EXPLORATION OF THE BIODIVERSITY AND FUNCTIONS OF THE FOREST MICROBIOME : A FOCUS ON THE TEMPERATE REGIONS

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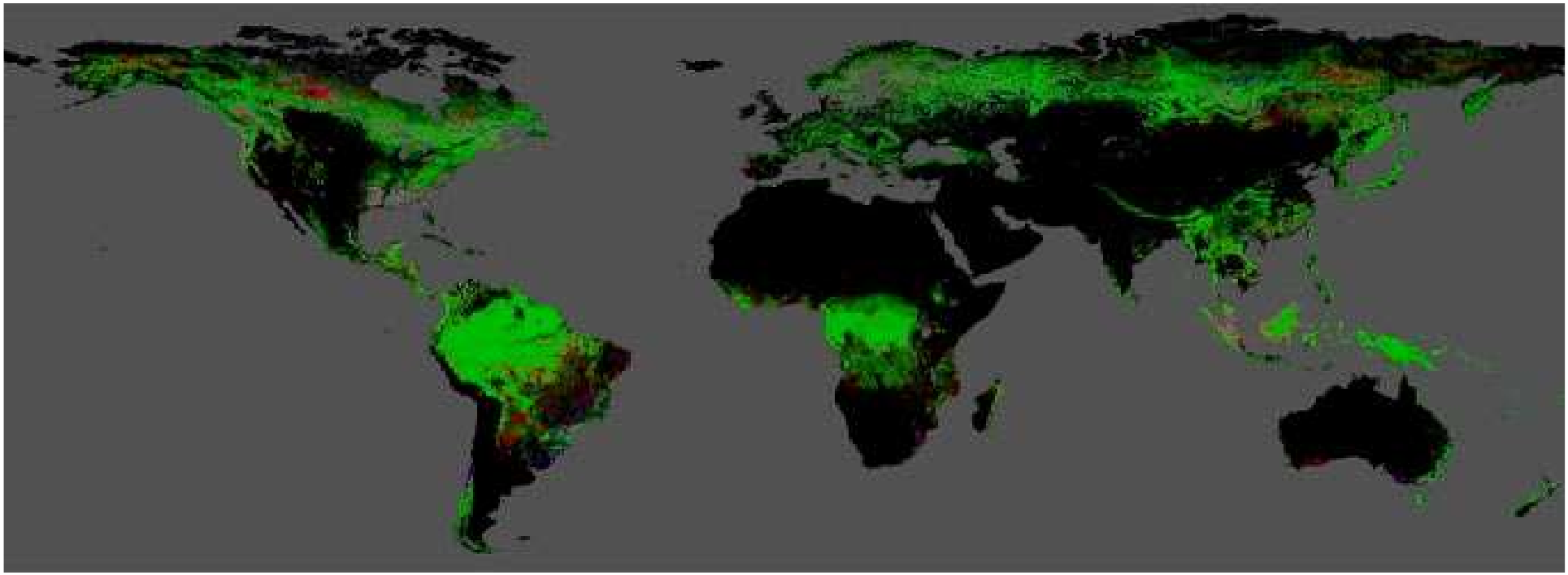
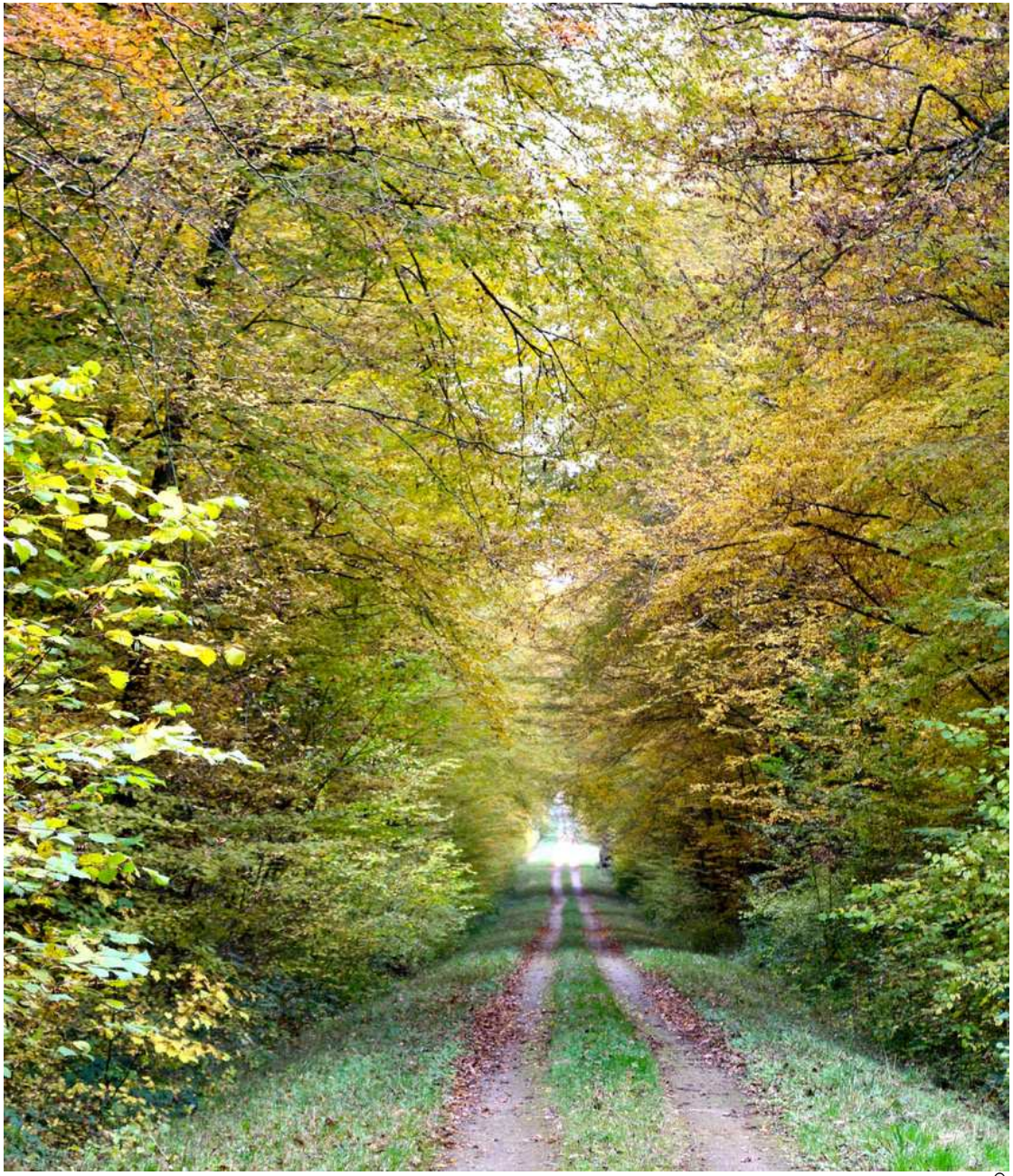
WHY TO FOCUS ON FOREST ECOSYSTEMS ?



Hansen *et al.*, Science 2013

30 % of the terrestrial ecosystems

WHY TO FOCUS ON FOREST ECOSYSTEMS ?



