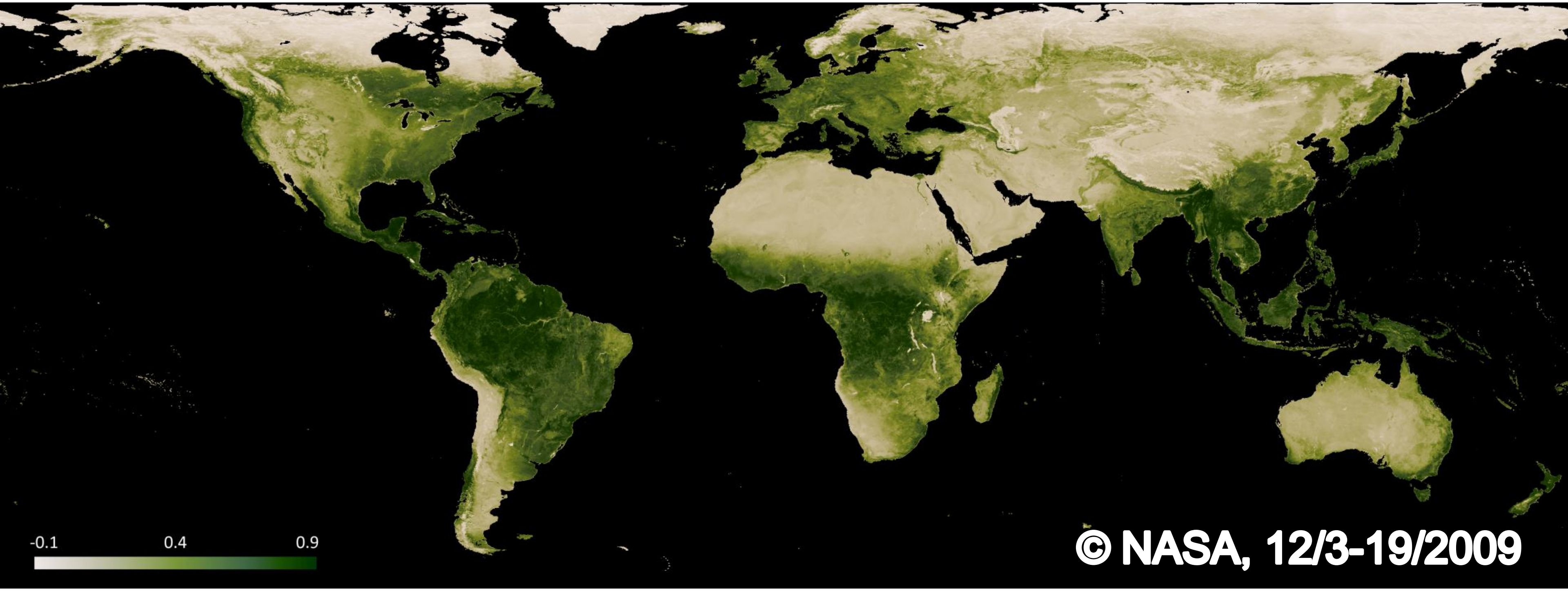
Microbial associations and interactions at the plant-climate interface

INRA SCIENCE & IMPACT

Corinne Vacher & Charlie Pauvert
Biogeco, INRA-Bordeaux

The surface of plant leaves is a microbial habitat (phyllosphere)

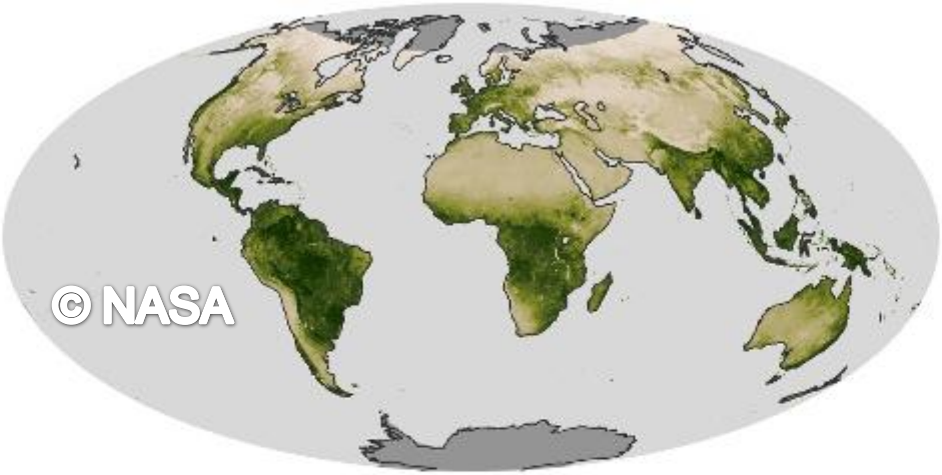
The phyllosphere is a vast dynamic area



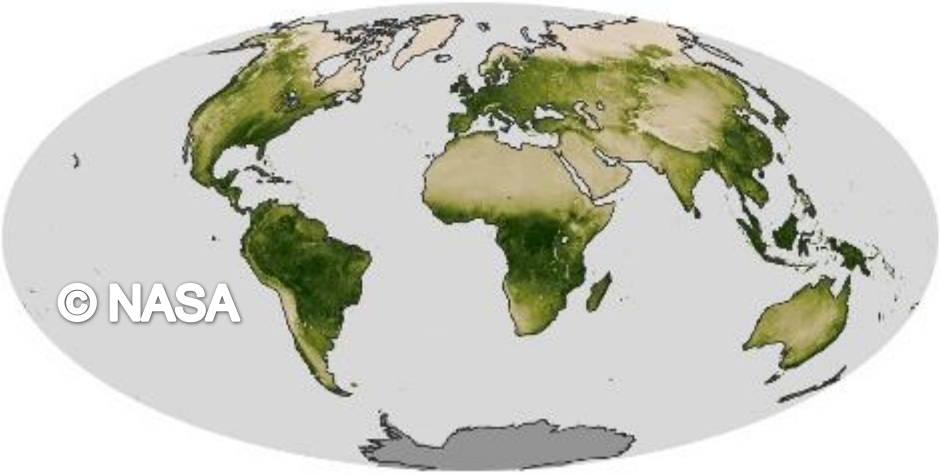
Upper + lower leaf surface ~ 1 billion km^2 ~ land surface x 2

