Visualizing the biogeochemical interface in soils

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▶ **To cite this version:**

Charlotte Védère, Claire Chenu. Visualizing the biogeochemical interface in soils. Doctoral. Soil systems: Analytical methods for integrating the chemical, biophysical interface in soils., Uppasala (on-line), Sweden. 2021. hal-04537247

HAL Id: hal-04537247
https://hal.inrae.fr/hal-04537247

Submitted on 8 Apr 2024

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Visualizing the biogeochemical interface in soils

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Soils group at Grignon

Research lines

- Optimizing organic waste recycling in agriculture
- Pesticides and other organic contaminants dynamics
- Soil organic matter dynamics
- Assessing ecosystem services provided by soils

Visualizing soils?

- Heterogeneous and complex environments. Observations and fluxes at the plot or profile scale controlled by microscale conditions and processes

- Soils specificities
  - Non-coherent material
  - Heterogeneous constituents: size, composition, softness
  - Importance of the moisture state

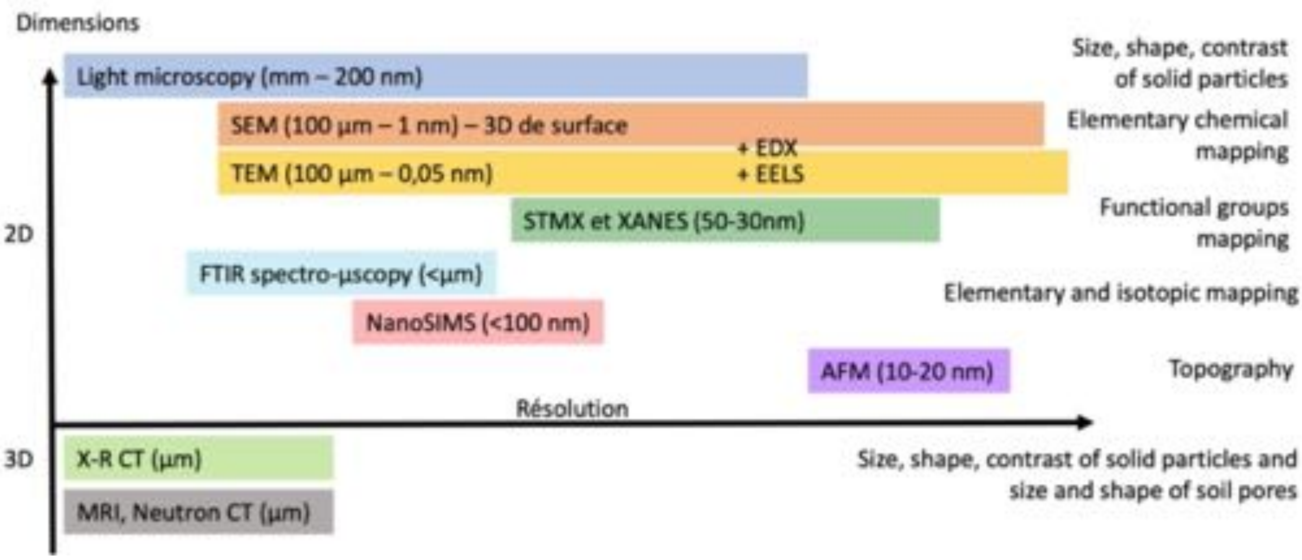
- Biogeochemical interface

minerals air

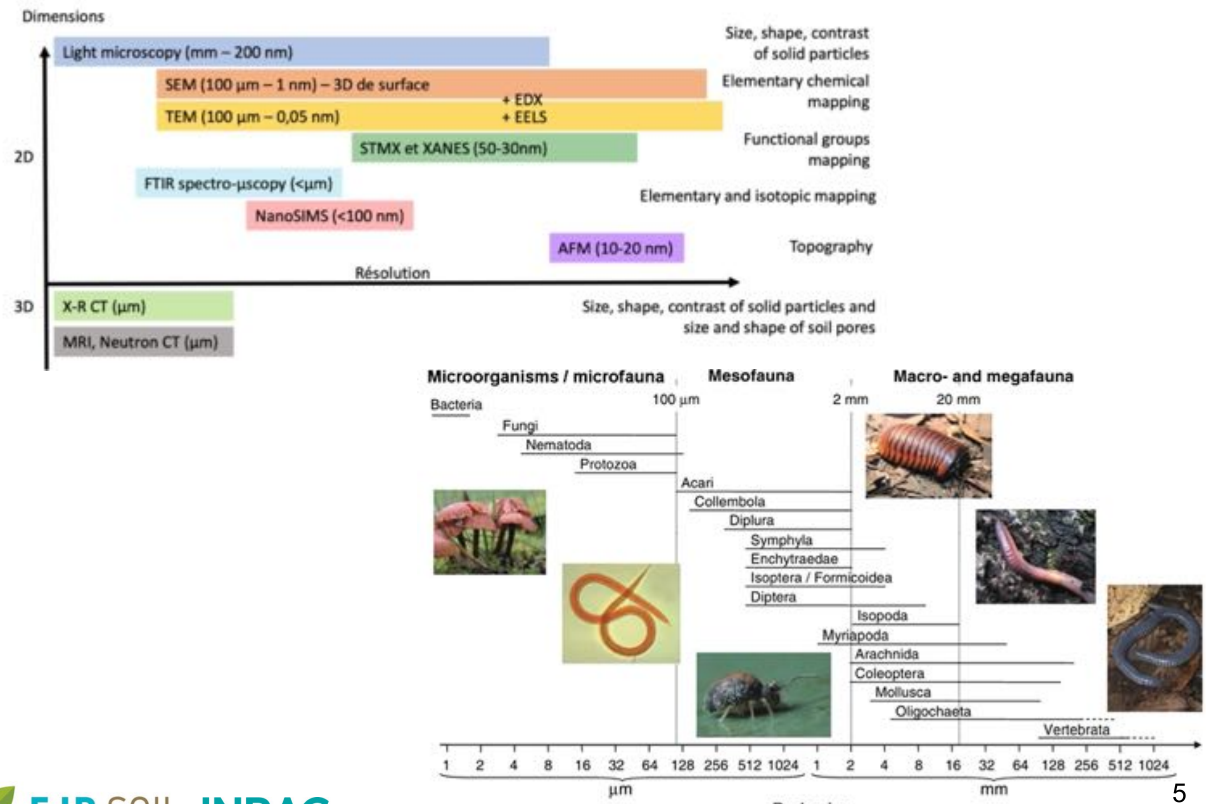
biota water

organic matter

Scales and methods



Scales & methods



Visualisation tools *(main ones..)*

category	name	principle and radiation used	image	resolution
Ligth microscopy	Stereomicroscopy	incident light	3-D	≈ 10 µm
	Ligth microscopy bright field	transmitted light	2-D	< 1 µm
	Epifluorescence microscopy	transmitted light -> fluorescence	2-D	0.2 µm
	Confocal (laser) microscopy	transmitted light -> fluorescence	3-D	0.16 µm
Electron microscopy	Scanning electron microscopy (SEM)	reflected electrons	3-D	10 nm
	Transmission electron microscopy (TEM)	transmitted and diffracted electrons	2-D	0.2 nm
X-ray spectro microscopy	Soft X-ray spectro microscopy in the water window (STXM)	transmitted X-rays	3-D	30 nm
InfraRed spectroscopy	VNIR	absorbed IR light	2-D	50 µm
	Raman microscopy	difused monochromatic light	2-D	µm
	FTIR microscopy	absorbed IR light	2-D	µm
Secondary ion mass spectrometry	nanoSIMS	ions beam -> sample ions collected	2-D	150 nm
X-ray computed tomography	X-ray µCT	attenuation of transmitted X-rays	3-D	1 µm
Scaning probe microscopy	Atomic force microscopy (AFM)	cantilever scans the surface of the sample	3-D	1 nm

Outline

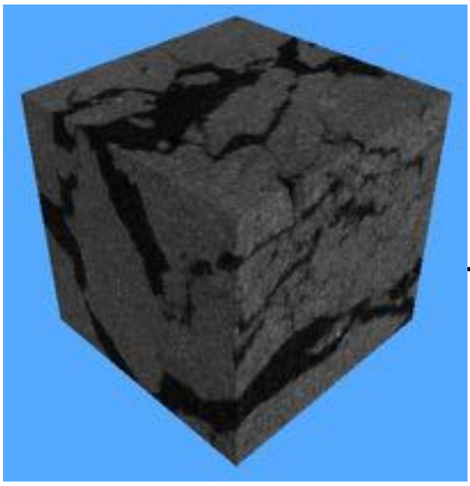
1. Visualisation of the soil habitat
   a. Overview of available methods
   b. Visualizing soil structure – Soil moisture dependence
   c. Visualizing soil organic matter
2. Localizing and identifying soil inhabitants: microorganisms
3. Microscale information on the physiological state and activity of soil microorganisms
4. Visualizing the biogeochemical interface

1- Visualizing the soil habitat

Visualisation tools

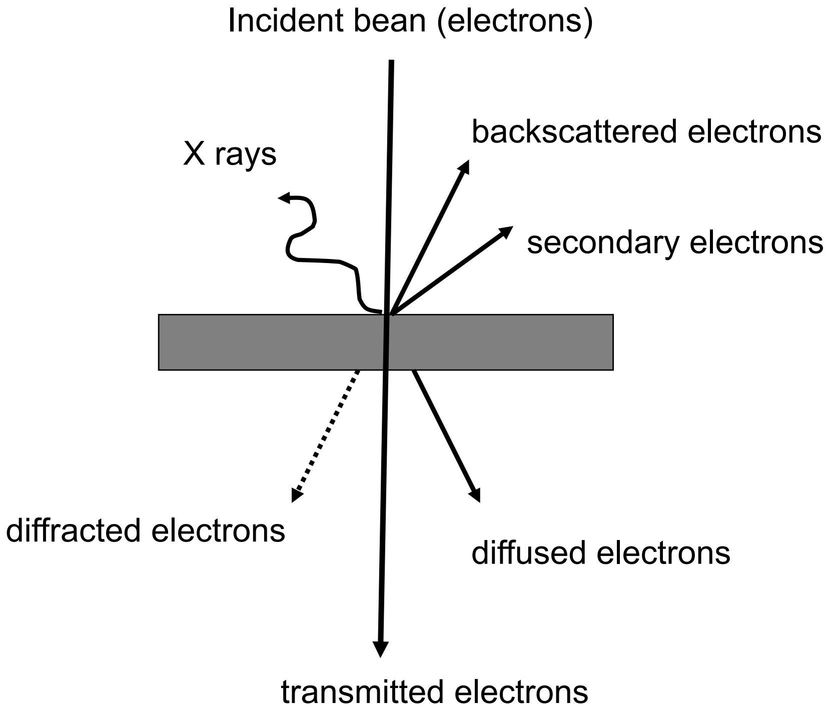
category	name	principle and radiation used	image	resolution	information on soil constituents
Ligth microscopy	Stereomicroscopy	incident light	3-D	≈ 10 µm	size, shape, color, contrast of solid particles + specific stains or labels for OM
	Ligth microscopy bright field	transmitted light	2-D	< 1 µm	
	Epifluorescence microscopy	transmitted light -> fluorescence	2-D	0.2 µm	
	Confocal (laser) microscopy	transmitted light -> fluorescence	3-D	0.16 µm	
Electron microscopy	Scanning electron microscopy (SEM)	reflected electrons	3-D	10 nm	size, shape, topography of solid particles. + EDX elemental analysis
	Transmission electron microscopy (TEM)	transmitted and diffracted electrons	2-D	0.2 nm	size, shape, contrast of solid particles + stains OM + elemental analysis EDX + functional groups OM (EELS)
X-ray spectro microscopy	Soft X-ray spectro microscopy in the water window (STXM)	transmitted X-rays	3-D	30 nm	elemental mapping + functional groups OM (NEXAFS)
InfraRed spectroscopy	VNIR	absorbed IR light	2-D	50 µm	functional groups mapping
	Raman microscopy	difused monochromatic light	2-D	µm	functional groups mapping
	FTIR microscopy	absorbed IR light	2-D	µm	functional groups mapping
Secondary ion mass spectrometry	nanoSIMS	ions beam -> sample ions collected	2-D	150 nm	elements & isotopes mapping
X-ray computed tomography	X-ray µCT	attenuation of transmitted X-rays	3-D	1 µm	size, shape, contrast of solid particles + stains OM
Scaning probe microscopy	Atomic force microscopy (AFM)	cantilever scans the surface of the sample	3-D	1 nm	topography of solid particles

