derived traits (CT porosity, vertical tortuosity or the total branch length). Fungal communities were also correlated with clay but to a lesser extent with the X-ray CT derived characteristics. Weighted correlation network analysis (WCNA) was used to detect modules within the microbial community and determine co-ocurrence patterns between bacteria and fungi. Three modules were detected differing in fungal and bacterial composition. X-ray CT derived traits were significant correlated with one of the modules. This module is formed by 29 Fungi, predominantly form the phylum Ascomycota, and 12 Bacteria from different phyla (Acidobacteria, Proteobacteria and Bacteroidetes). A non-modular group formed mostly by bacteria was also correlated with the X-ray CT derived traits but with opposite sign.

This exercise shows how fungi and bacteria formed distinct associations or modules correlated with soil physical traits which cannot be detected when the two kingdoms are studied as separate functional groups. Further studies focused on these modules would help to link microbial and physical functions in soils.

Control of transport processes on microbial dynamics and pesticide degradation from µm to mm scales

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Keywords: spatial distributions, microbial degradation, transport processes

Soil microorganisms perform several major desirable and undesirable ecosystem functions by degrading a part of soil organic matter. Deciphering the implied mechanisms at microbial spatiotemporal scales is a key point to understand the variability of soil functions at larger scales. Among all factors controlling the degradation of soil organic matter, spatiotemporal access of bacteria to their substrates appears to be particularly relevant in soils, where substrate and bacteria distributions are heterogeneous and sparse enough to see this habitat as a "desert" for bacteria. Unlike 2,4-D, a pesticide that can easily diffuse through soil water films, 2,4-D degraders have been shown by Pinheiro *et al.* [2015] to be immobile at field capacity at fixed saturation. Infiltration can however move the degraders and enhance biodegradation, suggesting potential positive feedbacks between dispersion and biodegradation.

The objective of this work is to formalize how spatiotemporal organizations control the encounter between degraders and soluble organic µ-pollutants such as 2,4-D.

We develop a reactive transport model at cm scale (Babey et al., 2017) aimed at investigating the impacts of initial distributions of 2,4-D and its bacterial degraders on its biodegradation. Sorption, diffusion, advection, and microbial processes are calibrated on cm-scale experiments performed on the degradation of 2,4-D in natural repacked soil cores (Pinheiro et al., 2015, Pinheiro et al., not yet published). We develop a spherical model at a finer resolution to explore finer initial distributions.

In a context where 2,4-D can diffuse but bacteria cannot, passive advection-dispersion of 2,4-D and its degraders cannot explain the steep increase in 2,4-D degradation efficiency observed in irrigated experiments. This lack of effect of bacteria dispersal can be explained by a higher dilution of 2,4-D concentrations perceived by the degraders that are remote from 2,4-D initial spot that leads to a decrease of initial degradation rate, entirely counterbalancing the lower competition for the substrate perceived by the degraders that are remote from other degraders. Other hypotheses are needed to explain the experimental observations, such as active crowding-effect inhibition.

Strong biodegradation heterogeneities can emerge from the interaction of the non-linear spatial and biological processes. Therefore degrader dispersion would be favorable only in specific situations. Our results give guidelines to design future experiments for validation of the explored processes. Better understanding of the contact probability between substrates and microbial degraders can help us to evaluate more precisely the biological activity and dynamics of microbial degraders.

Babey T, Vieublé-Gonod L, Rapaport A, Pinheiro M, Garnier P, de Dreuzy J-R. Spatiotemporal simulations of 2,4-D pesticide degradation by microorganisms in 3D soil-core experiments. Ecol Model. 2017 Jan;344:48–61.

Pinheiro M, Garnier P, Beguet J, Martin Laurent F, Vieublé Gonod L. The millimetre-scale distribution of 2,4-D and its degraders drives the fate of 2,4-D at the soil core scale. Soil Biol Biochem. 2015 Sep;88:90–100.

Does pore scale biogeography exist in different soil types ?