F. Martin®

Several ecosystem services:

A reserve of biomass and energy ?

Water cycling and air quality?

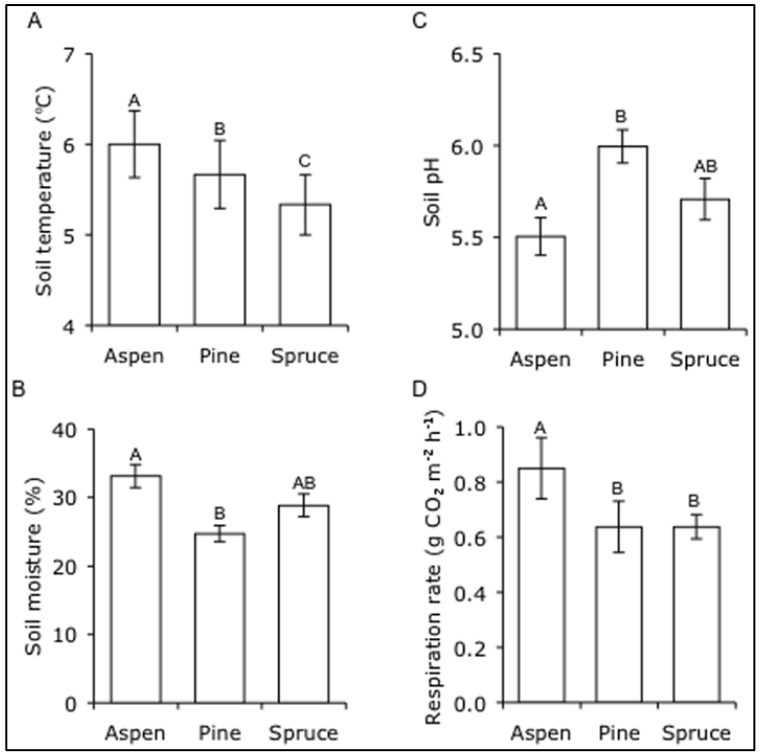
A cultural and recreational use?

A reservoir of biodiversity ?

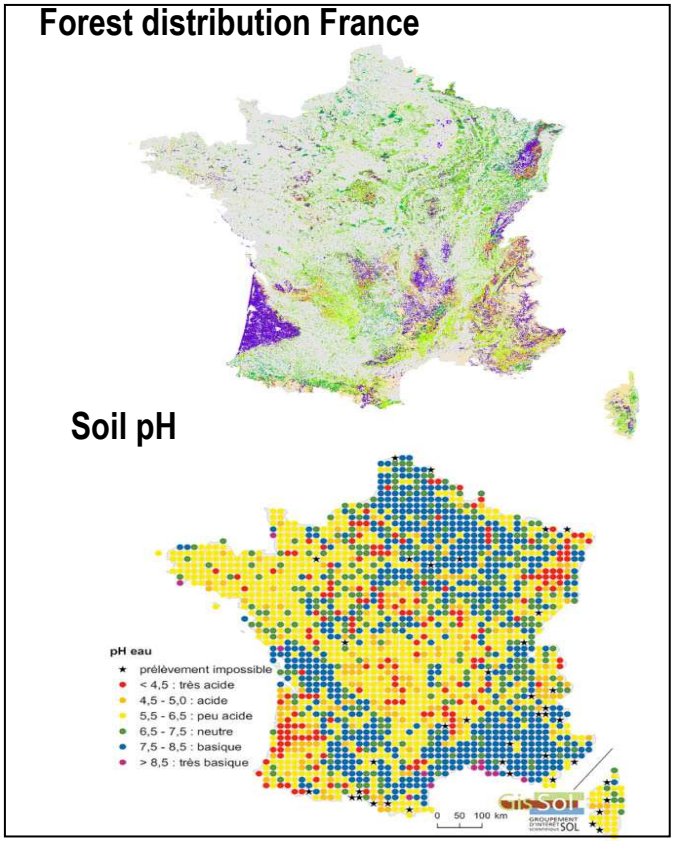
TWO IMPORTANT DIFFERENCES WITH OTHER TERRESTRIAL ECOSYSTEMS

Presence of perennial plants: the trees (40 to 120 years)

Long term impact on their environment

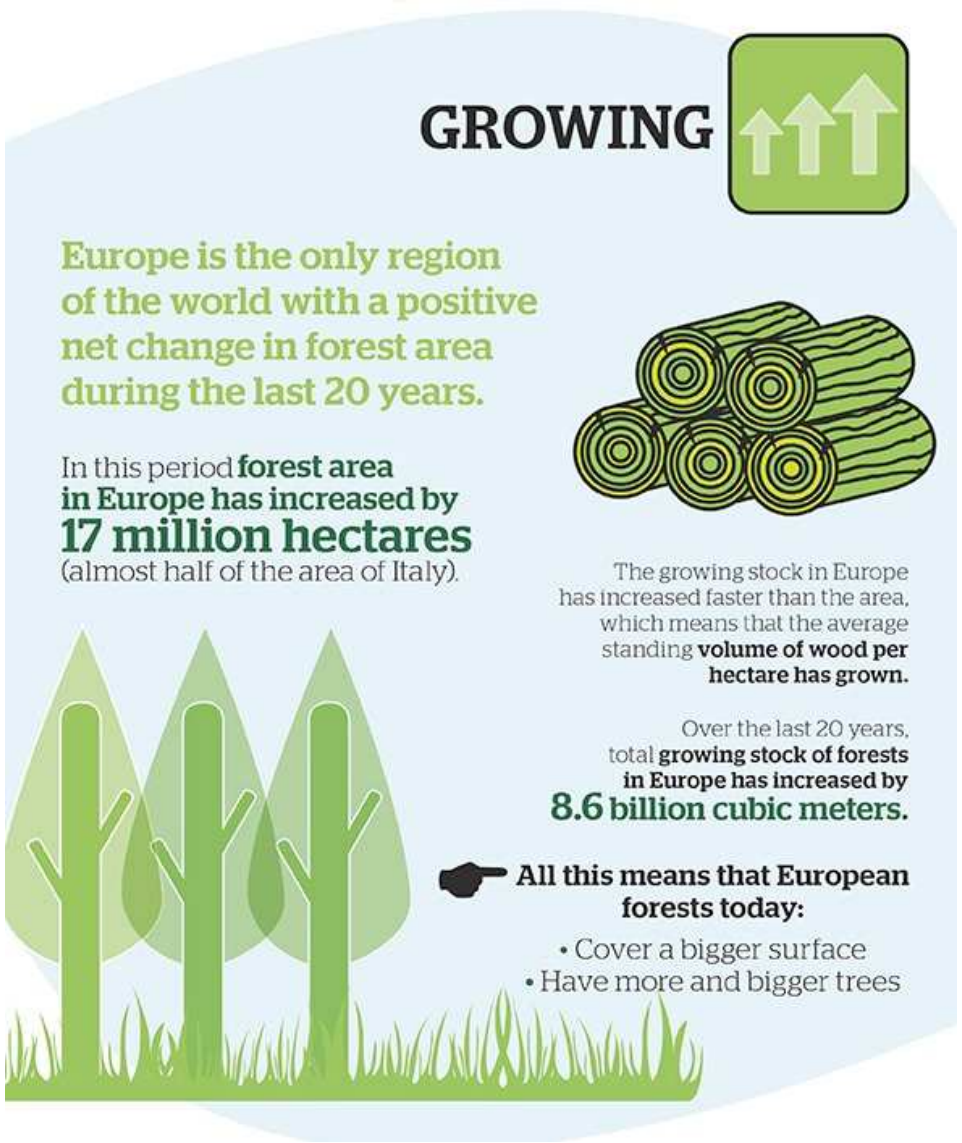


Distribution on nutrient-poor soils
(usually non fertilized, except in certain regions or countries..)