1.2 Visualizing soils structure. Moisture dependence



Wednesday 1st dec
Non-invasive 3-D imaging methods
Mats Larsbo

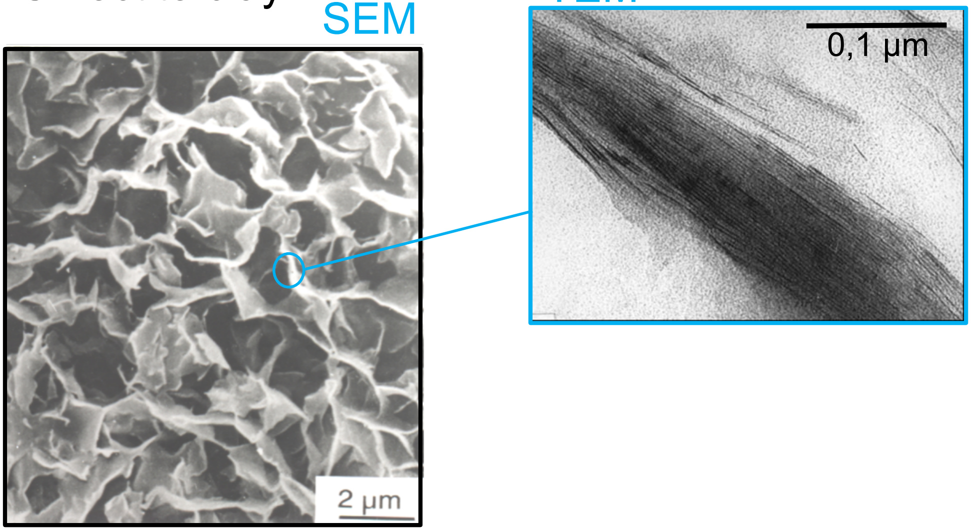
Electron microscopy : Electron - sample interaction



Smectite clay

SEM

TEM



ω= 3,7 g water g^{-1} solid

Ψ = -0.032 bar

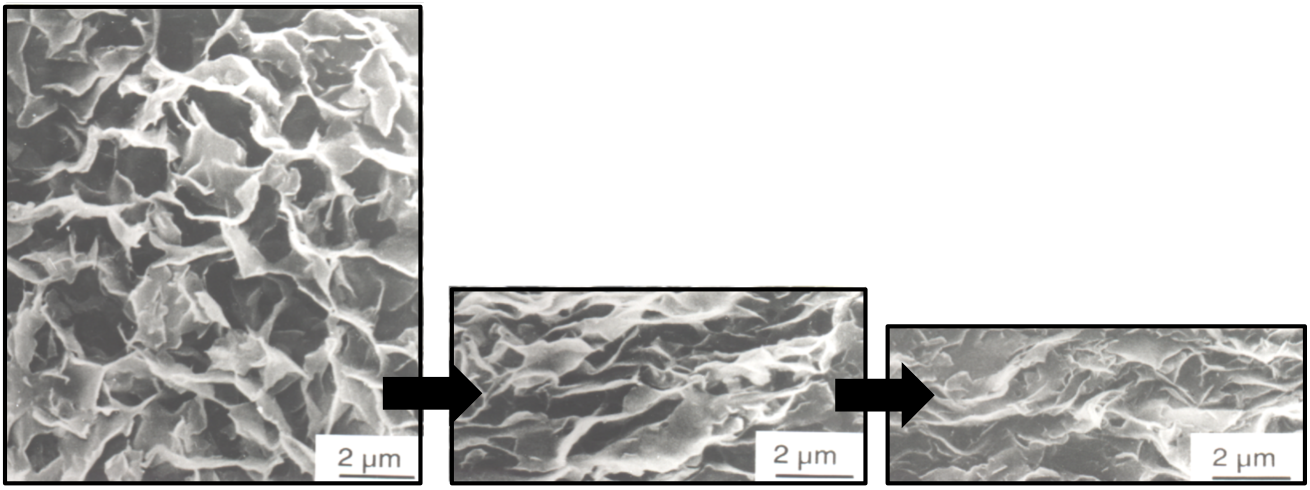
Ψ = pF 1.5

Tessier, 1990

Soil microstructures are fragile

Smectite clay

SEM



ω= 3,7 g water g^{-1} solid

Ψ = -0.032 bar

Ψ = pF 1.5

ω= 1,7 g water g^{-1}

Ψ = -1 bar

Ψ = pF 3

ω= 0,82 g wter g^{-1}

Ψ = -10 bars

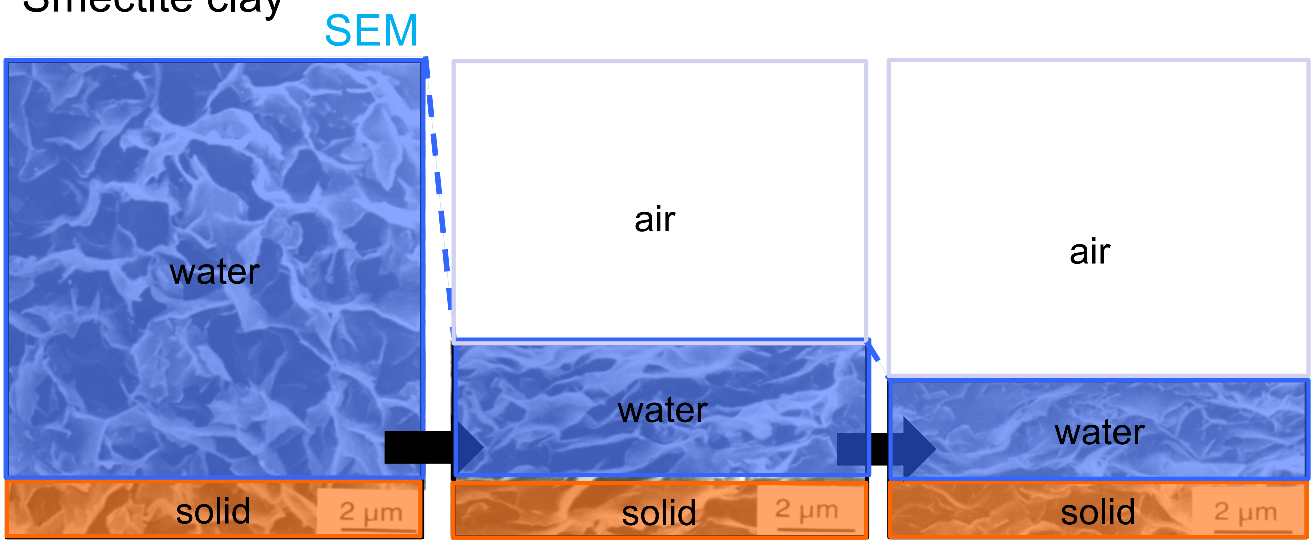
Ψ = pF4

Low Temperature SEM

Tessier, 1990

Soil microstructures are fragile

Smectite clay



ω= 3,7 g water g^{-1} solid
Ψ = -0.032 bar
Ψ = pF 1.5

ω= 1,7 g water g^{-1}
Ψ = -1 bar
Ψ = pF 3

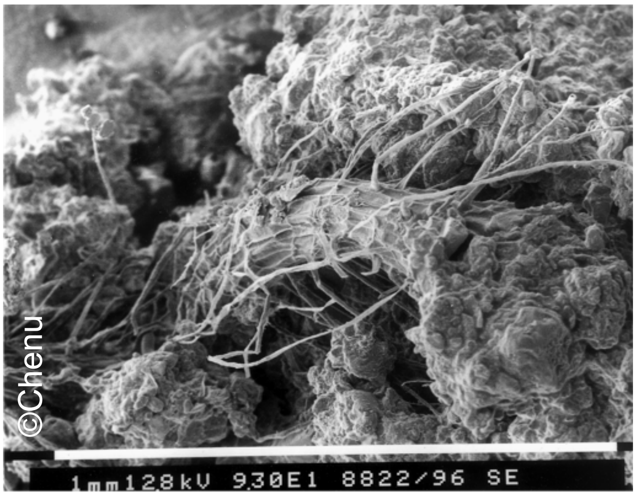
ω= 0,82 g wter g^{-1}
Ψ = -10 bars
Ψ = pF4

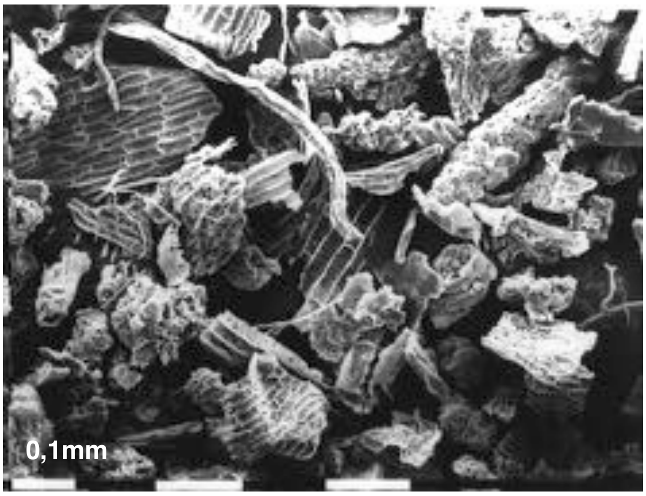
Low Temperature SEM
Tessier, 1990

1.3 Localizing organic matter in soils

SEM how to identify soil constituents ?