Vorholt 2012

The phyllosphere is a vast dynamic area



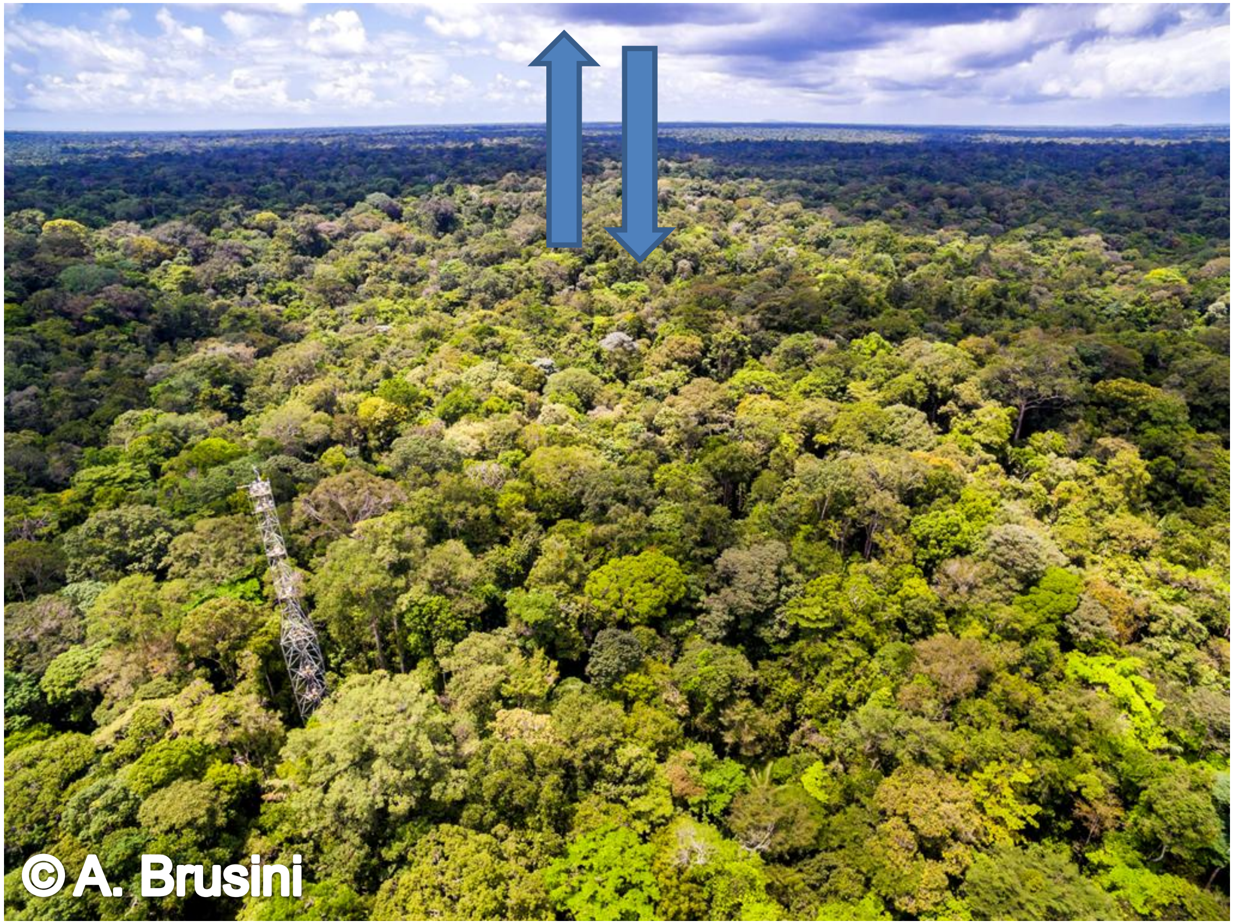
January 2015



July 2015

- Seasonal changes (phenology)
- Anthropic disturbances (deforestation, urbanization, desertification)