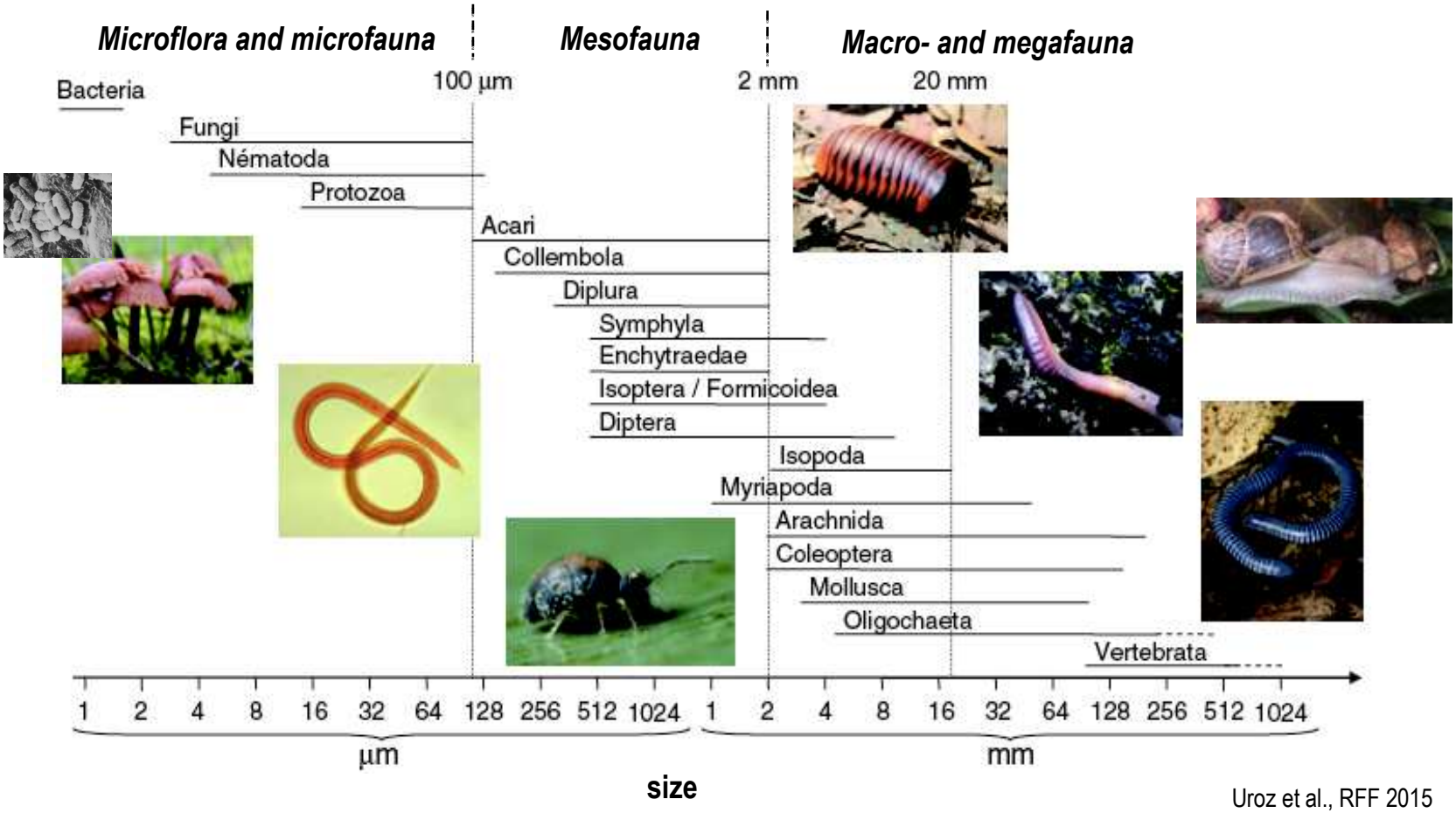
Forest ecosystems: perennial plants (need longer time to grow and to establish), nutrient-poor and rocky soils (usually non amended), forest exploitation (intensification of forest harvest, use of heavy machines, tree species choice)