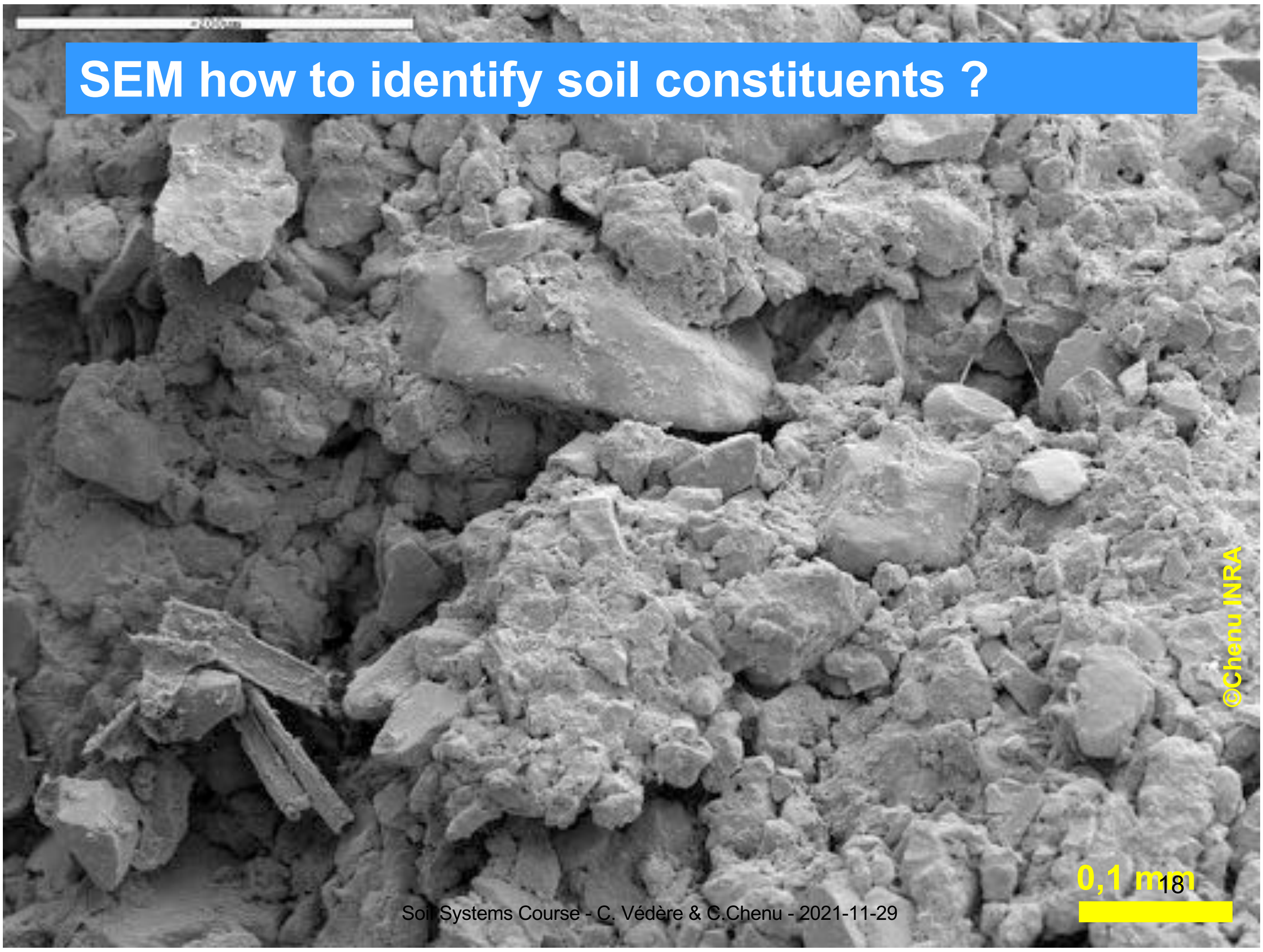
- morphology



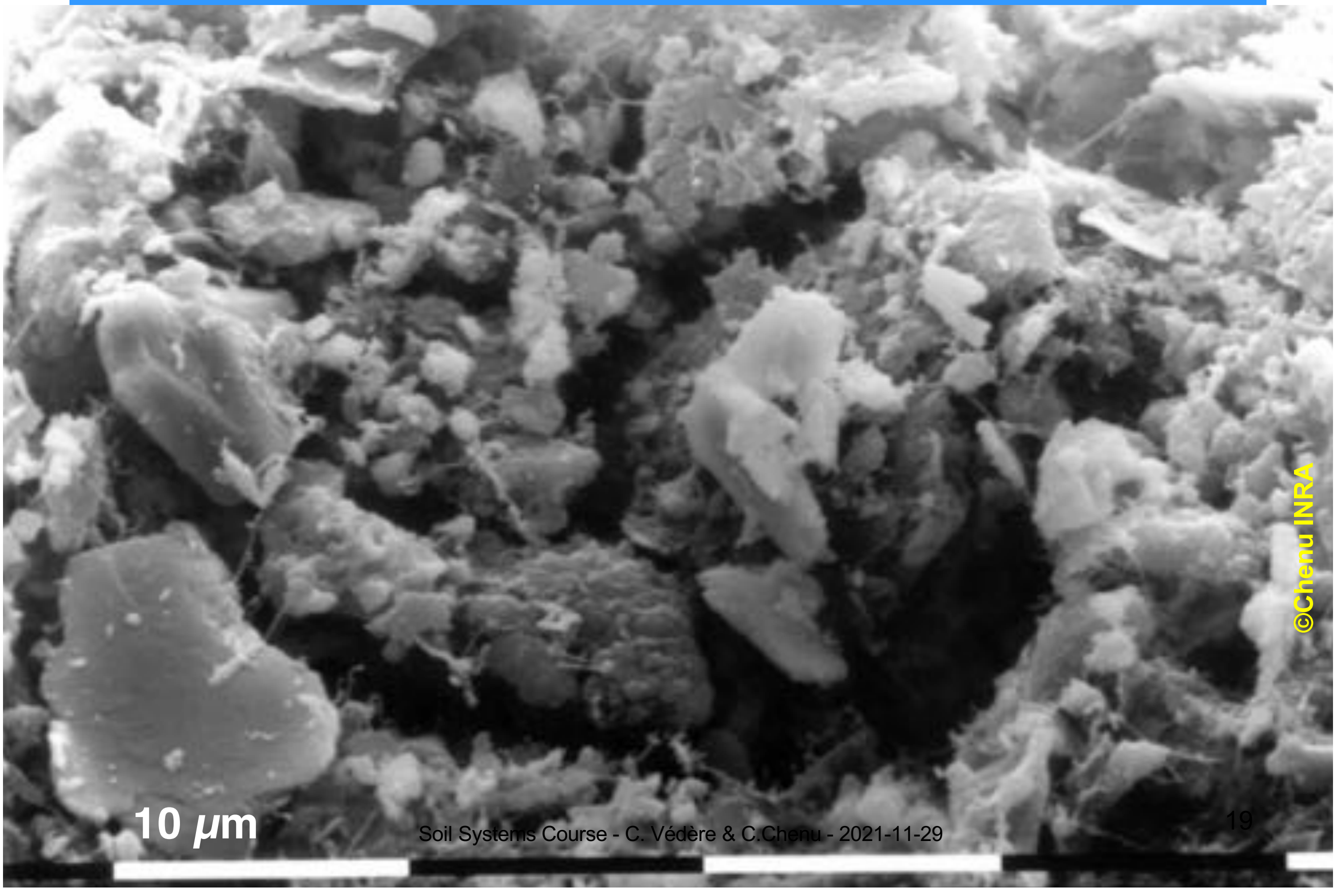


Besnard et al. 1996 EJSS

SEM how to identify soil constituents ?



SEM how to identify soil constituents ?

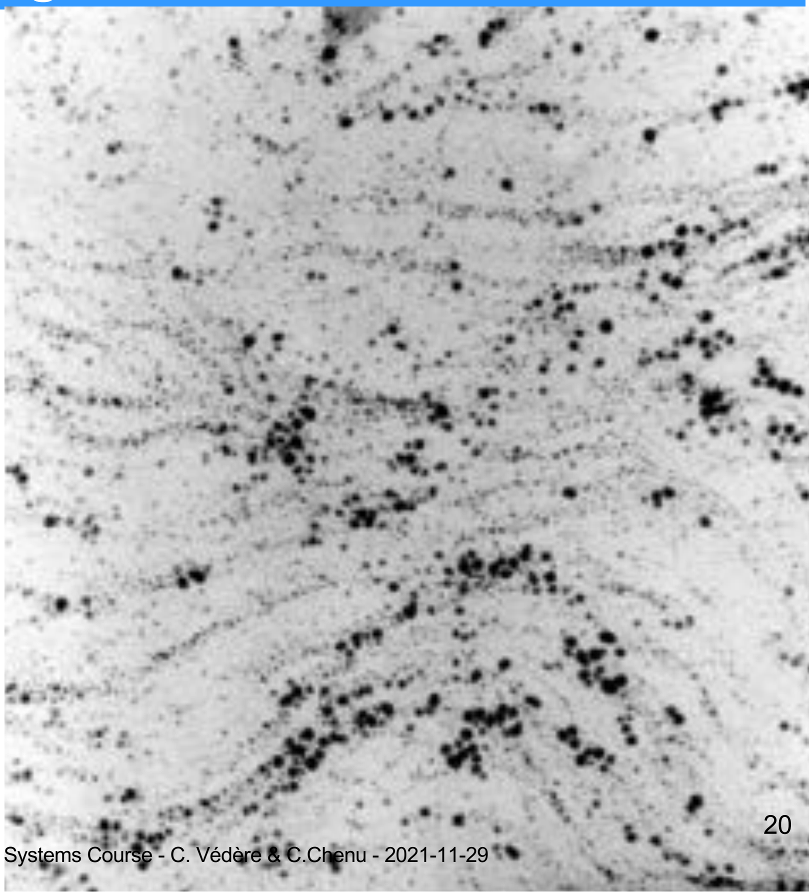


TEM: staining organic matter to visualize it

Fungal polysacharide
pF2, 0.01MPa
w≈ 20g/g (gel)