The phyllosphere is an exchange surface



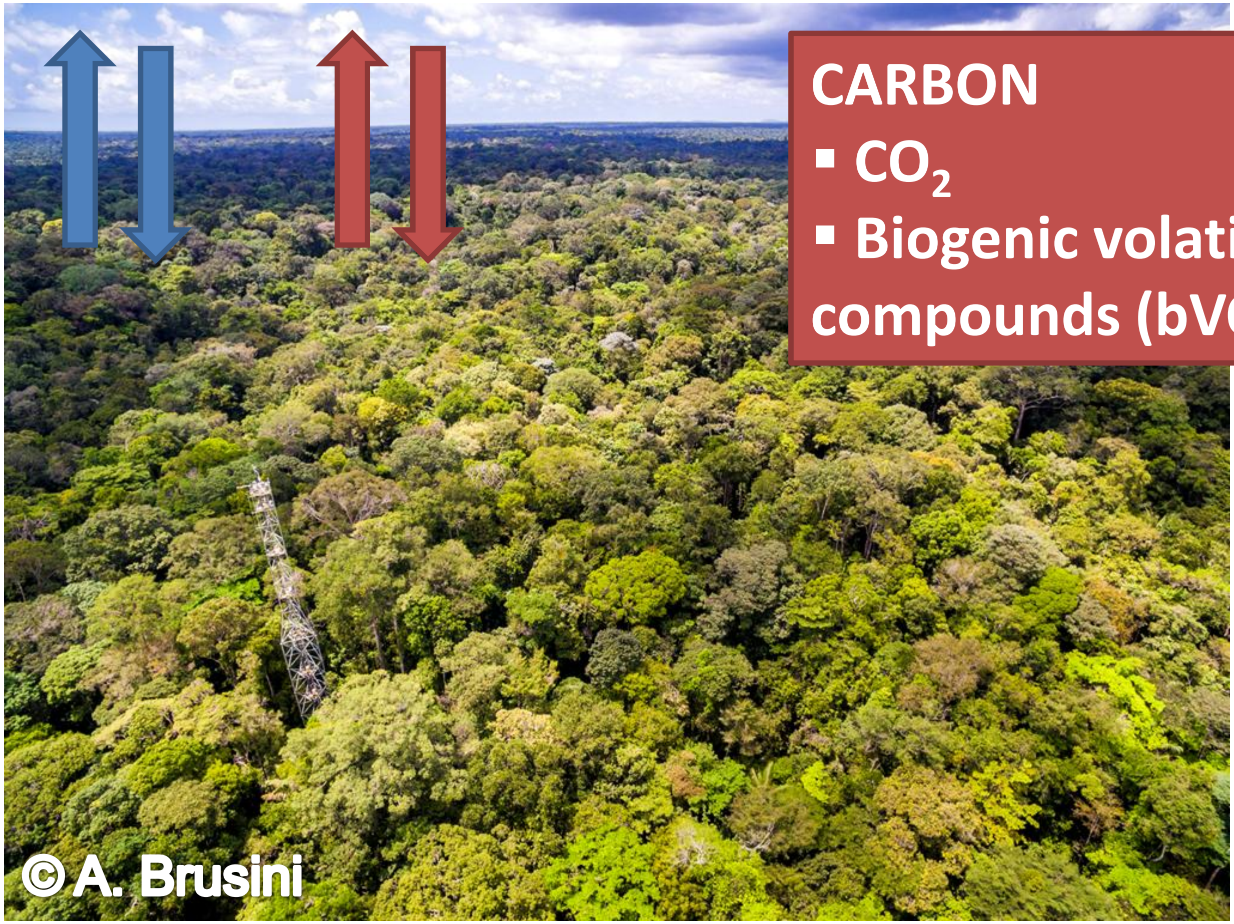
Guyaflux tower, Paracou, French Guyana

The phyllosphere is an exchange surface

WATER
- Water vapor
- Precipitations

Adapted from Suni et al. 2015

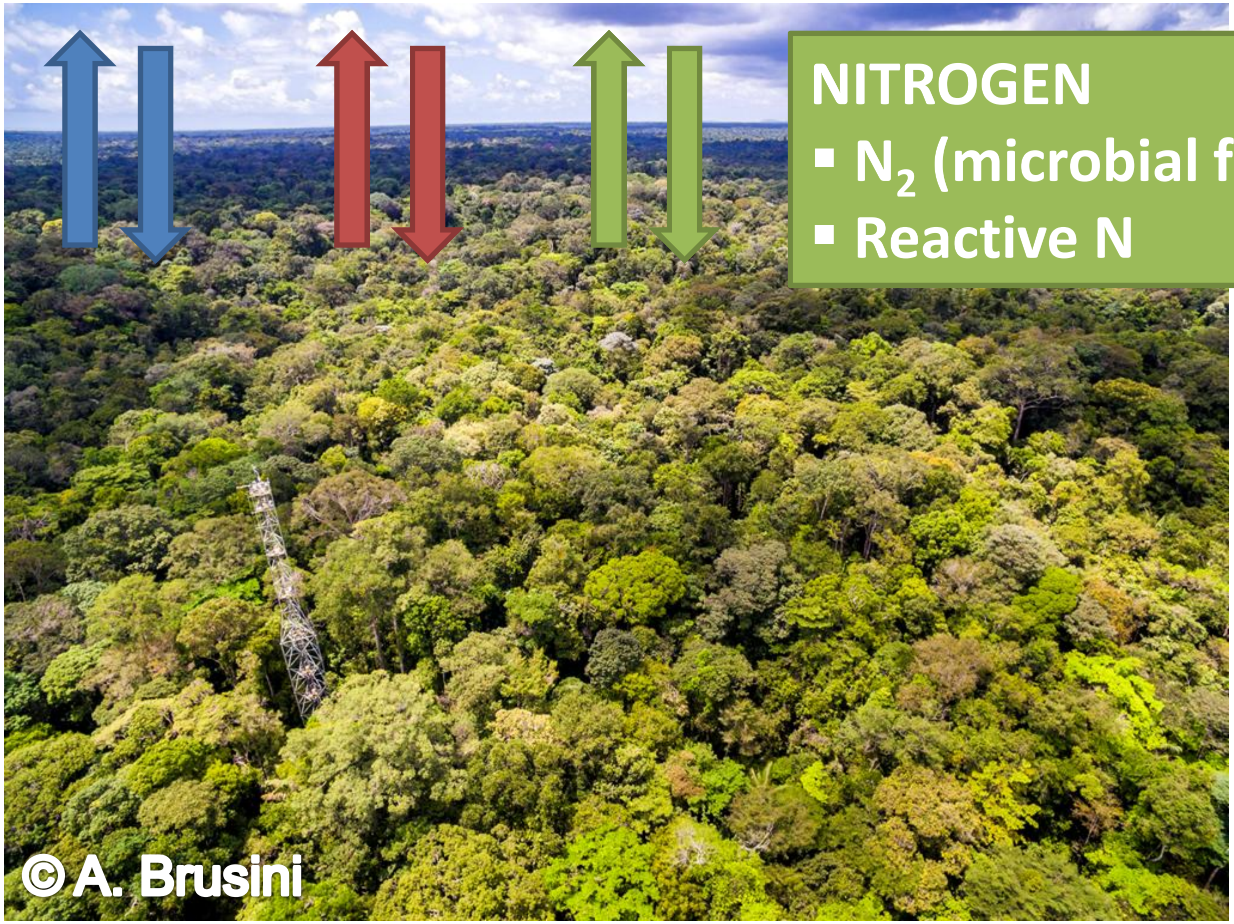
The phyllosphere is an exchange surface



CARBON
- CO_2
- Biogenic volatile organic compounds (bVOCS)

Adapted from Suni et al. 2015

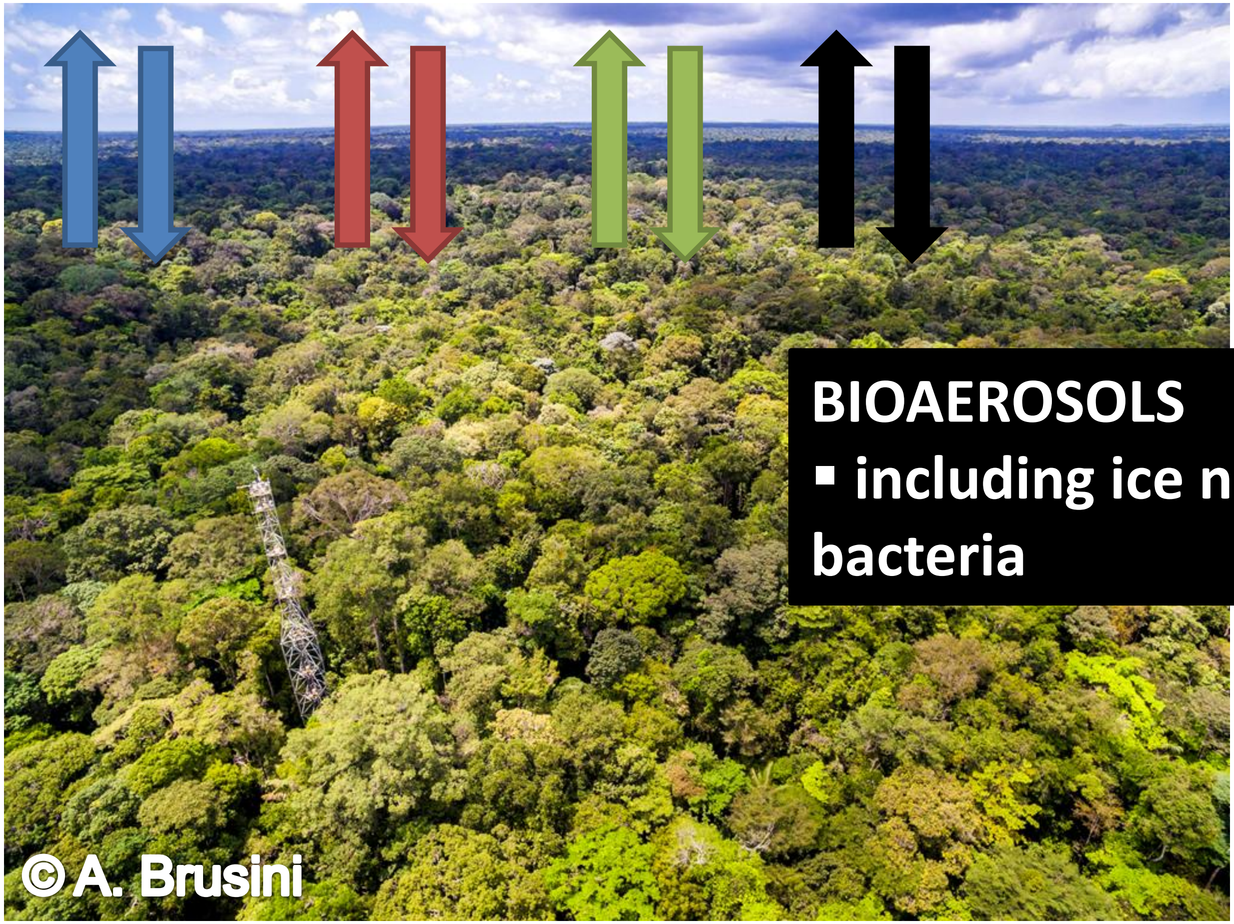
The phyllosphere is a surface of exchange