IMPORTANCE TO MANAGE A GROWING RESOURCE IN EUROPE



FAO

WHAT WE CAN SEE AND WHAT WE DO NOT EXPECT



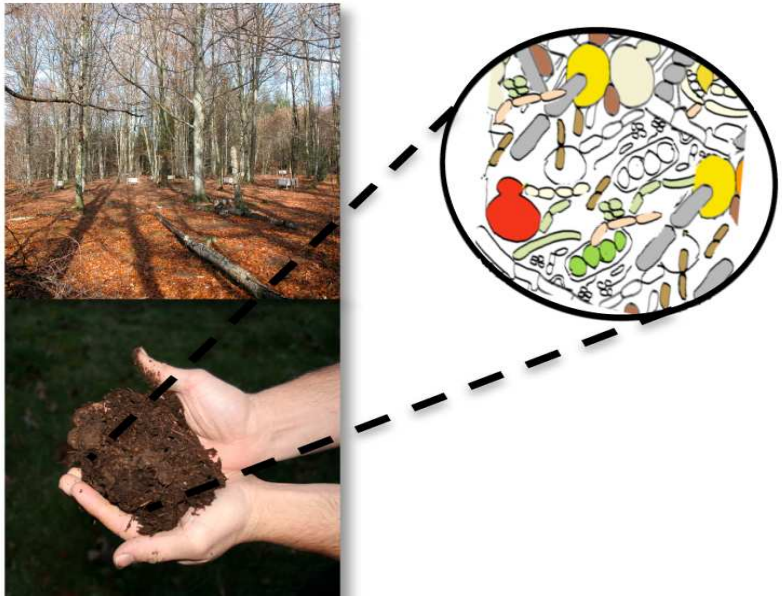
Uroz et al., RFF 2015

size

Diversity

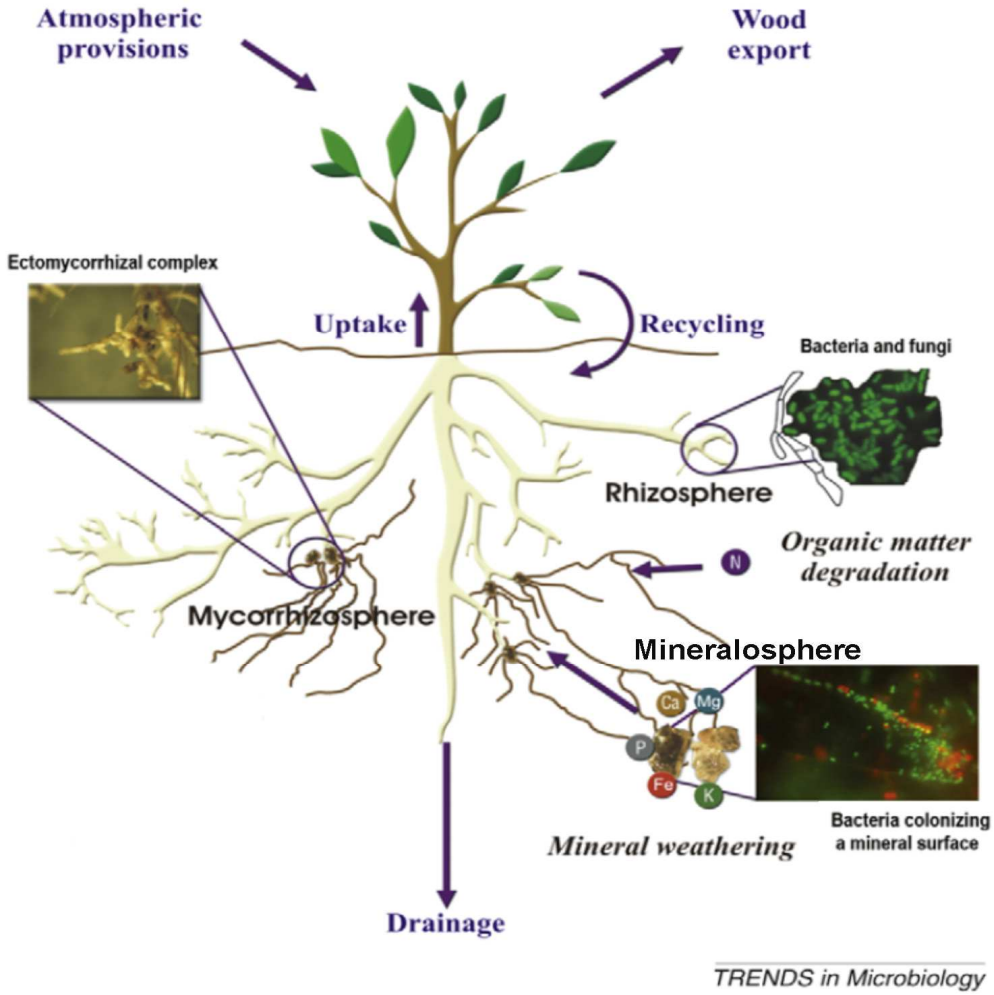
All participate in nutrient cycling, tree nutrition and health…
Through their direct actions and their complex interactions…

WHICH DIVERSITY? WHICH FUNCTIONS FOR SOIL MICROORGANISMS?



Determining the diversity of all these organisms, their functions and their interactions will permit to better understand ecosystem functioning

A FOCUS ON THE DIVERSITY AND THE ROLE OF FOREST SOIL MICROBES



Archaea, **bacteria, fungi**

Organic matter decomposition
N cycling
Mineral wethearing

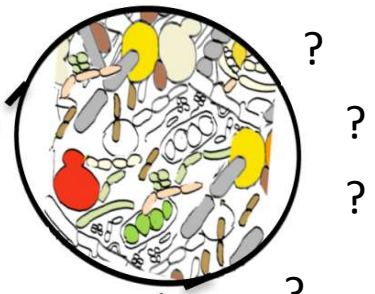
Improve nutrient access, tree nutrition and health

WHICH IMPACT OF GLOBAL CHANGES ON FOREST SOIL MICROORGANISMS?

Land use

Forestry practices and intensification

Climate change



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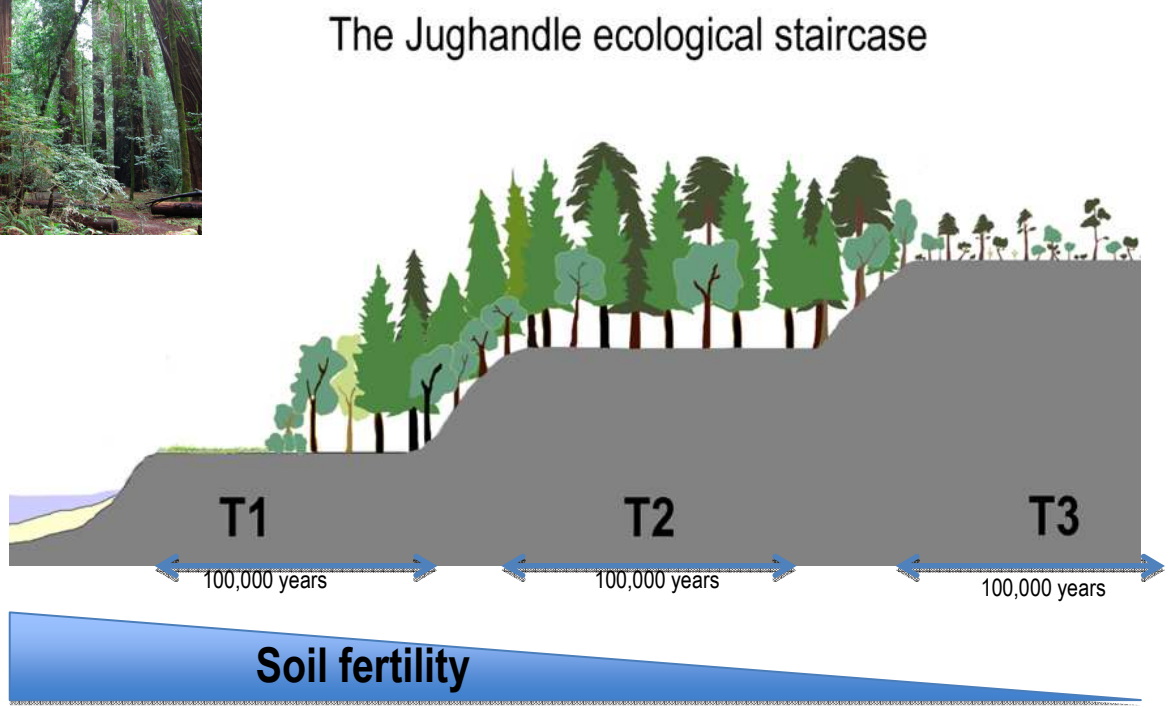
ENVIRONMENTAL RETROGRESSION: A CASE STUDY

Impact of soil age (fertility)

Every 100,000 years a new terrace
constituted of the same mineral parental material

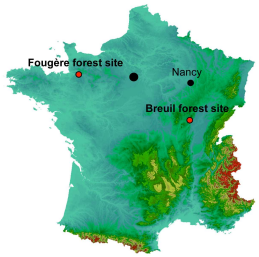
'Normal' forest

Pygmy forest

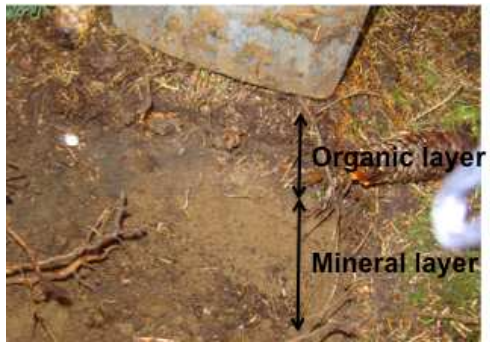


The soil in relation to its fertility level can strongly impact the land cover (retrogression stage)