Embedded in epoxy resin
Thin sections
Thierry staining

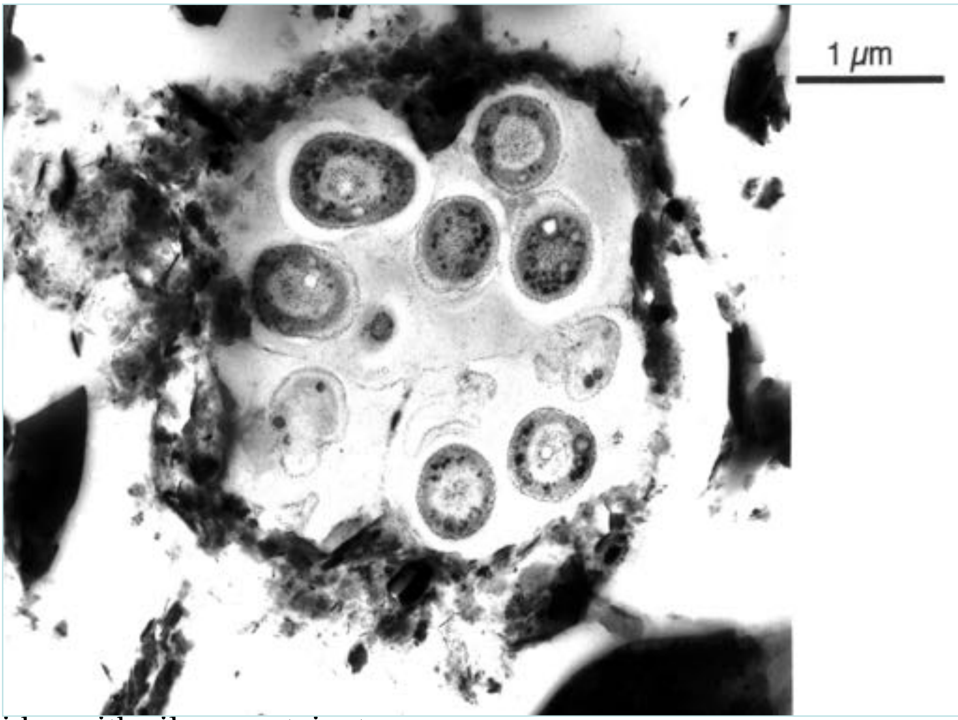
0.1 µm



Chenu & Jaunet, 1992

EJP SOIL European Joint Programme INRAE

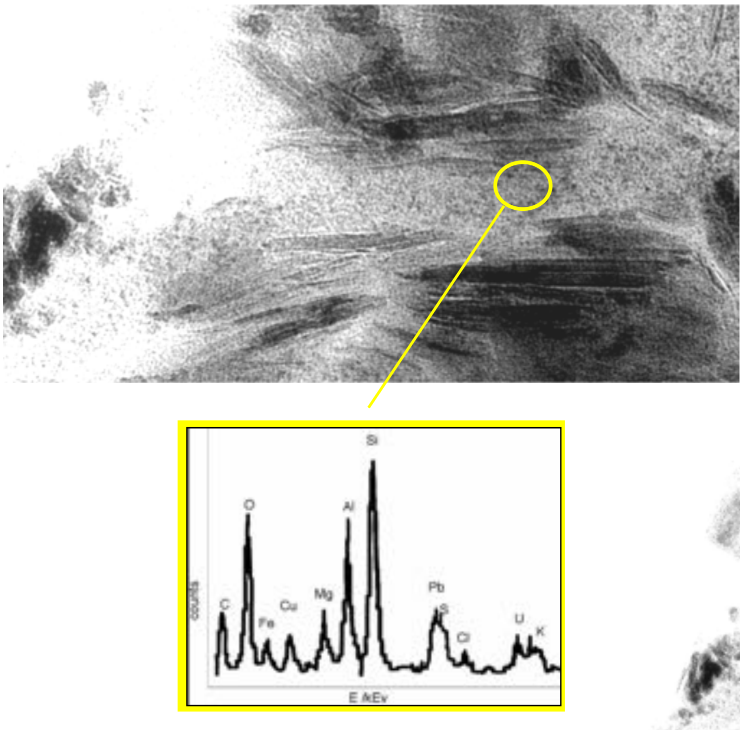
Visualizing the microbial habitat : Bacterial microaggregate



TEM
Staining of polysaccharides with silver proteinate

Robert & Chenu, 1992

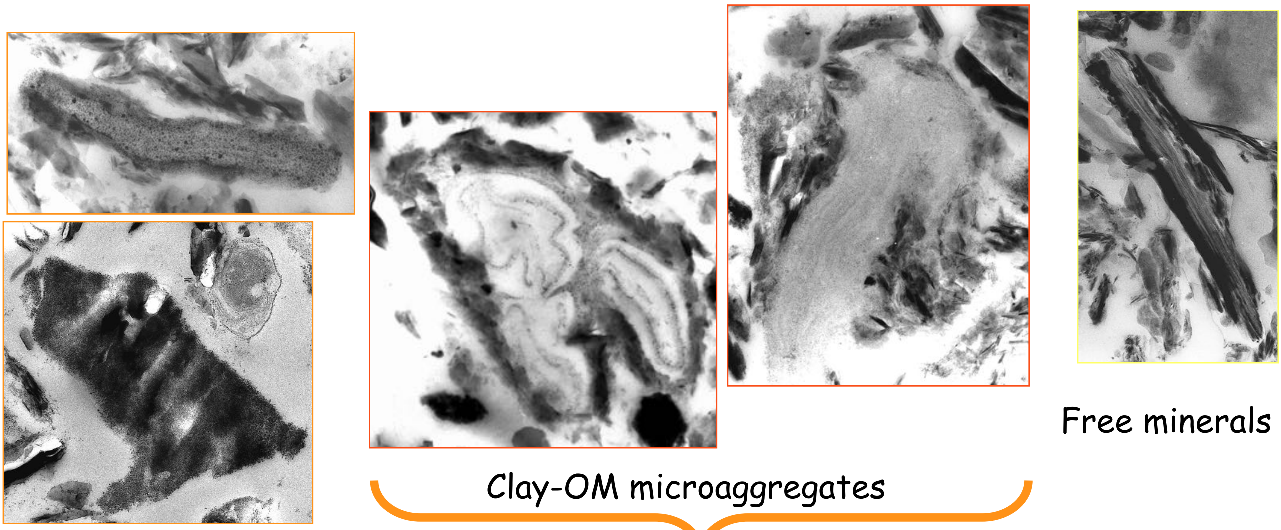
Clay-OM complex



Chenu & Plante, 2006, EJSS

TEM Clay sized organo-mineral complexes

- <2 µm size fraction after complete dispersion of soil



Free minerals

Clay-OM microaggregates

Free OM

Clay-bound OM

F< 2 µm

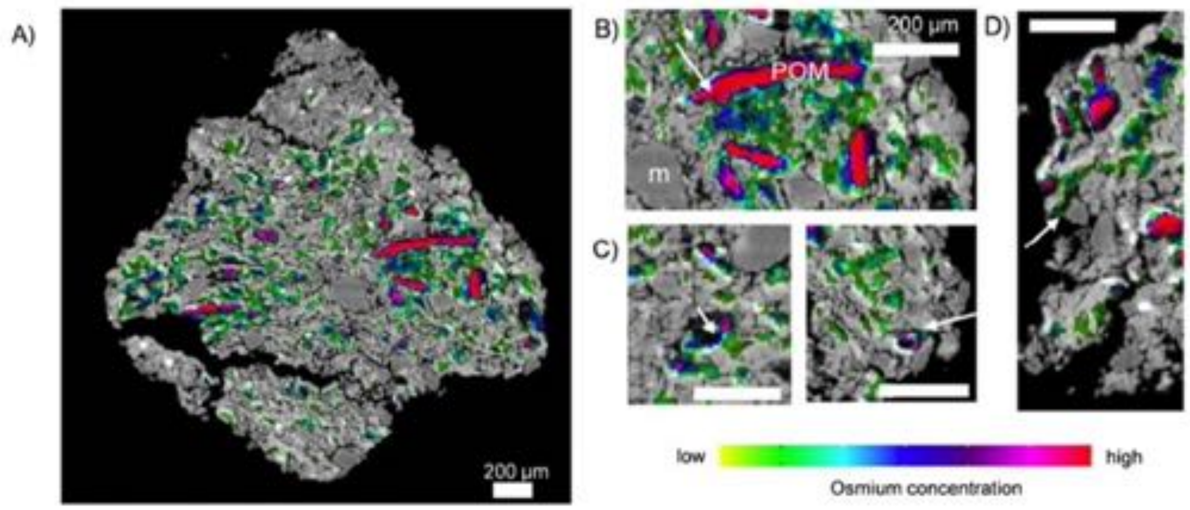
Chenu & Plante, 2006

1 µm

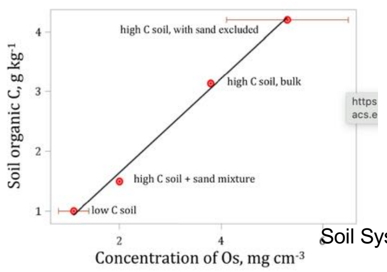
OM in clay-sized fraction is morphologically heterogeneous

Staining organic matter to visualize it in CT tomography

Os	N=76	Osmium tetroxyde	Unsaturated bonds: C=C aliphatic, aromatic and heterocyclic



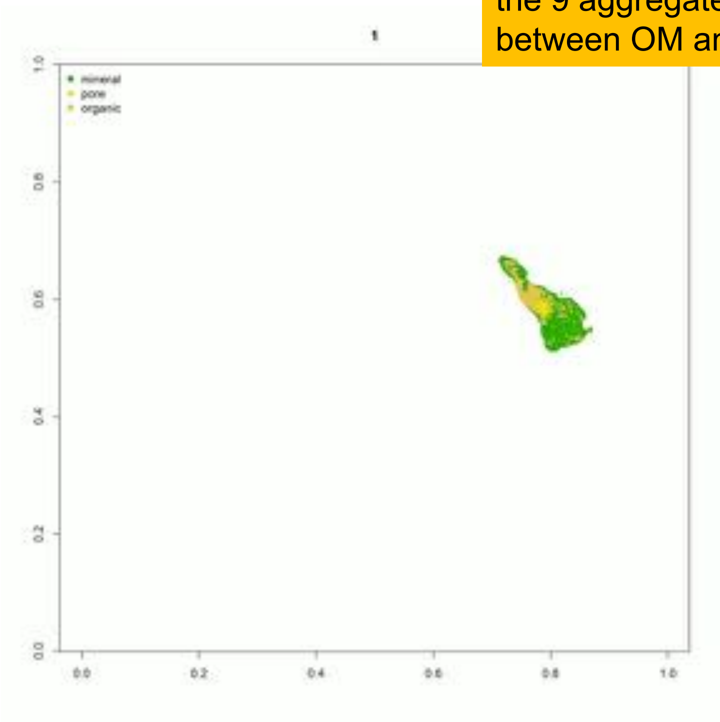
Peth et al., 2014 SBB



Zheng et al., 2020

Mapping soil organic matter in an aggregate

No correlation between the respiration rate of the 9 aggregates and transition probability between OM and pore voxels

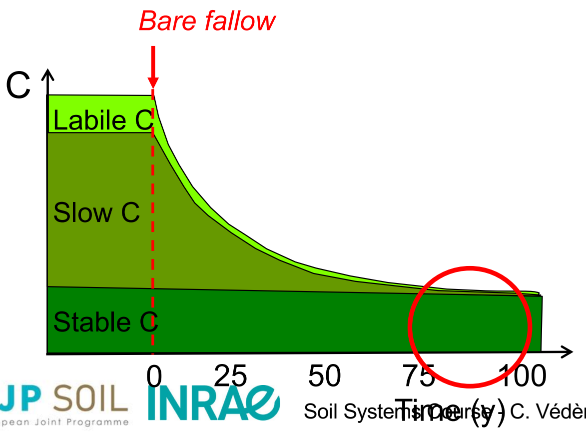


Rawlins et al. 2016 Soil

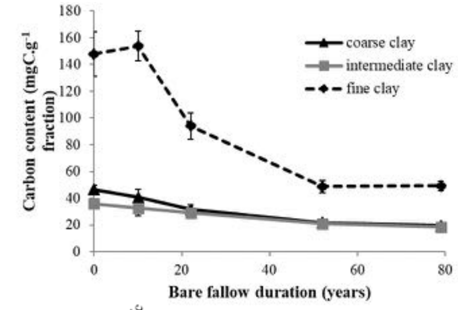
DOI [10.5446/18549](10.5446/18549)