NITROGEN
- N_2 (microbial fixation)
- Reactive N

Adapted from Suni et al. 2015

The phyllosphere is a surface of exchange

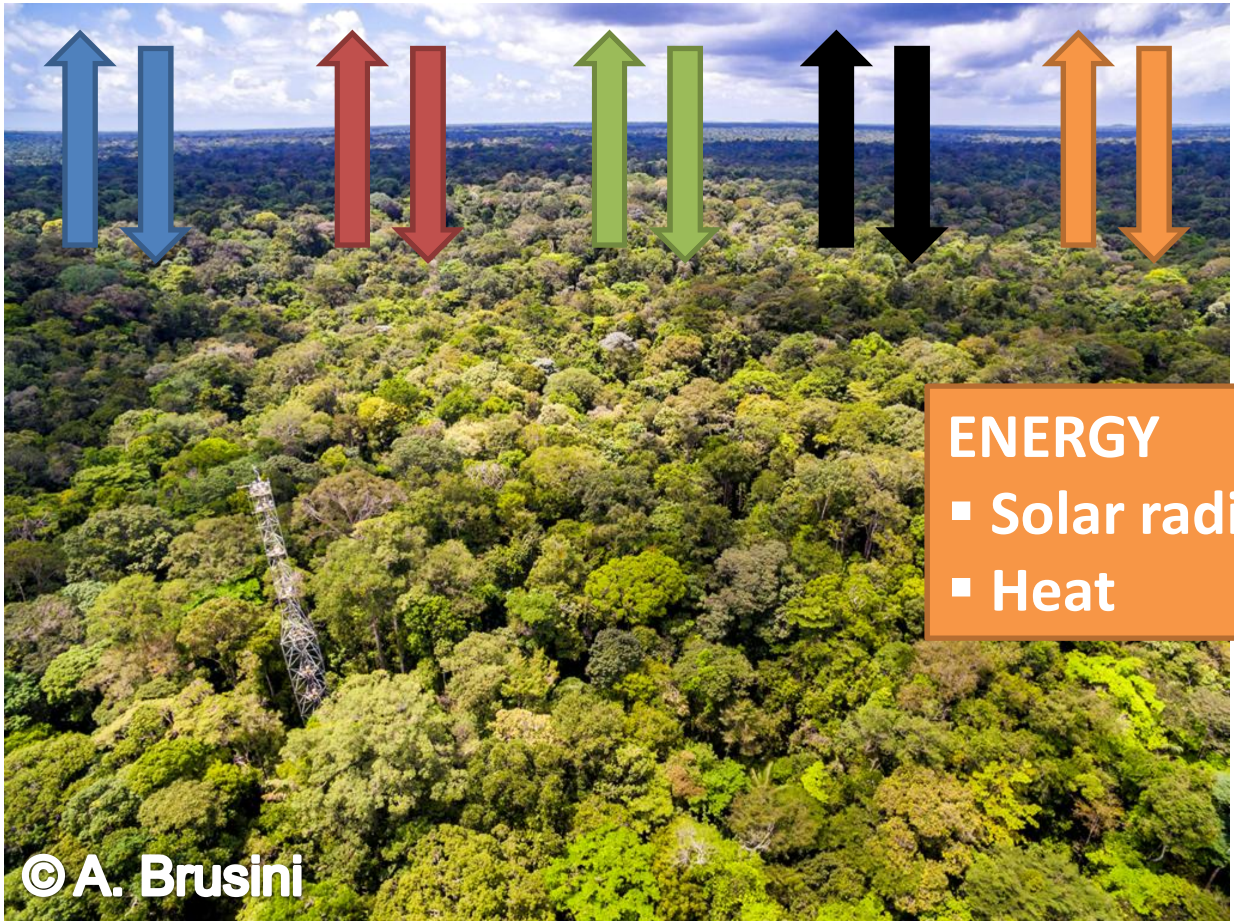


BIOAERSOLS

- **including ice nucleation bacteria**

Adapted from Suni et al. 2015

The phyllosphere is an exchange surface



ENERGY
- Solar radiation
- Heat

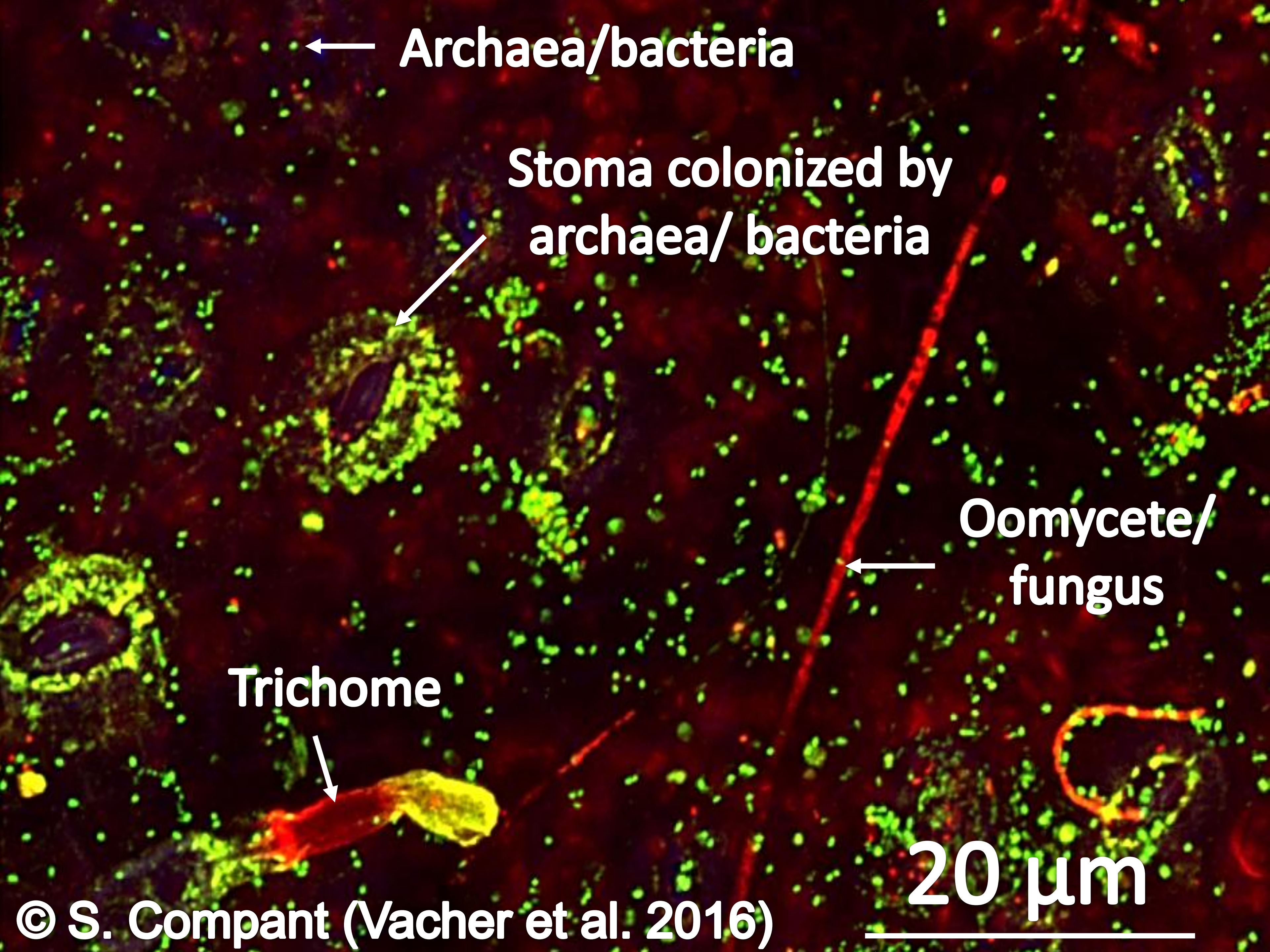
Adapted from Suni et al. 2015