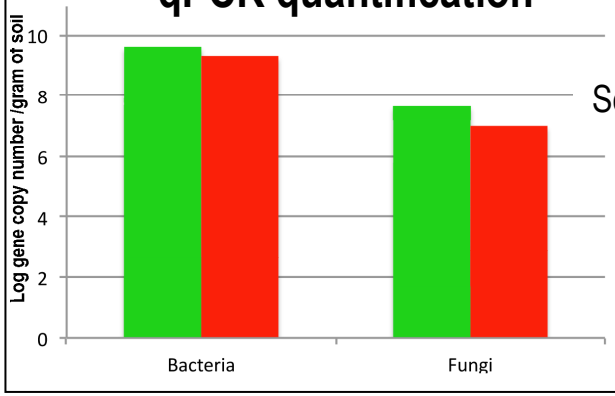
WHICH MICROBIAL DENSITIES IN FOREST SOIL ?



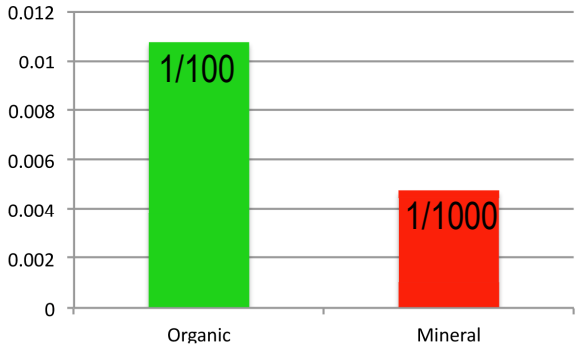
Example: A Norway spruce stand (Breuil-Chenue, Morvan)



qPCR quantification

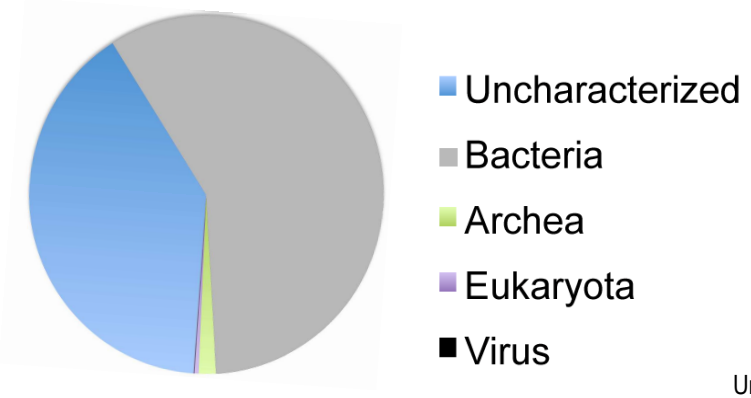


Ratio F/B



Shotgun metagenomic

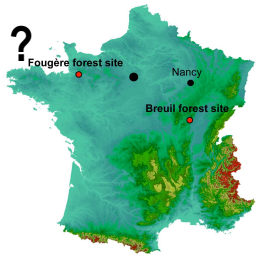
(454 pyrosequencing and Illumina directly on DNA)



Uroz *et al.*, PlosONE 2013

Quantitative and qualitative differences between bacteria and fungi, and between soil horizons…

DO TAXA AND FUNCTIONS UNIFORMELY DISTRIBUTED ACROSS SOIL HORIZONS ?

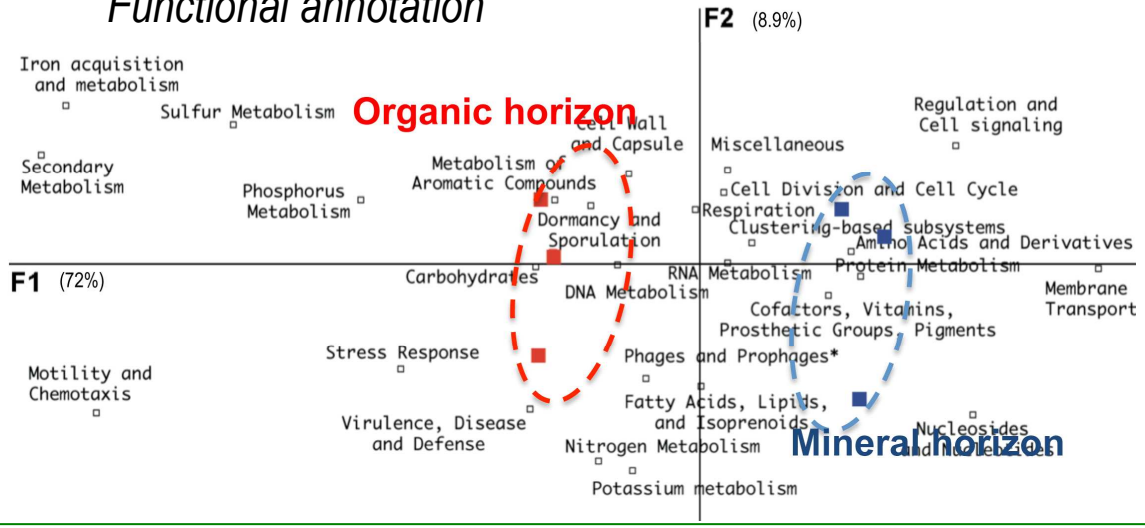


Phylogenetic annotation

DOMAIN		PHYLUM	
O>M	M>O	O>M	M>O
*Bacteria***	*Archaea****	*Proteobacteria***	*Firmicute***
*Eukaryota***		*Bacteroidetes***	*Chloroflexi****
		*Verrucomicrobia***	*Deinococcus-Thermus****
			*Euryarchaeota***
		*Chordata***	*Thaumarchaeota****
			*Crenarchaeota****
		*Ascomycota***	*Chlorobi**
			*Thermotogae**
		*Arthropoda**	*Aquificae***
			*Dictyoglomi****
			*Fusobacteria***