Long term persistence of C in soils

Long term bare fallow, Versailles, since 1928

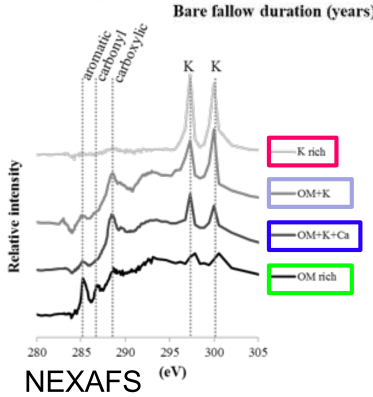


Long term persistence of C in soils: investigations at the submicron scale

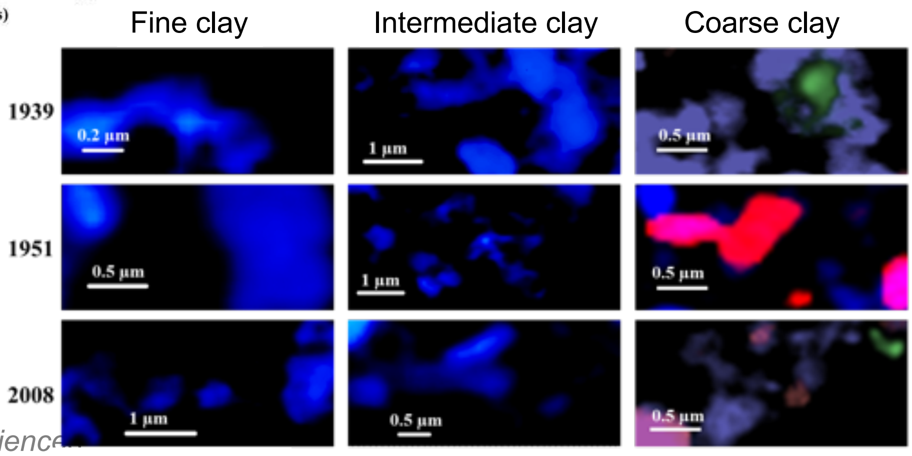


0.2-2 µm, Illite, kaolini
0.05-0.2 µm, Illite, (ka
<0.05 µm, interstratifie

- N-rich OM
- No change in average composition
- µPOM in coarse clay
- Illites loose their C
- smectite clay-C remain



NEXAFS



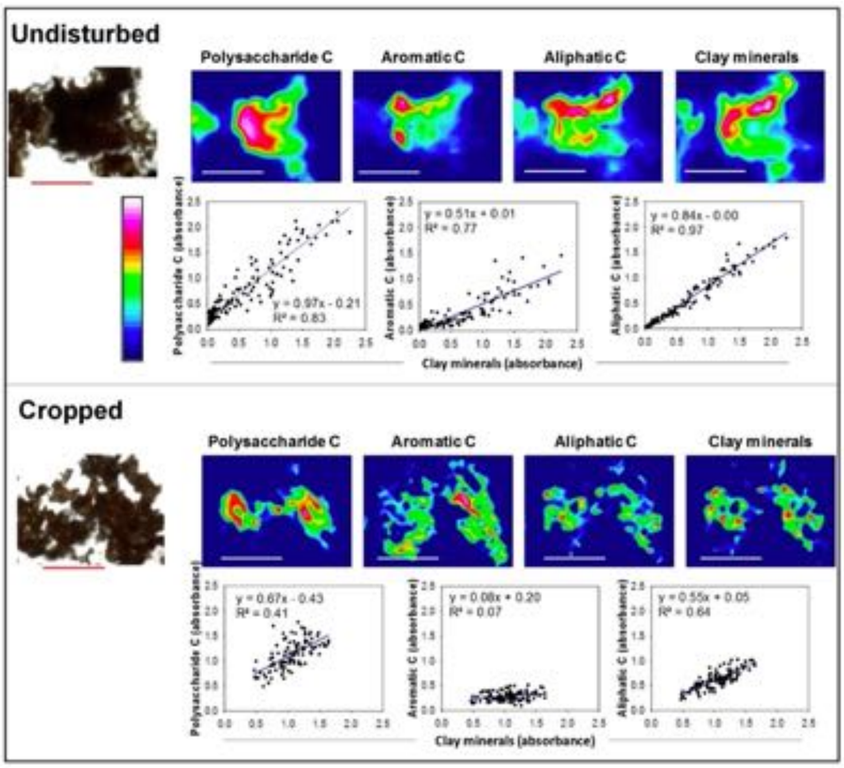
STXM-NEXAFS

OM+K+Ca OM+K OM rich K rich

Lutfalla et al., 2019, Biogeosciences

Synchrotron-based infra-red microspectroscopy

Thin sections of oxisol microaggregates



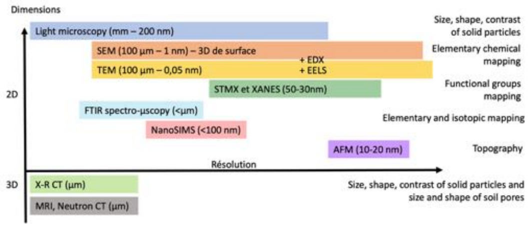
Hernandez-Soriano, et al.., 2018. EST

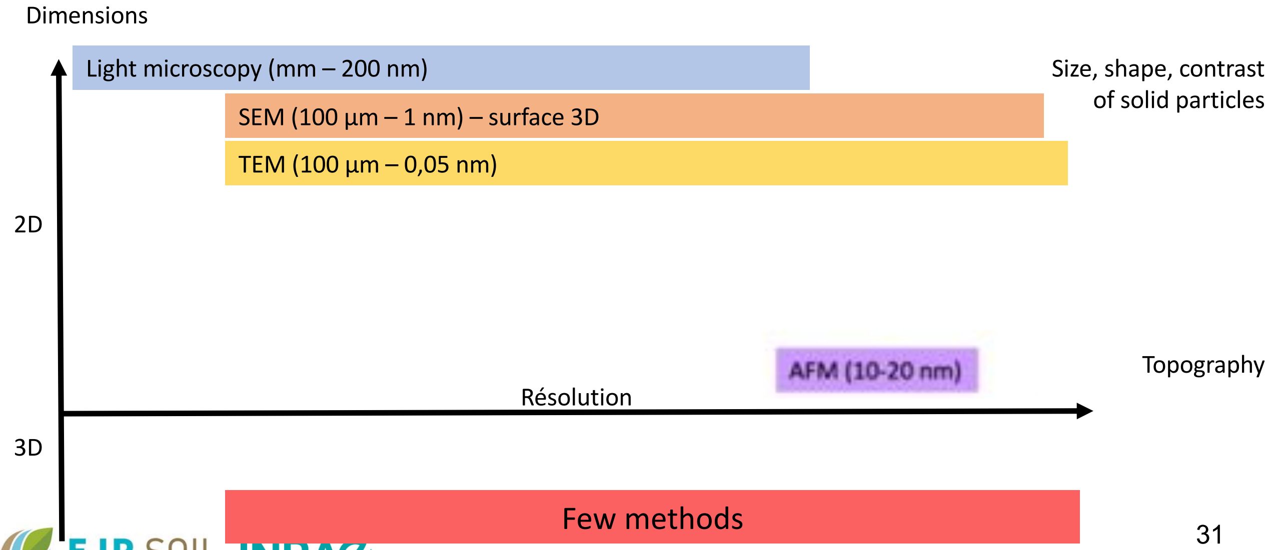
Information on soil organic matter

					Organic matter		
category	name	principle and radiation used	image	resolution	shape	C map	functiona l groups
Ligth microscopy	Stereomicroscopy	incident light	3-D	≈ 10 µm	x		
	Ligth microscopy bright field	transmitted light	2-D	< 1 µm	x		stains
	Epifluorescence microscopy	transmitted light -> fluorescence	2-D	0.2 µm	x		stains
	Confocal (laser) microscopy	transmitted light -> fluorescence	3-D	0.16 µm	x		stains
Electron microscopy	Scanning electron microscopy (SEM)	reflected electrons	3-D	10 nm	x	x	
	Transmission electron microscopy (TEM)	transmitted and diffracted electrons	2-D	0.2 nm	x	EDX	EELS
X-ray spectro microscopy	Soft X-ray spectro microscopy in the water window (STXM)	transmitted X-rays	3-D	30 nm			NEXAFS
InfraRed spectroscopy	VNIR	absorbed IR light	2-D	50 µm			x
	Raman microscopy	difused monochromatic light	2-D	µm			x
	FTIR microscopy	absorbed IR light	2-D	µm			x
Secondary ion mass spectrometry	nanoSIMS	ions beam -> sample ions collected	2-D	150 nm		x	
X-ray computed tomography	X-ray µCT	attenuation of transmitted X-rays	3-D	1 µm	x	stains	
Scaning probe microscopy	Atomic force microscopy (AFM)	cantilever scans the surface of the sample	3-D	1 nm	x		

2- Localizing and identifying inhabitants : micro-organisms

Visualisation of microorganism



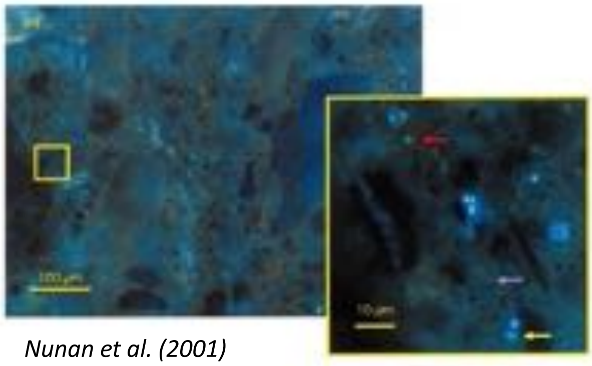


Visualisation of microorganism

Methods	Resolution	Visualisation	Size	Dimension	References
Light microscopy	mm – 200 nm	Shape, contrast (+ staining)	cm	2D	Otten et al. (2004); Thompson et al. (2005)
Fluorescence microscopy	mm – 200 nm	Shape, contrast (+ staining)	cm	2D or 3D (confocal)	Postma and Altemüller (1990); Baschien et al. (2001); Nunan et al. (2001); Schmidt et al. (2018); Juyal et al. (2020)
Scanning electron microscopy (SEM)	100 µm – 1 nm	Shape, topography	mm – cm	Surface 3D	Gaillard et al. (1999); Chenu et al. (2001); Schmidt et al. (2012); Zumstein et al. (2018); Witzgall et al. (2021)
Transmission electron microscopy (TEM)	100 µm – 0.05 nm	Shape, contrast (+ staining)	mm – cm	2D	Chenu and Plante (2006); Vidal et al. (2016)
Atomic force microscopy (AFM)	10 – 20 nm	Topography, resistance	mm	Surface 3D	McMaster (2012); Huang et al. (2015)

Visualisation of microorganism

Light microscopy → few contrast
Epifluorescence/confocal + fluorescent stain



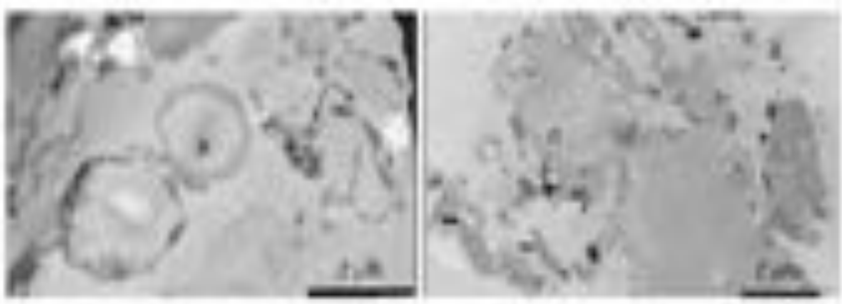
Nunan et al. (2001)