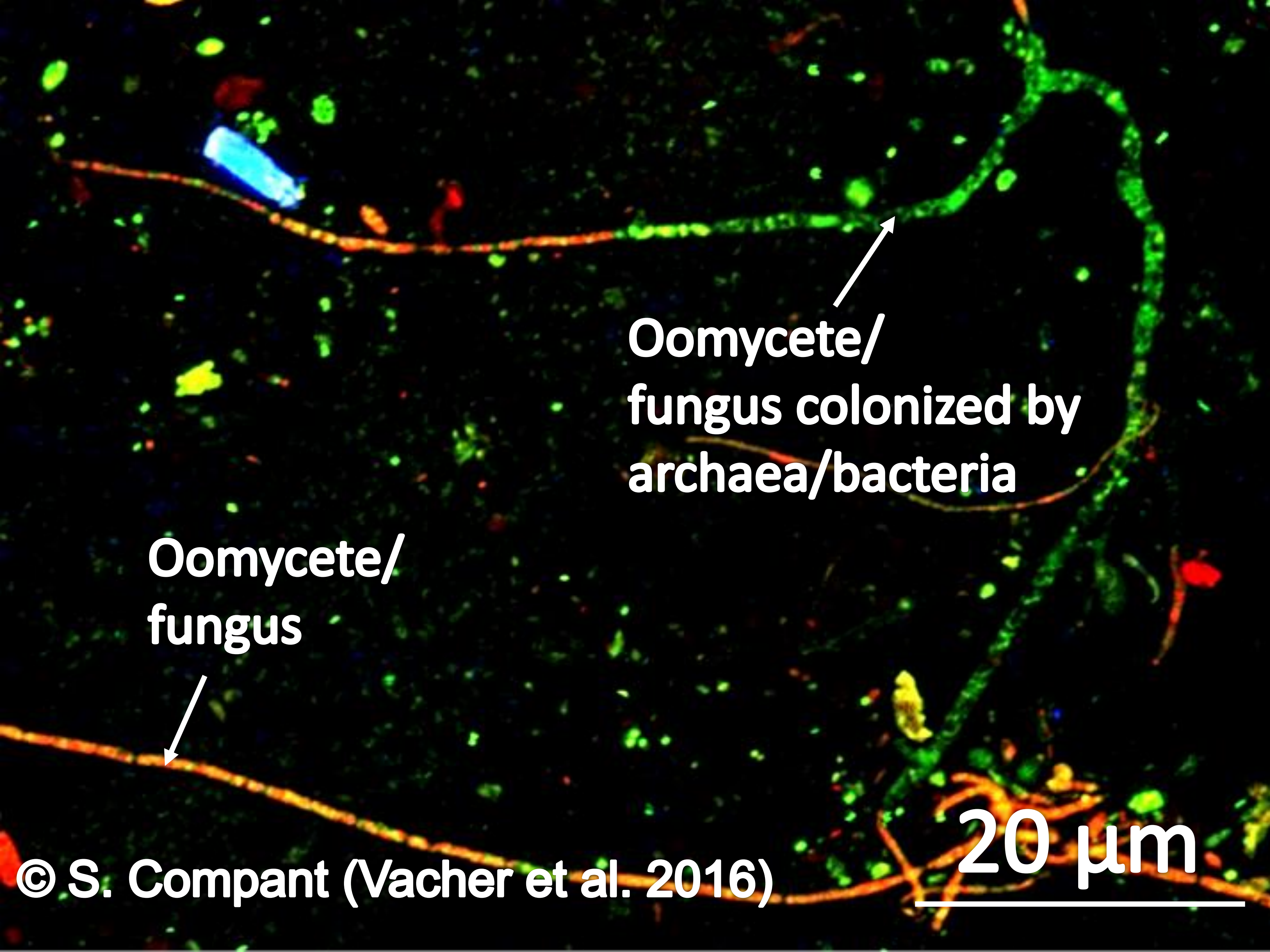
Atmospheric chemistry
Climate

Plant health and productivity
Carbon sequestration

Atmospheric chemistry
Climate

Plant health and productivity
Carbon sequestration


Archaea/bacteria
Stoma colonized by archaea/ bacteria
Oomycete/ fungus
Trichome
20 µm
© S. Compant (Vacher et al. 2016)


Oomycete/
fungus colonized by
archaea/bacteria
Oomycete/
fungus
20 µm
© S. Compant (Vacher et al. 2016)