Specific microbial taxa are enriched in relation to the soil properties

Functional annotation

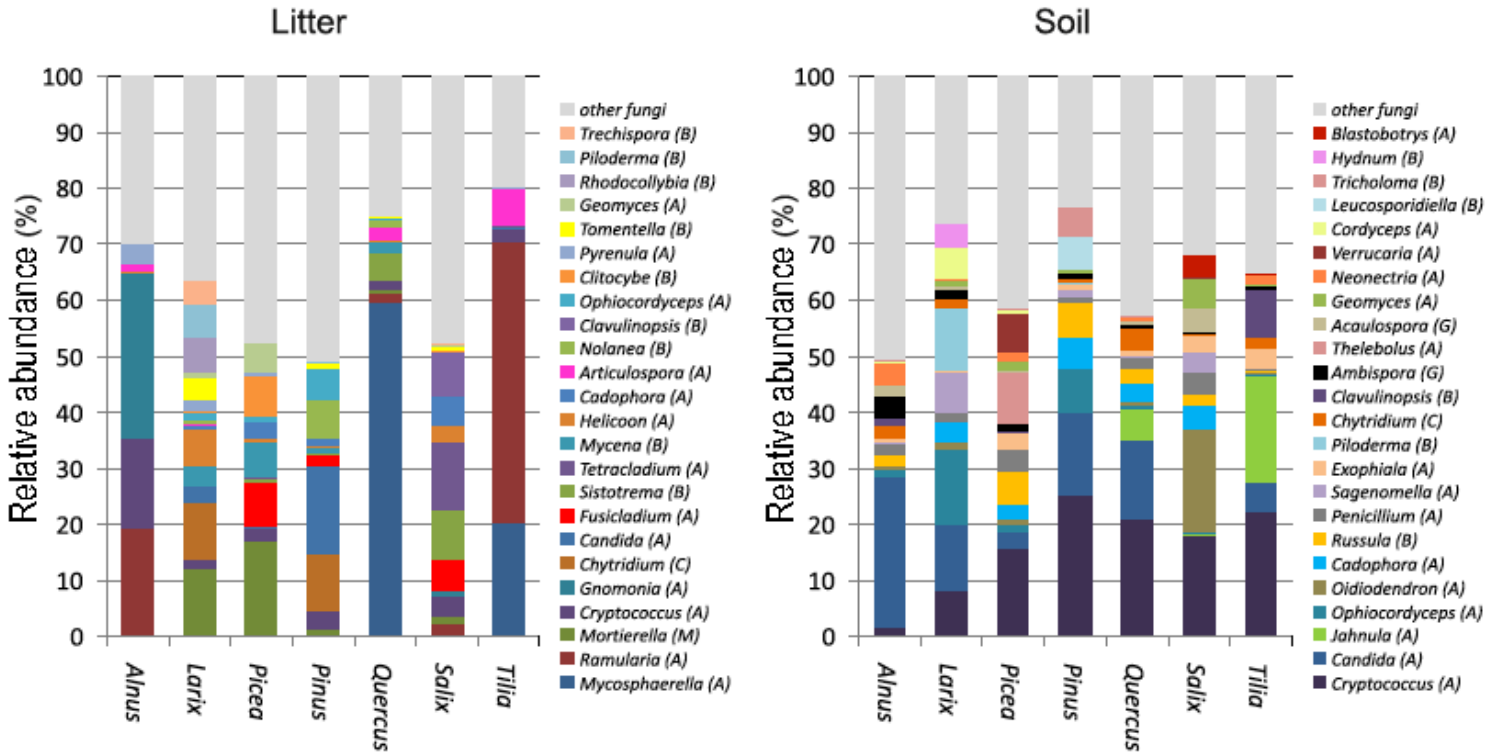


Specific microbial functions are enriched in relation to soil properties

Uroz *et al.*, PlosONE 2013

DO MICROBES IMPACTED BY THE TREE SPECIES ?

Relative composition of the fungal communities in the soil and in the litter below different tree species

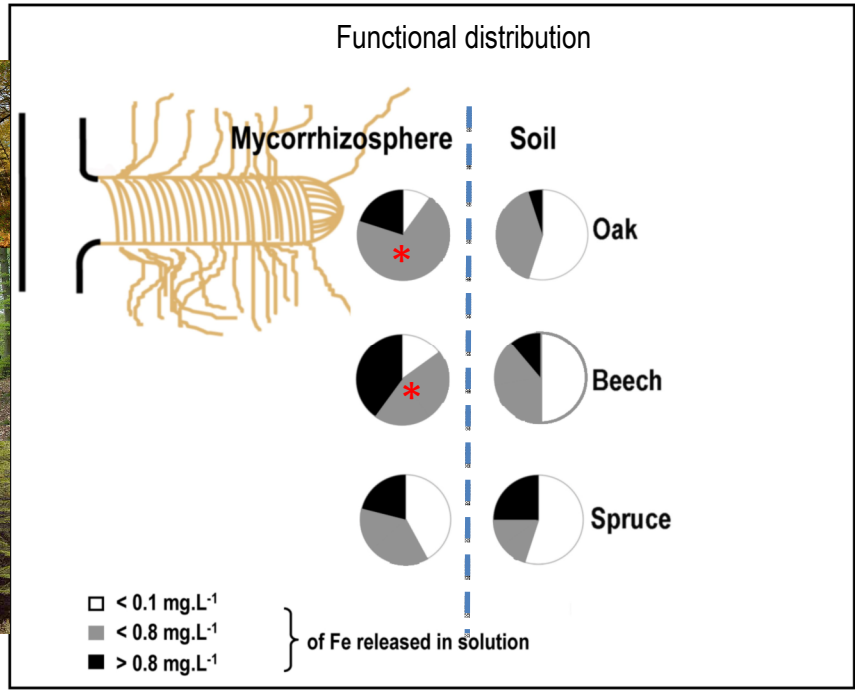


Urbanova et al., SBB 2015

- For a same tree species, similar diversity and richness values in the litter and soil, but different taxonomic composition.
- Strong variation of composition of the communities according to the the tree species.

DO MICROBES IMPACTED BY THE TREE SPECIES ?

Tree species effect on the mineral weathering bacterial communities



Calvaruso *et al.*, AEM 2010

- Enrichment of effective mineral weathering bacterial communities in beech and oak, but not below spruce
- Similar bacterial diversity between the different tree species, but different between rhizosphere and bulk soil
- Trends confirmed by culture-independent approaches (16S rRNA pyrosequencing)

DO INTENSIFICATION OF ORGANIC MATTER REMOVAL IMPACT MICROBES ?

Douglas fir

Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests

4 levels of removal:

REF: No removal

OM1: Trunk (=51 % of net C removal)

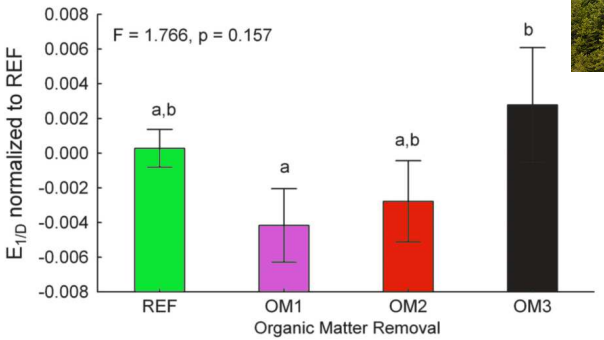
OM2: All the part of the trees (=65%)

OM3: All the parts of the trees + tree residuals (=84%)

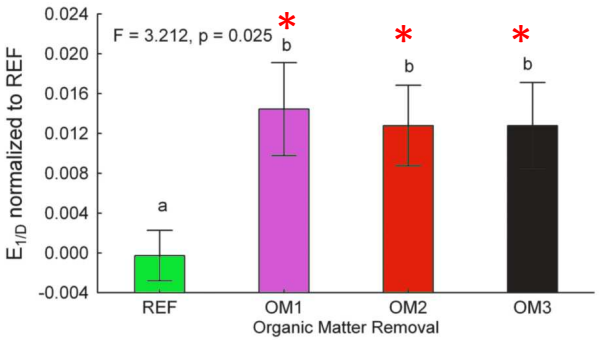
10 years after…

Molecular analysis of the microbial communities (16S rRNA and ITS pyrosequencing)