Target	Stains	Acquisition	References
Nucleic acids	Acridine orange, SYBR Green I, DAPI, Europium chelate	Epi-fluorescence	Postma and Altemüller (1990); Schlüter et al. (2019)
Proteins	FITC		Chen et al. (2007)
Polysaccharides	Phenol aniline blue, Phenolic tryptophan blue, DTAF, Calcofluor White		Morgan et al. (1991); Nunan et al. (2001); Chen et al. (2007)

Visualisation of microorganism

Scanning electron microscopy

Transmission electron microscopy



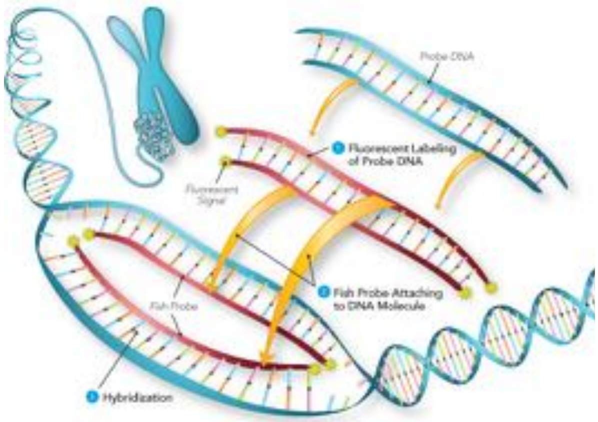
<u>Little contrast</u>

Chenu & Plante (2006)

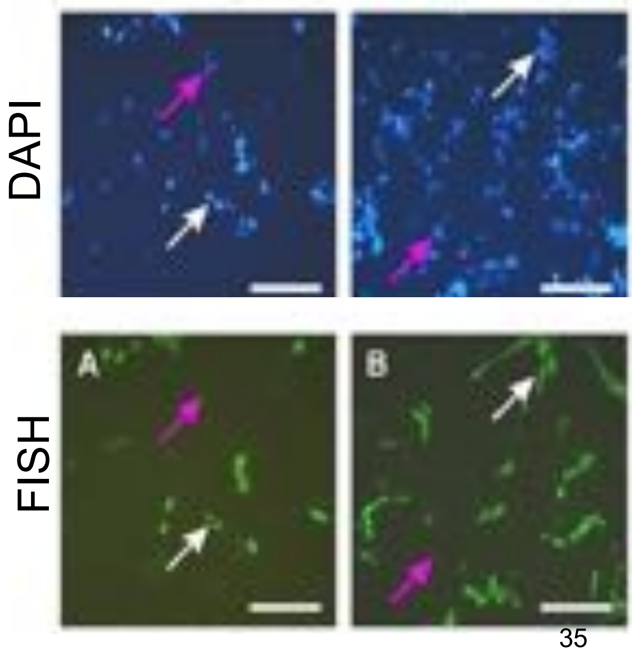
Visualisation of microorganism

Target and identifying microorganisms

Fluorescence In situ Hybridization (FISH)
→ use an oligonucleotide probe coupled to a fluorescent marker which binds to a specific sequence of RNA or DNA within the microbial cell.



1 – DAPI : total µ
2 - µ stained with FISH : targeted µ

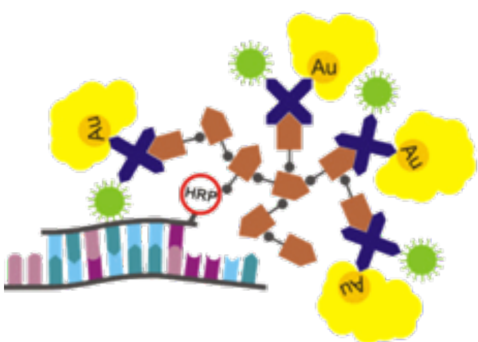


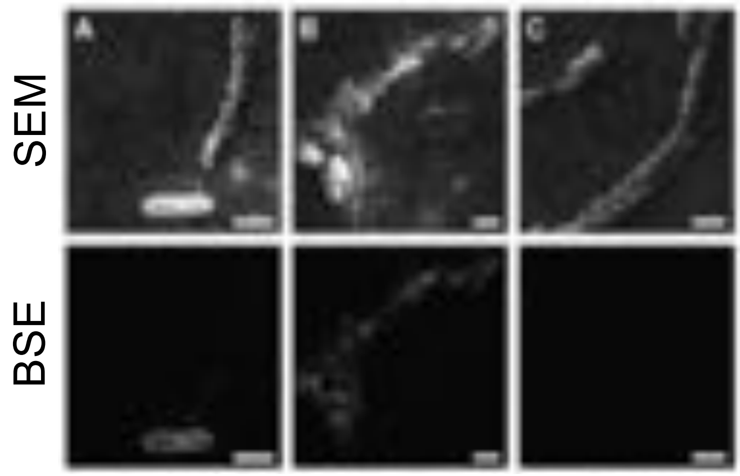
Schmidt et al. (2012)

Visualisation of microorganism

Target and identifying microorganisms

Electron microscopy → few contrasts
Gold-Fluorescence in situ hybridization
→ FISH + gold





Schmidt et al. (2012)

3- Microscale information on the physiological state and activity of soil microorganisms

Microbial activities

Physiological state:

Fluorescence microscopy + marqueur spécifique (dead/dormant/actives)
(Blagodatskaya & Kuzyakov, 2013)

Physiological state	Stains	Aquisition	References
Dead microorganisms with altered membranes	Propidium iodide (PI)	Epi-fluorescence or confocal microscopy	Maraha et al. (2004)
Active microorganisms showing enzymatic oxidation or hydrolysis by active cells	2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC),		Maraha et al. (2004); Busse et al. (2009)
	Fluorescein Diacetate (FDA)		Maraha et al. (2004)
Dormant microorganisms	Total – (Active + Dead microorganisms)		

Microbial activities

Assimilation:

Informations	Methods	Acquisition methods	References
Assimilation: **Metabolically active microorganisms**	Autoradiography	Light and electron microscopy	Lee et al. (1999); Ouverney and Fuhrman (1999); Holz et al. (2019); Becker and Holz (2021)
	SIMS, NanoSIMS	Scan with mass spectrometer	Cliff et al. (2002); Herrmann et al. (2007); Vidal et al. (2018)

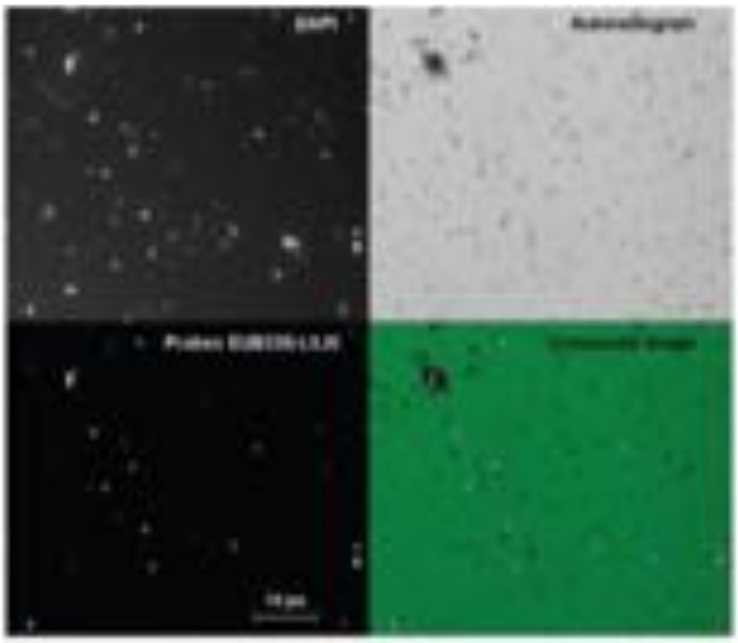
Microbial activities

Assimilation:

Autoradiography
Radioisotope labelled substrates + autoradiography.

When placed in contact with an emulsion or a photographic film the distribution of radioactive source is recorded and therefore the zones of substrate source and assimilation can be located

Micro-autoradiography
eletron microscopy

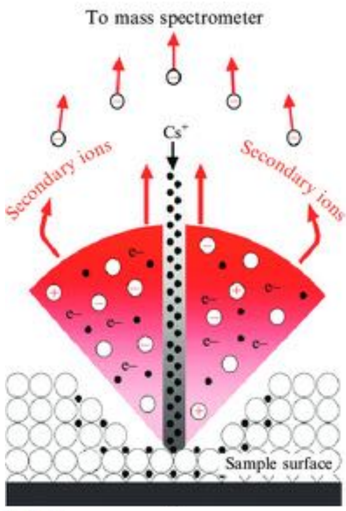


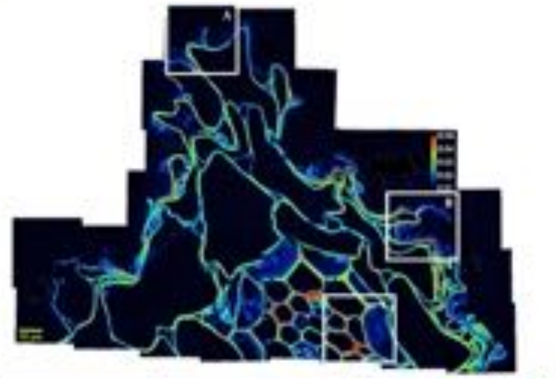
Roger et al. (2007)

Microbial activities

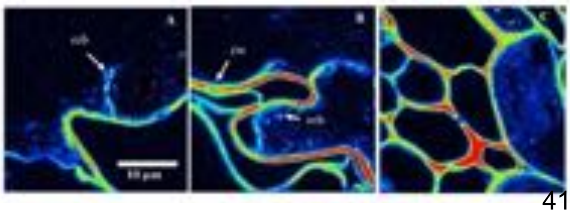
Assimilation:

NanoSIMS
Elementary and isotopic map of the soil sample
Very fine resolutions: < 100 nm





Stable isotopic label (^{13}C) of plant roots
Visualisation of the assimilation of 13C by the microorganisms in the soil *(Vidal et al., 2018)*



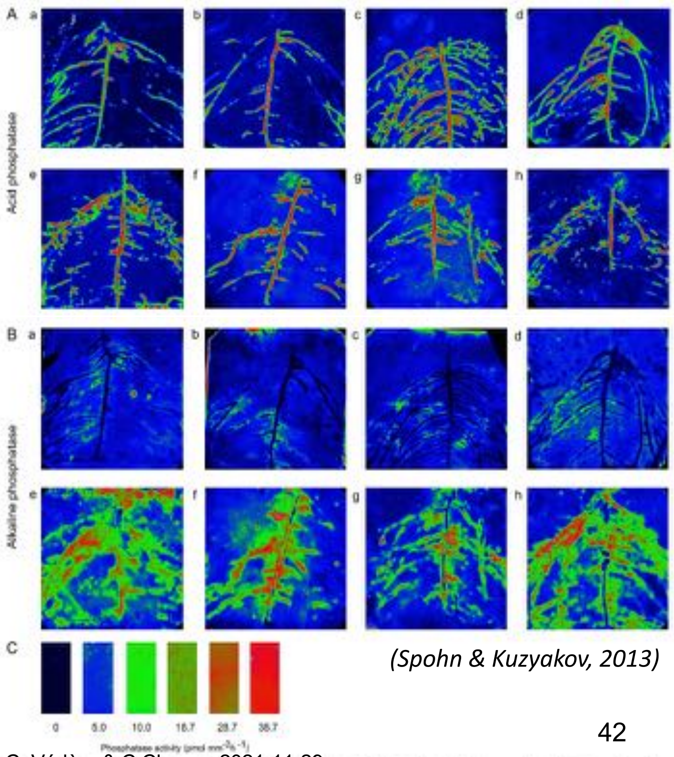
Microbial activities

Enzymatic activities:

Zymography
Spatial distribution of enzymes on the surface of a soil sample.