Functions of phyllosphere microorganisms

Plant commensals	++++++
Plant mutualists	++
Plant parasites	+

Functions of phyllosphere microorganisms

Plant commensals →

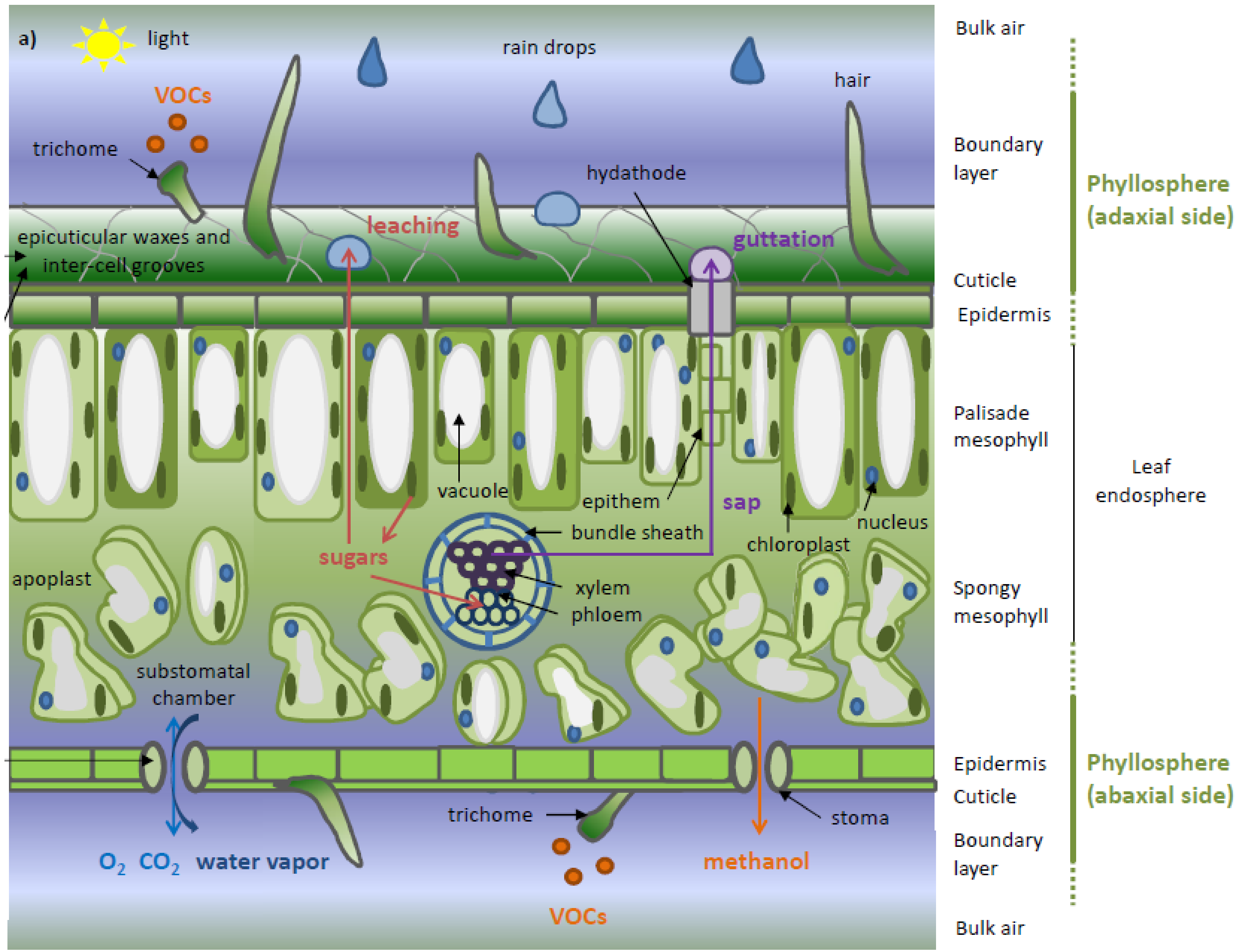
Plant mutualists

Plant parasites

Use the leaf as a support (e.g. phototrophic microorganisms)

Use the nutrients available on the leaf (pollen, honeydew, leachates, VOCs)

Decompose senescent leaves and litter (e.g. dormant saprobes)



Vacher et al. 2016

Functions of phyllosphere microorganisms

Plant commensals

Plant mutualists →

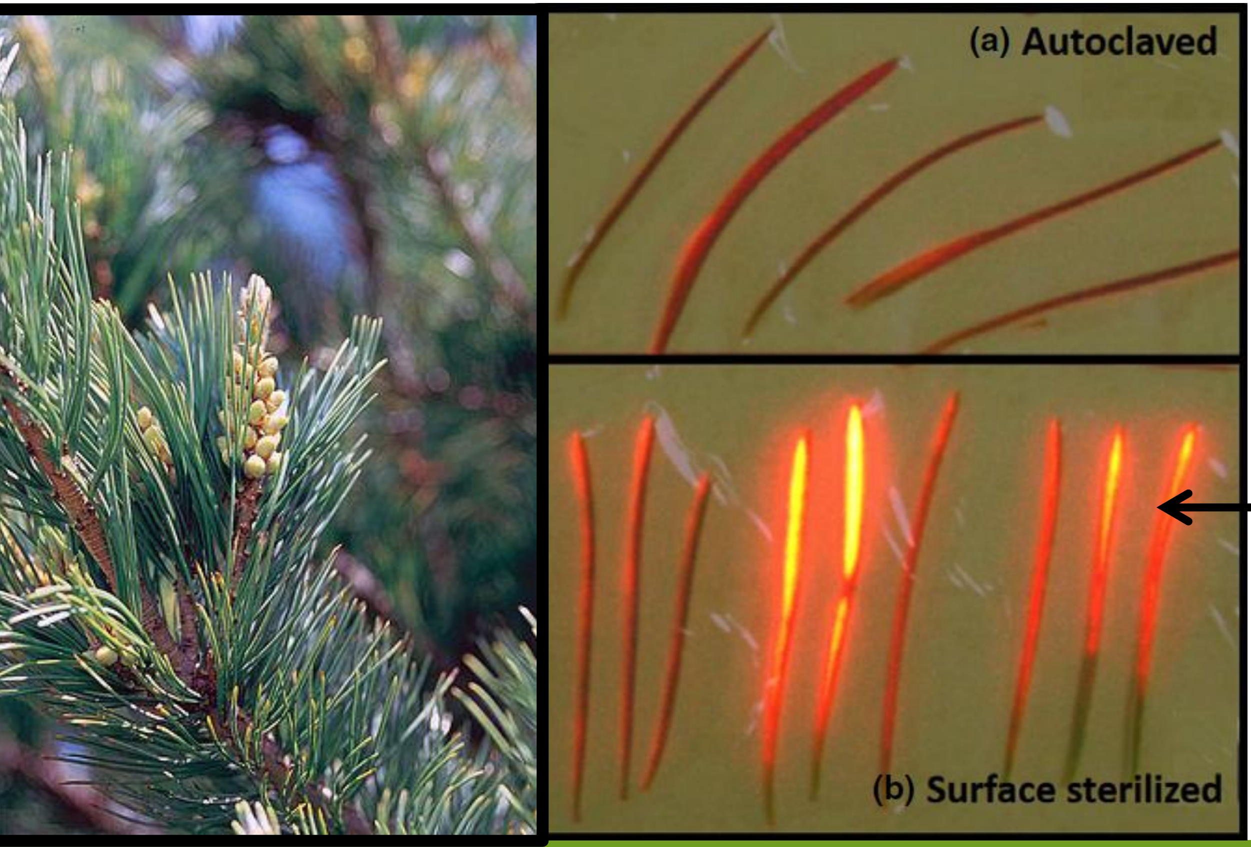
Trophic mutualists (e.g. diazotrophic bacteria)

Protective mutualists (against pathogens, herbivores, drought)