Bacteria



Fungi



Hartmann et al. ISME J 2012

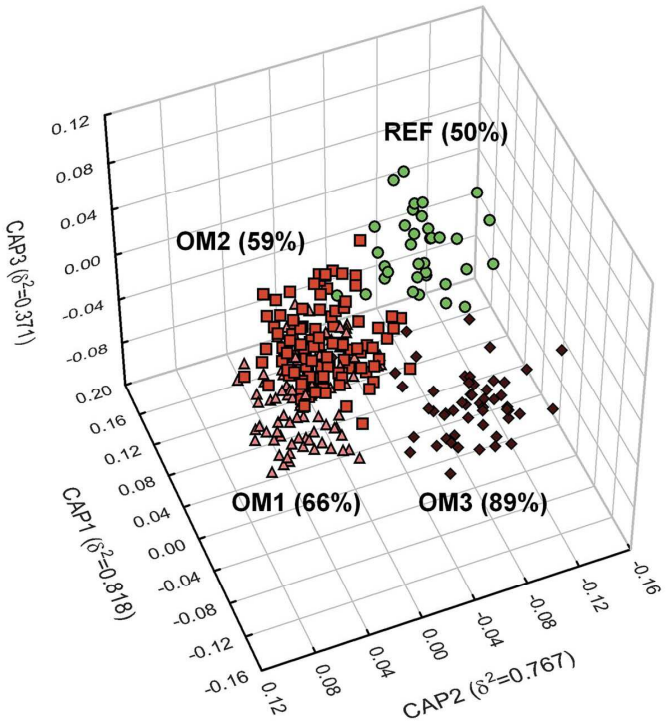
- Bacteria and fungi are not impacted in the same way by soil organic matter removal.

- Stronger effect on the evenness of the fungal communities for each level of OM removal.

DO INTENSIFICATION OF ORGANIC MATTER REMOVAL IMPACT MICROBES ?

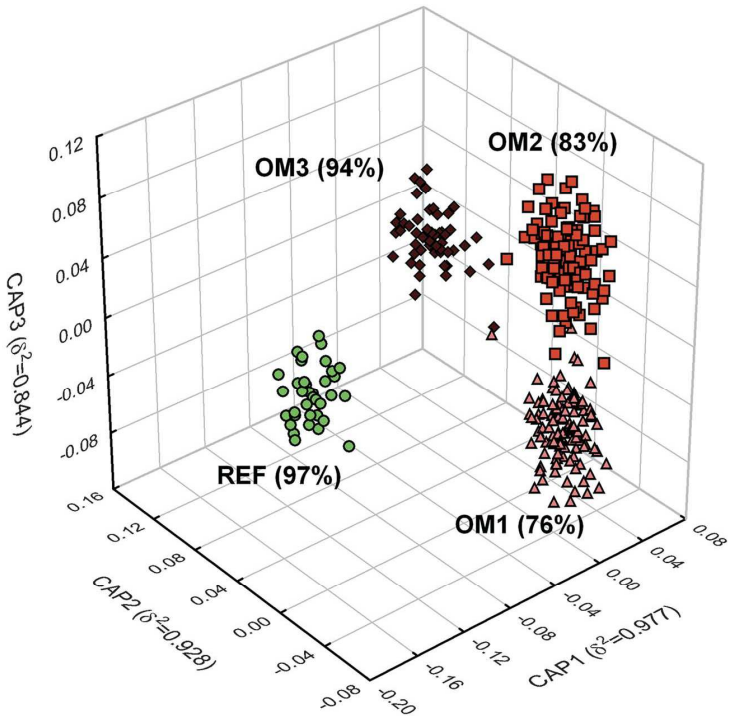
Multivariate analyses of the community structure

Bacteria



trace=1.96; p<0.0001

Fungi



trace=2.75; p<0.0001

Hartmann et al. ISME J 2012

- Ten years after the harvest, intensification effects are still visible, both on the structure of the bacterial and fungal communities.
- For bacteria, weak differences between low and medium OM removal levels
- Stronger effect on the fungal communities.

DO SOIL COMPACTION IMPACT MICROBES ?

Resistance and resilience of the forest soil microbiome to logging-associated compaction

Ex: Ermatingen, Switzerland

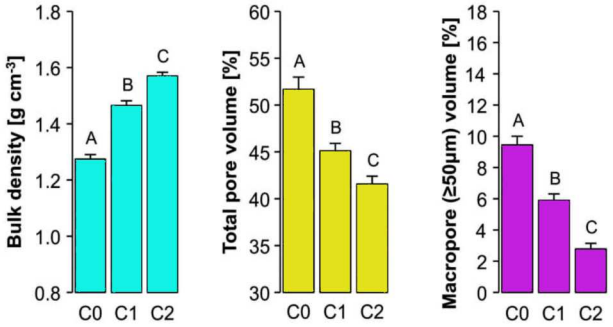
3 levels of compaction tested :

C0: Unaffected area (reference)

C1: Few passes of 24 t skidders

C2: Several passes of 24 t skidders

Physico-chemical properties



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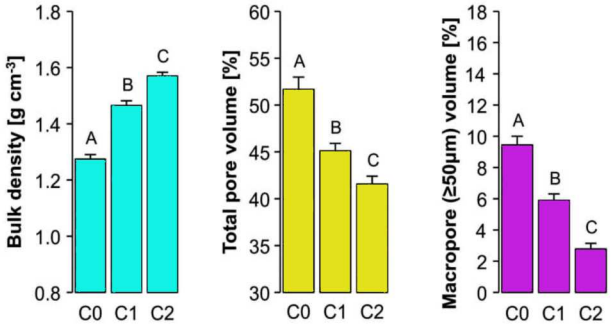
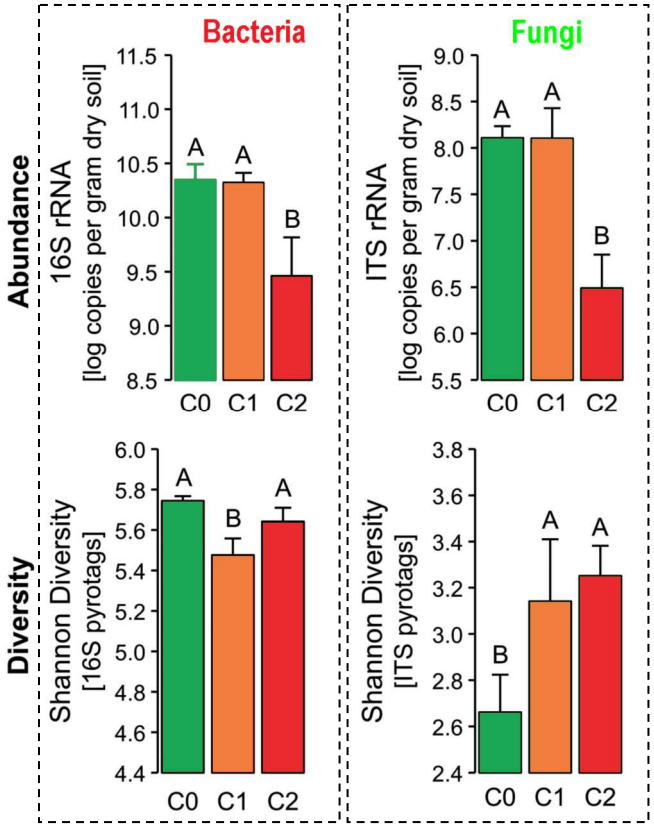