A gel or membrane + substrate which changes colour when it comes into contact and reacts with a specific enzyme.

Resolution: ~ tens of µm and often used at mm scale



(Spohn & Kuzyakov, 2013)

4- Visualising the biogeochemical interface

4- Visualising the biogeochemical interface

4.1- Small-scale spatial distribution of microorganisms in the detritusphere

Visualise the biogeochemical interface

Small-scale spatial distribution of microorganisms in the detritusphere

Organic matter
High spatial heterogeneity

(Chenu & Plante, 2006; Peth et al., 2014)

µ-organisms
fill a little part of soil and high spatial heterogeneity

(Chenu & Stotzky, 2002; Ranjard et Richaume, 2001; Chenu et al, 2001; Young et al., 2008)

Modulation of accessibility by:
Porosity
Contrasted colonization by µ
Soil moisture

ACCESSIBILITY → Biodegradation

(Pinheiro et al., 2015 Negassa et al., 2015; Coppens et al., 2006)

Transfers
- Advection
- Diffusion
- Growth
- Mobility

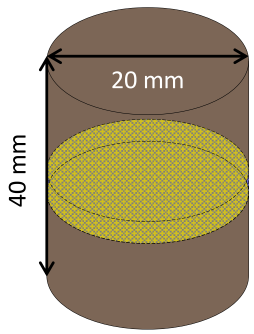
Incorporation of residues:
→ Hot spot of µ activity and biogeochemical cycling
→ Distribution gradient of µ

Water and spatial µ relation:
Diffusion and advection influence µ spatial repartition

Visualise the biogeochemical interface

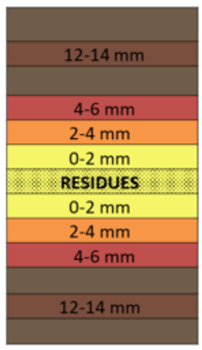
Small-scale spatial distribution of microorganisms in the detritusphere

Cosms:
Crop Luvisoil
Sieving: 2-5 mm
Matric potential:
pF1.5: Ø < 100µm
pF2.5: Ø < 10µm
pF3.5: Ø < 1µm
(Védère et al., 2020)



Detritusphere: central layer of maize residues + metal mesh (200µm)

Incubation: in closed chambers 20° C



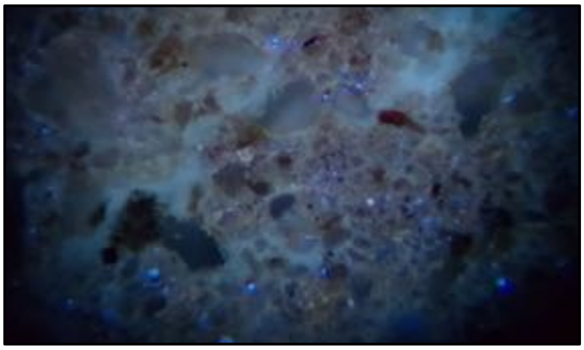
Interest zones: at increasing distance from the residues

Sampling time: day 3, 7, 15 and 45 → 36 cosms
Difficult to know when something will happen and the preparation is destructive → multiplication of samples

Small-scale spatial distribution of microorganisms in the detritusphere

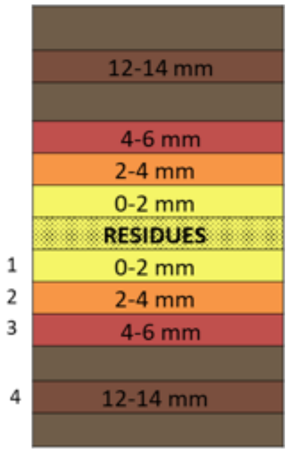
Thin sections:
Fluorescent stain: Calcofluor white (polysaccharides) *(Nunan et al., 2011)*

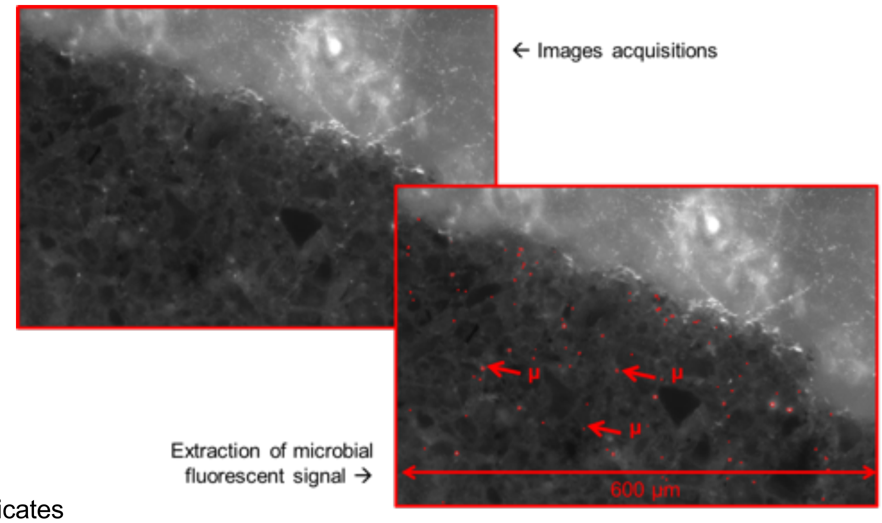
Acquisition:
Stereomicroscope: Zeiss AXIO. Zoom. V16
Objectif: PlanNeoFluar Z 2.3x
Camera: AxioCam HR R3
Lamp: Zeiss HXP 120C



Visualise the biogeochemical interface

Small-scale spatial distribution of microorganisms in the detritusphere





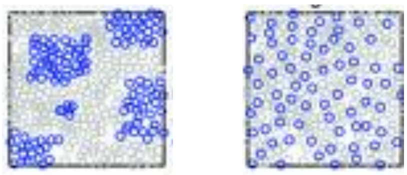
STUDIED ZONES:
4 Images / cosm x 2 replicates
Image mosaic (1862 images / mosaic)
Surface: 4mm wide x 2mm heigh
Magnification: x258
Resolution: 0,25μm

Visualise the biogeochemical interface

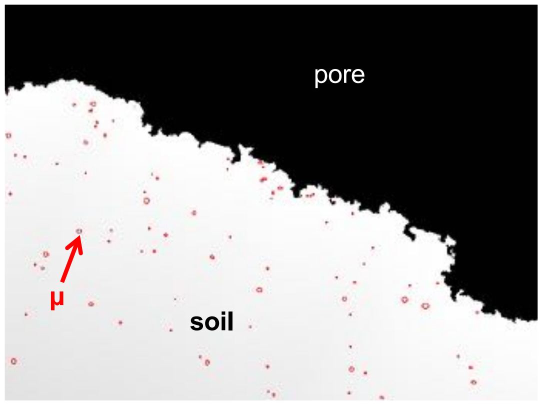
Small-scale spatial distribution of microorganisms in the detritusphere

Distribution of colonies
According to Ripley's Index
(dispersion of point)

agregated dispersed

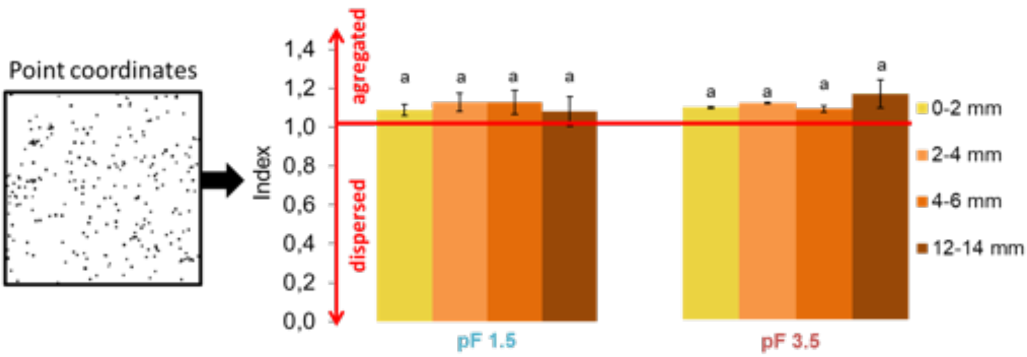


Distribution around macropores
Distribution of colonies around
macroporosity (> 0.05 mm²)



Visualise the biogeochemical interface

Small-scale spatial distribution of microorganisms in the detritusphere



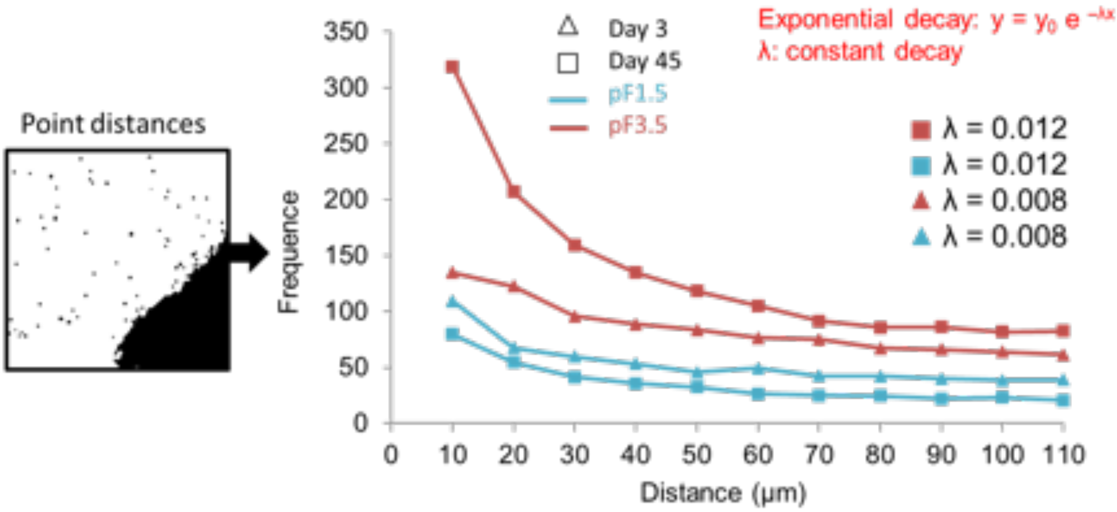
Index > 1 → distribution of colonies is agregated
Colonies show agregated tendencies but no differences between any modalities

Microorganisms dispersion can be stimulated in wet soil *(Dechesne et al. 2010)*
→ Not observed, with a very different experimental set-up (sand matrix)

More patchy organization in a deep horizon soil than surface *(Nunan et al. 2003)*
Far from the residue, poor nutrient environnement = more agregation
→ Not observed