Plant parasites

Diazotrophic bacteria

Pine needles (*Pinus flexilis*) exposed to radioactive N_2



Uptake of atmospheric N_2 by endophytic bacteria

Moyes et al. 2016

Functions of phyllosphere microorganisms

Plant commensals

Plant mutualists

Plant parasites →

Primary pathogens
- ➢ Cause disease in healthy plants

Latent/secondary pathogens
- ➢ Cause disease in stressed or senescent plants

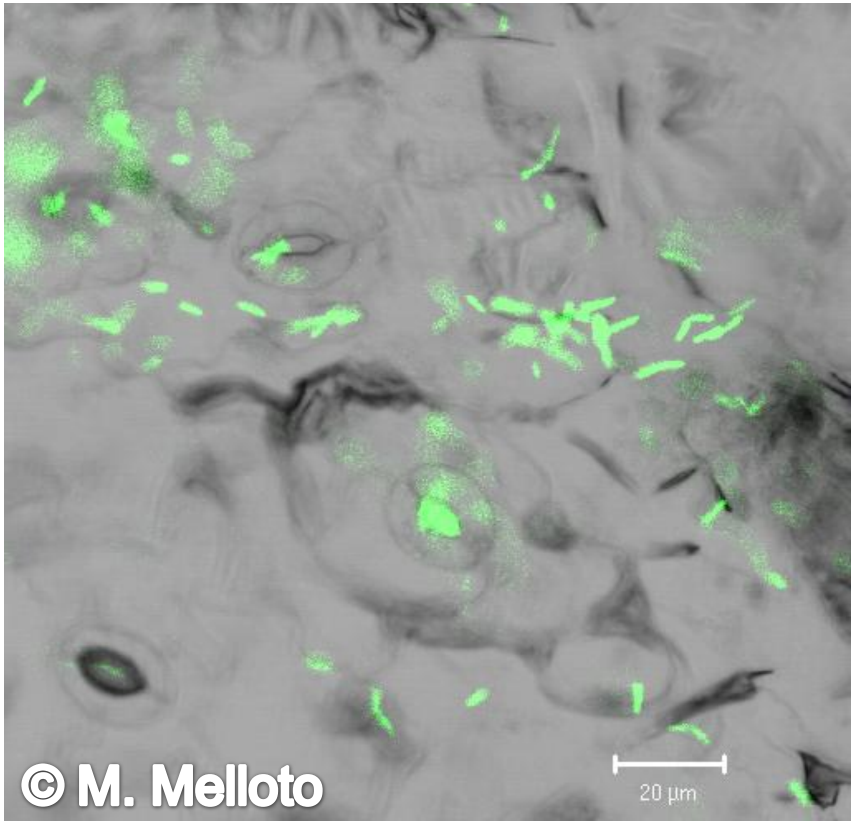
Impact of primary pathogens on leaf physiology

Reduction in **photosynthetic activity**

Emission of **volatile organic compounds**

Copolovici et al. 2015

Impact of primary pathogens on leaf physiology

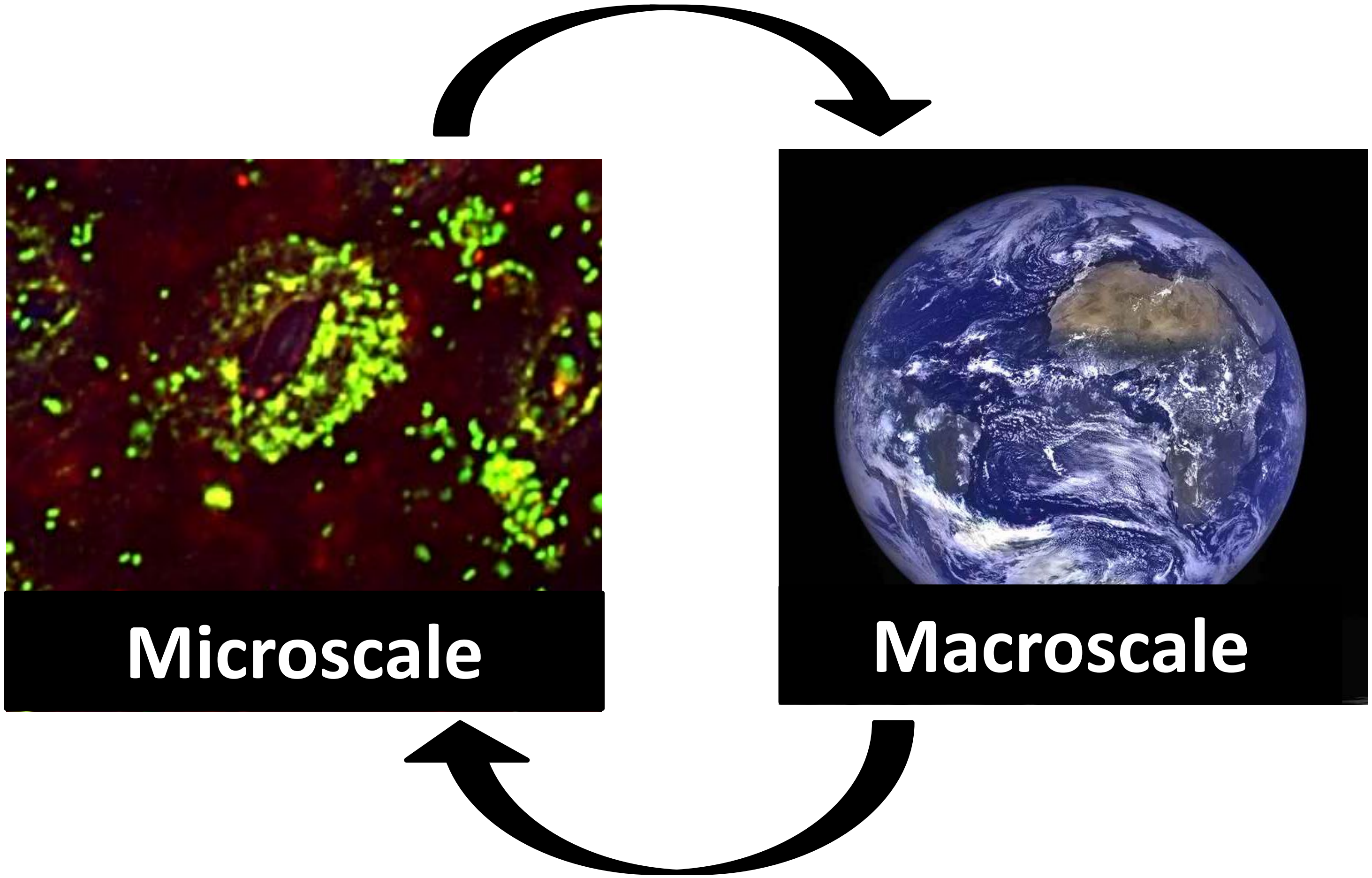


Pseudomonas syringae triggers **stomatal aperture** by producing a virulence factor (coronatine).