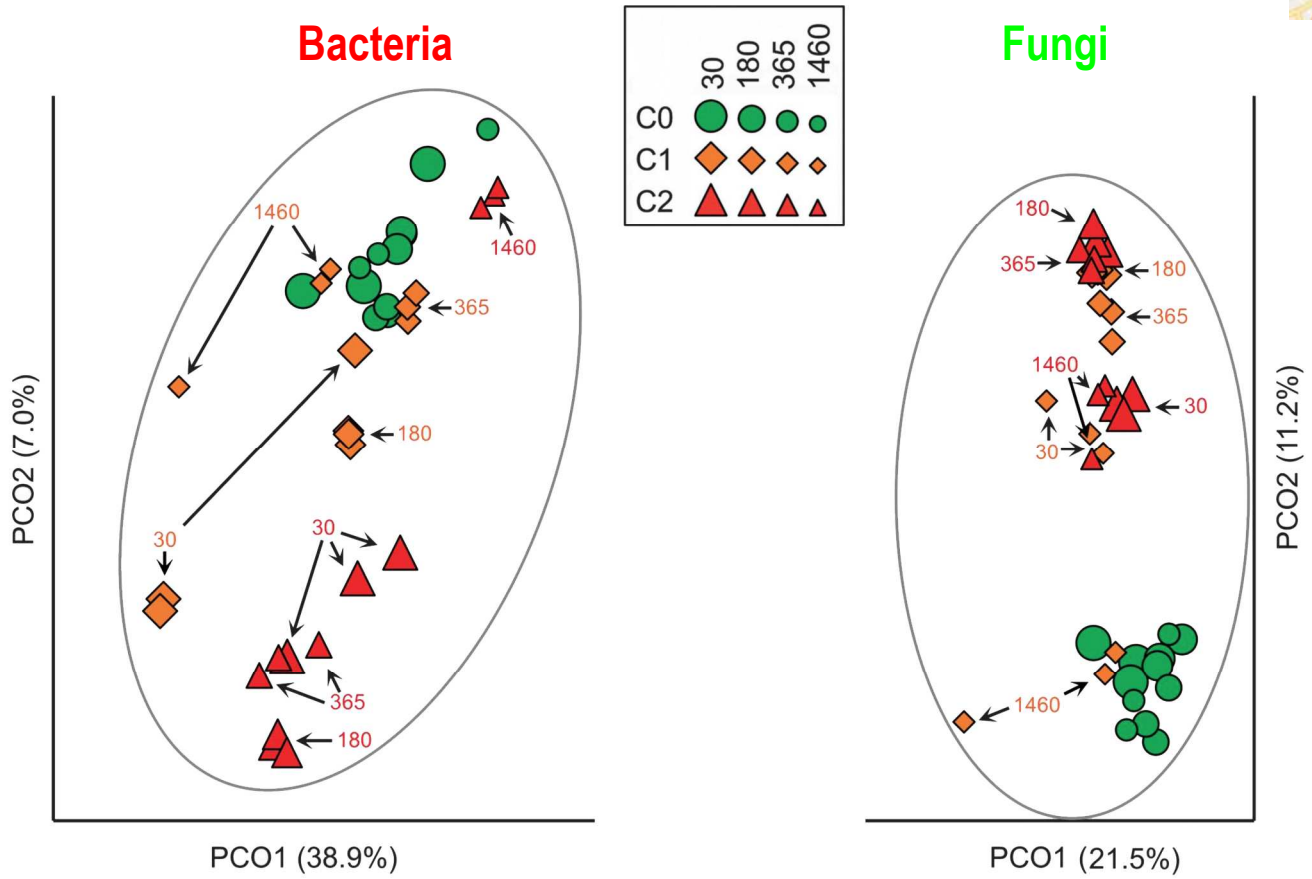


Hartmann et al. ISME J 2014

- **Soil compaction strongly modified the soil properties…even after 4 years. [moving from oxic to anoxic conditions]**
- **Strong modification of the densities of microbes**
- **Different patterns for bacterial and fungal diversity**
- **Long-term (years) effect on the biological functioning of the soil**

DO SOIL COMPACTION IMPACT MICROBES ?

Multivariate analyses of the community structure (16S rRNA and ITS)

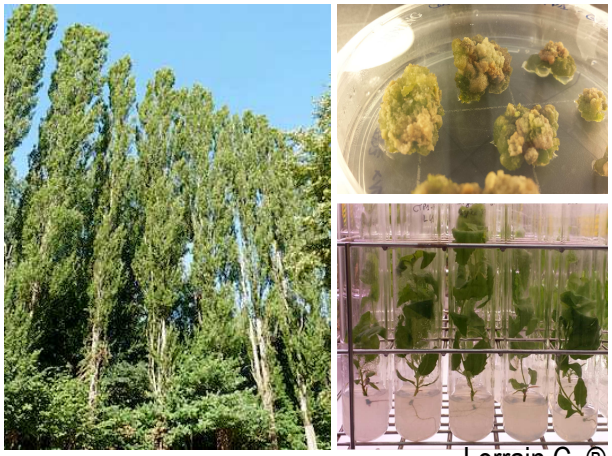


Hartmann et al. ISME J 2014

- **Soil compaction strongly modified the structure of the microbial communities**
- **Resilience seems to occur for bacterial communities (4 years after=1460 days), but not for fungi**
- **Long term effect of the compaction on the microbial communities**

TREE MICROBIOME OR TREE MICROBIOMES ?

Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome



Lorrain C. ®

Comparison of wild-type and mutant in lignin production. Mutation of the Cinnamoyl-CoA reductase (CCR), an enzyme central to the lignin biosynthetic pathway

Populus tremula x Populus alba

Endophytic communities

Leaves

Density: WT<GMO
Structure: WT≠GMO

Stem

Density: WT<GMO
Structure: WT≠GMO

Root

Density: WT<GMO
Structure: WT≠GMO

Rhizosphere communities

Density: WT=GMO
Structure: WT=GMO

Beckers et al. PNAS 2016

A single mutation strongly impacts the endophytic microbiomes, but not the rhizosphere microbiome

CONCLUSIONS

- Higher bacterial diversity than fungal diversity in forest soil (ratio B/F lower than in grassland or arable soils..)
- Vertical distribution of the microbiome at both taxonomic and functional levels
- A strong tree species effect … Importance to consider this aspect for the forestry practices
- A long-term effect of the forestry practices and of their intensification (i.e., soil compaction, organic matter removal,…)
- Presence of complex microbial communities (=TREE MICROBIOME) in the different interfaces developed by the trees.

ACKNOWLEDGEMENTS: UMR1136 IAM (M. BUEE, A. DEVEAU, F. MARTIN) / UR1138 BEF (M-P. TURPAULT, J. RANGER)…

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