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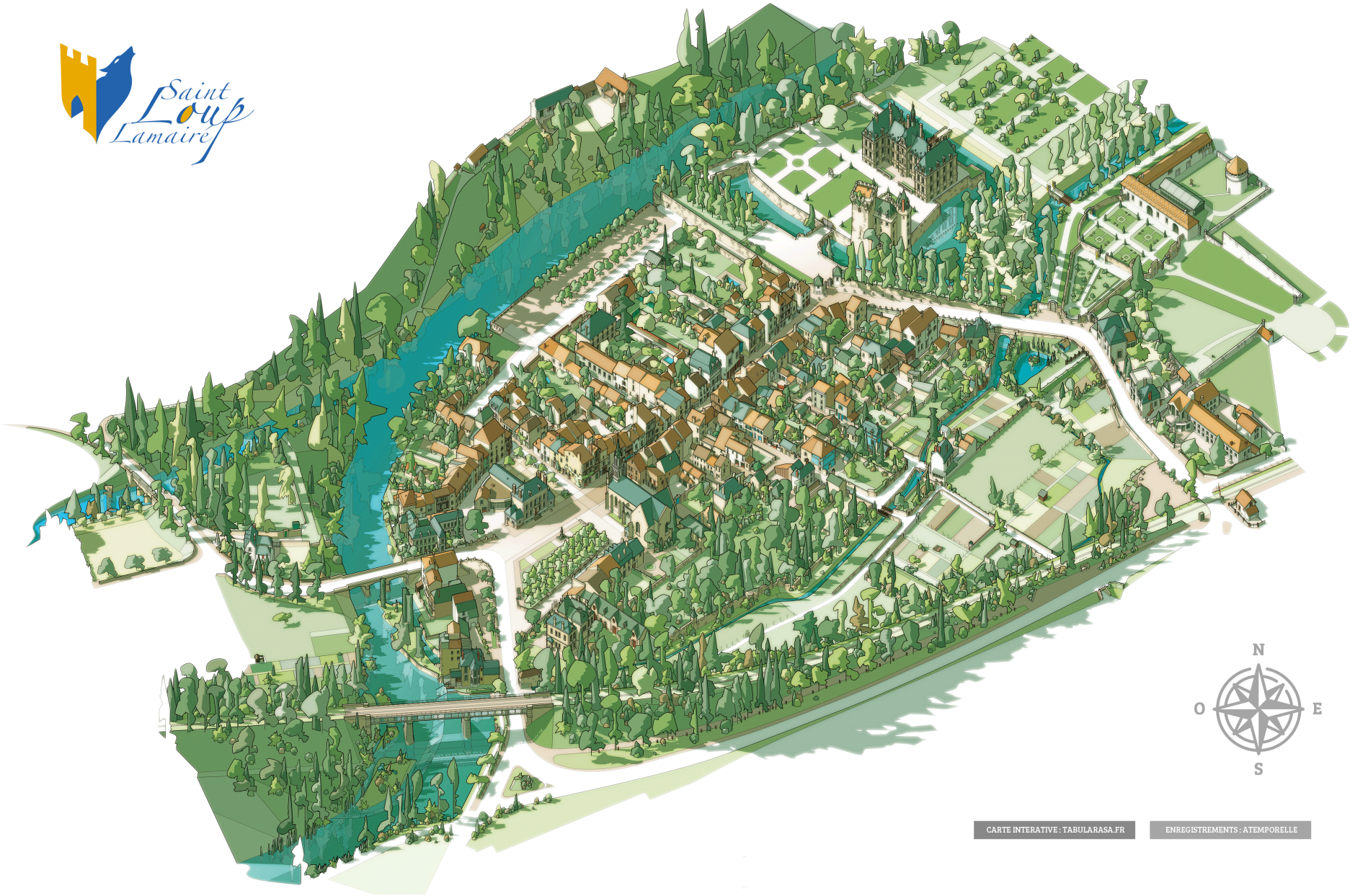
2- IEES, UPMC, CNRS, IRD, INRA, UPEC, Paris, France

Microbial activity is largely controlled by the abiotic conditions prevailing in their habitats, which are very heterogeneous at the microscopic scale. A few studies have demonstrated a microbial biogeography at the pore scale. Different regions in the soil pore network can be considered to be different microbial habitats and these different regions have been shown to be well correlated with organic carbon mineralization rates. We aimed to test whether such a functional biogeography exists for different soil types and whether it is consistent across soils.

We selected six topsoils with contrasted texture, soil organic matter content and pH (2 cambisols, 3 luvisols and 1 podzol under different managements). We added a 13C labelled, easily mineralisable organic substrate, pyruvate, to soil samples previously equilibrated at different matric potentials, in order to place the substrate preferentially in soil pores with neck diameters of 3 to 10 µm or 30 to 10µm, according to the Jurin-Laplace law. The soil samples were then incubated at pF 1.5 for 3 weeks and CO2 and 13C-CO2 were monitored. At the end of incubation, total and 13C-PLFA were extracted and analysed.

Basal mineralisation, expressed as % total organic C was affected by soil type, mainly related soil pH and the quantity and quality of the organic matter. The 6 soils exhibited contrasted microbial community composition, as shown by their PLFA profiles. The addition of pyruvate did not induce any priming effect in soils, except in the long term bare fallow soil, where the mineralization of SOM was presumably limited by energy. In the long term bare fallow soil, pyruvate mineralization was the same whatever the region it was placed in, suggesting other controls of its mineralization than the characteristics of pore scale habitats. In four soils out of six, the mineralization of pyruvate was more rapid when it was initially placed in large pores (30 to 100µm) than in small pores (3 to 10 µm), suggesting that pore scale biogeography may be a general feature in soils and that coarser pores are more favourable habitats for soil organic matter mineralization.

Saint Loup Lamairé



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