Visualise the biogeochemical interface

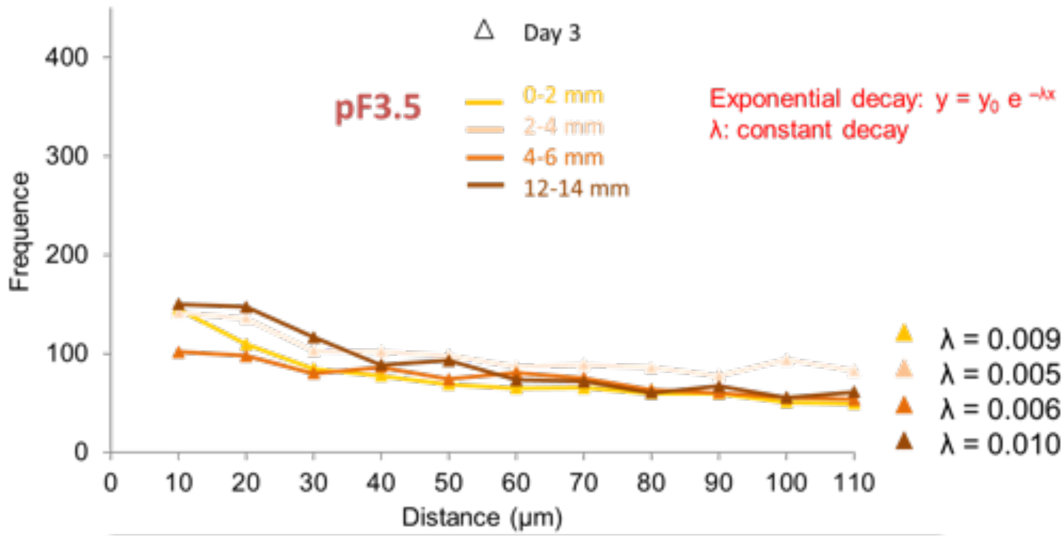
Small-scale spatial distribution of microorganisms in the detritusphere



Repartition gradients:
- Higher decrease at day 45 than day 3 for both pF
- More pronunced at pF3.5

Visualise the biogeochemical interface

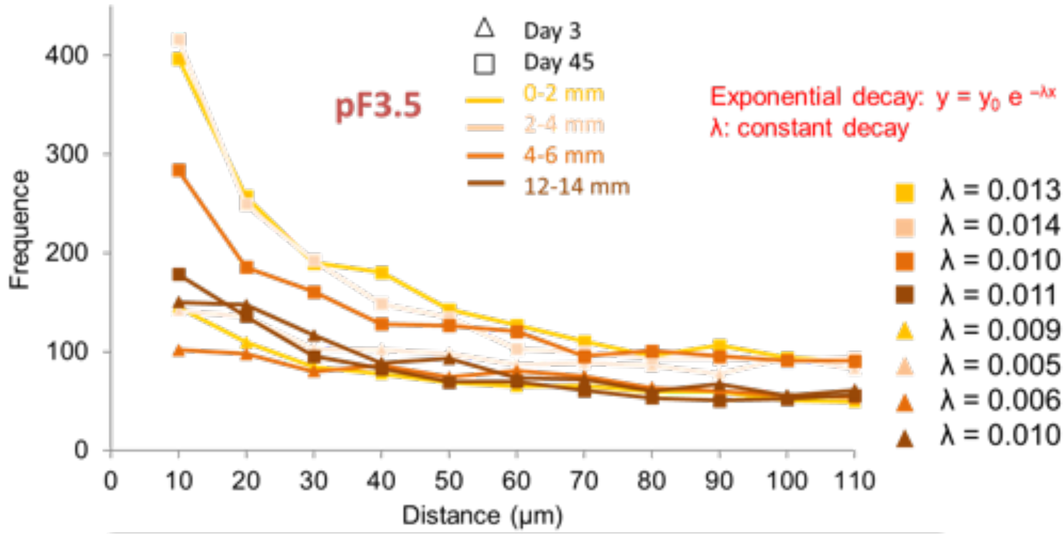
Small-scale spatial distribution of microorganisms in the detritusphere



Repartition gradients:
- Higher decrease at day 45 than day 3
- After 45 days, decrease are function of the distance of the residue

Visualise the biogeochemical interface

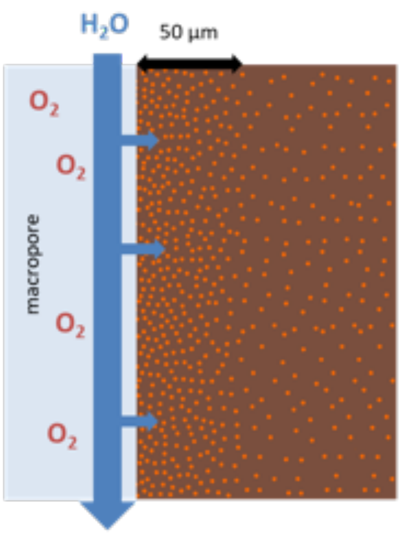
Small-scale spatial distribution of microorganisms in the detritusphere



Repartition gradients:
- Higher decrease at day 45 than day 3
- After 45 days, decrease are function of the distance of the residue

Visualise the biogeochemical interface

Small-scale spatial distribution of microorganisms in the detritusphere



Macroporosity structures µ spatial distribution.
Water saturated microporosity in the vicinity of macropores (O_2) → best localization for µ

Gradient is weaker far from residues

Gradient is weaker in humid conditions

Gradient is steeper after 45 days of incubation

Low soil moiture and soluble organic matter diffusion toward the porosity → favour the presence in time of µ at the contact of added OM

Visualise the biogeochemical interface

Small-scale spatial distribution of microorganisms in the detritusphere

Methodological questions:

→ Fluorescence microscopy is adapted to studying hotspots of microbial activity in soils

→ It allows the acquisition of information about soil microorganisms self repartition and their position according to soil porosity

Scientific questions:

→ Soil microorganisms aggregation was not influenced neither by matric potential and distance from the residues

→ Macroporosity organize µ repartition in gradients which are influence by matric potential

Other:

→ ^{13}C-labelled residues were used and could have allow to observe their in-situ biodegradation and localize the position of active-µ

- NanoSIMS acquisition has been processed but the ^{13}C was too much diluted in soil to be observed properly
- ^{13}C soil measurements allowed to observe its diffusion over 4 mm

4- Visualising the biogeochemical interface

4.2- Influence of soil structure on the spread of Pseudomonas fluorescens in soil at microscale (Juyal et al., 2020)

Visualise the biogeochemical interface

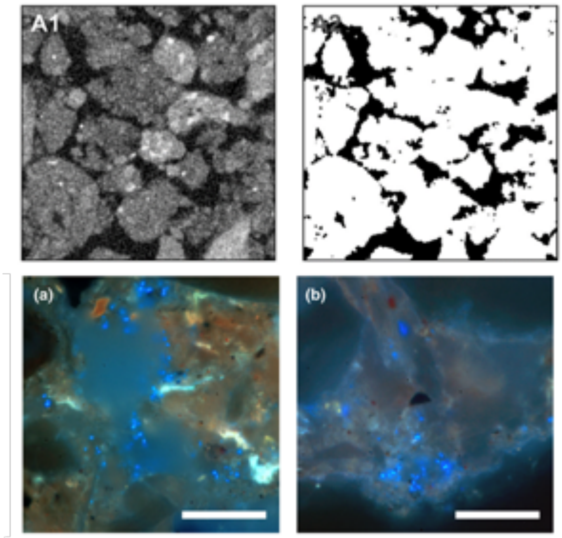
Influence of soil structure on the spread of Pseudomonas fluorescens in soil at microscale (Juyal et al., 2020)

- (1) Pore architecture
X-ray µTomography (3D)

- (2) Spatial distribution of bacteria
Fluorescence microscopie (2D)

Soil cosms with contrasted bulk densities:
1,3 et 1,5 $g.cm^{-1}$

+ bacteria inoculum



Juyal et al., 2019

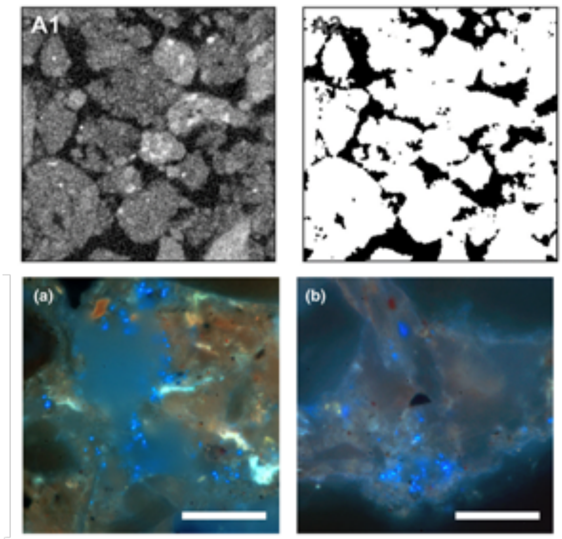
Visualise the biogeochemical interface

Influence of soil structure on the spread of Pseudomonas fluorescens in soil at microscale (Juyal et al., 2020)

(1) Pore architecture - X-ray µTomography (3D)
→ Connectivity: 1,3 > 1,5 $g.cm^{-1}$
→ pore-solid interface: 1,3 > 1,5 $g.cm^{-1}$

<u>(2)</u> Spatial distribution of bacteria - Fluorescence microscopie (2D)
BD: 1,3 $g.cm^{-1}$
→ colonies
→ Higher cell density
→ More cell diffusion

<u>(3) Relation: cells VS porosity</u>
→ pore-solid interface ↗ - cells diffusion ↗
→ Connectivity ↘ - cells diffusion ↘
→ More visible at BD:1,3 $g.cm^{-1}$



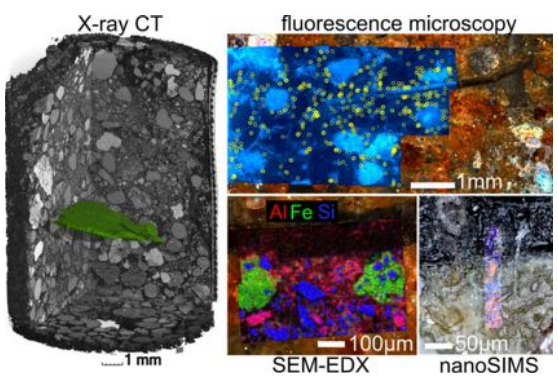
Juyal et al., 2019

4- Visualising the biogeochemical interface

4.3- Correlative Imaging Reveals Holistic View of Soil Microenvironments (Schlüter et al., 2019)

Visualise the biogeochemical interface

Correlative Imaging Reveals Holistic View of Soil Microenvironments (Schlüter et al., 2019)



- (1) Pore architecture
X-ray Tomography (3D)
- (2) Spatial distribution of bacterias
Fluorescence microscopie (2D)
- (3) Soil matrix characterisation
NanoSIMS, SEM-EDX (2D)

Conclusions - Take-home messages

Conclusion: Why do we need imaging tools?

Interests:
- Elaborate conceptual models : microbial activities, fate of OM, etc.
- Quantify soil organisation of OM and microbial repartition, etc.
- Get more reliable predictive models through a better understanding of mecanisms occuring in soil at microscale

Limits and focus:
- Acquisition strategies (destructive VS non-destructive)
- Sample preparation (material and methodological difficulties)
- Multiplication of sample in time
- Difficulties of combine methods at various scales and deep fields
- Image representativity
- Image processing anticipation

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