The commensal microbiota protects against infection by *Ps.*

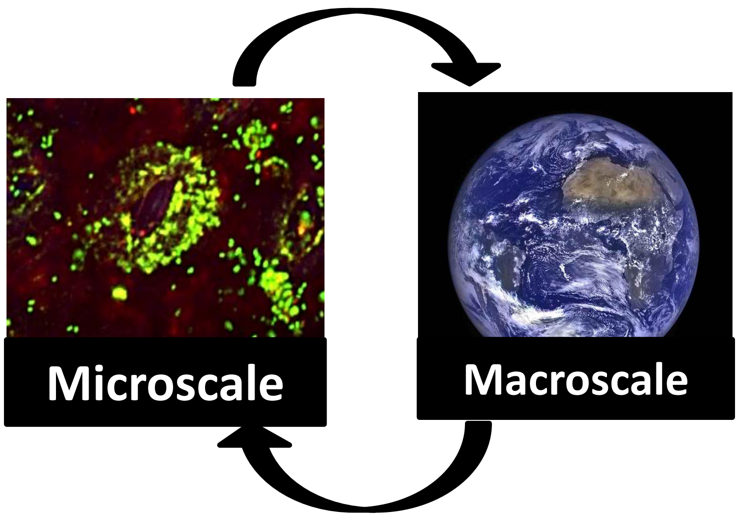
Melotto et al. 2006
Vogel et al. 2016

A current challenge is to better link the spatial scales



A current challenge is to better link the spatial scales

- Abundance
- Distribution
- Composition
- Diversity
- Dynamics
- Interactions

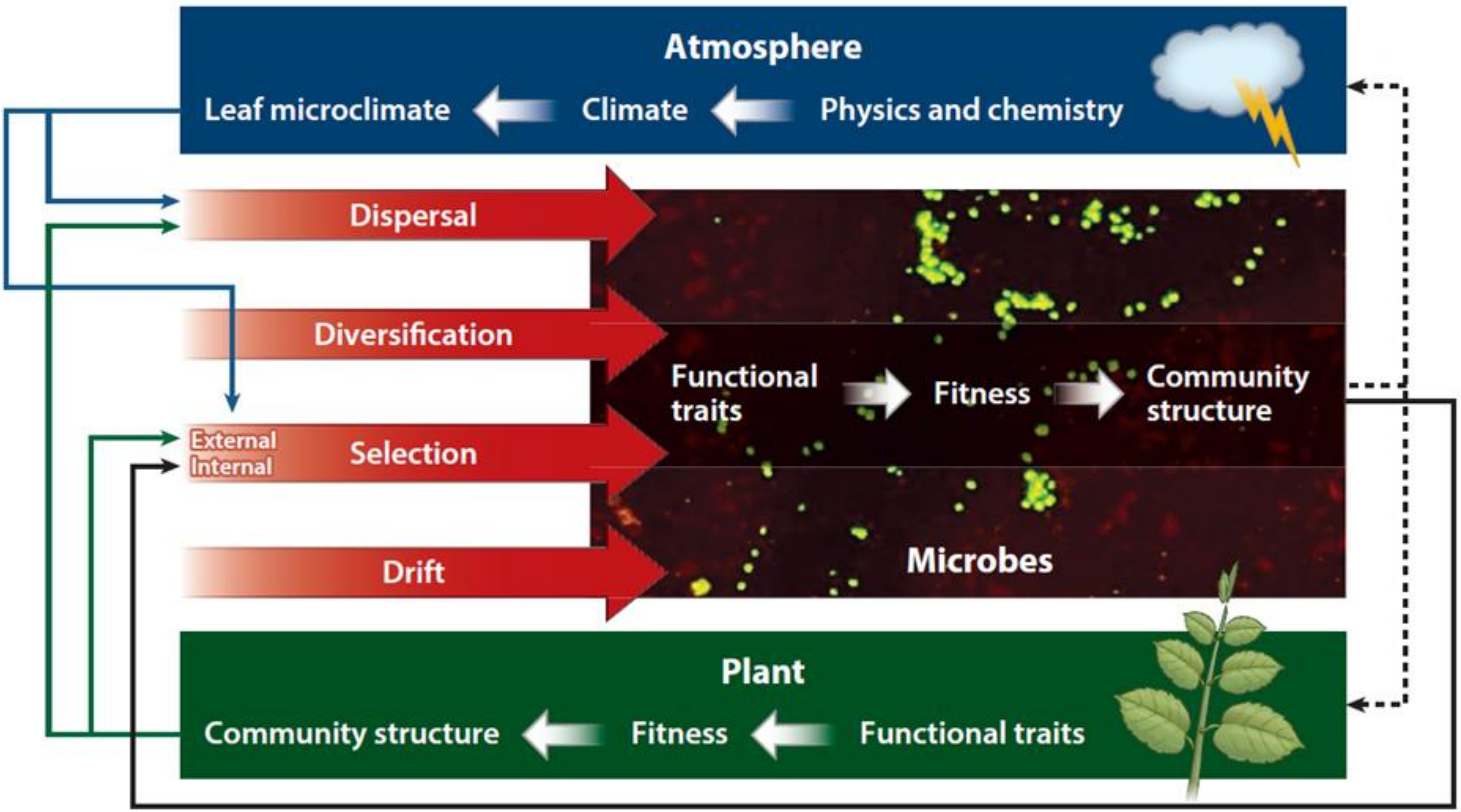


- Water cycle
- Nutrient cycle
- Climate regulation
- Pest/disease regulation

Atmospheric chemistry
Climate

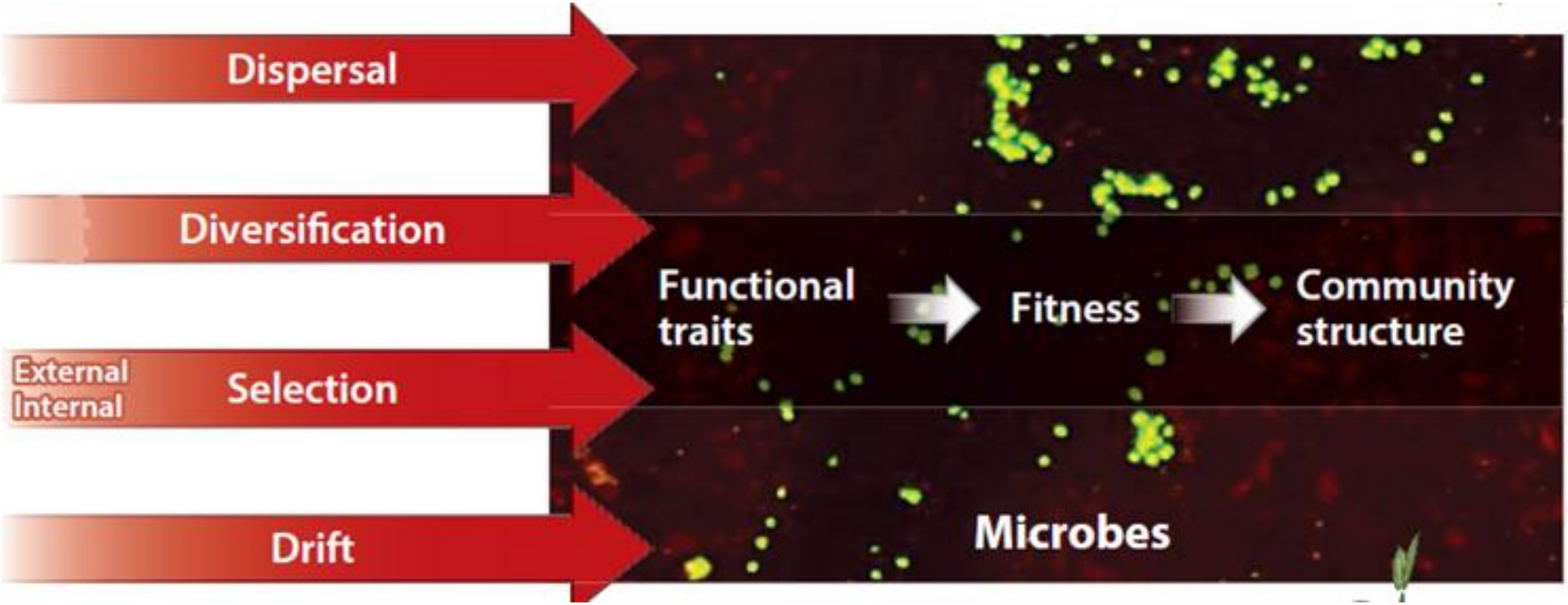
Framework ?

Plant health and productivity
Carbon sequestration



Vacher et al. 2016

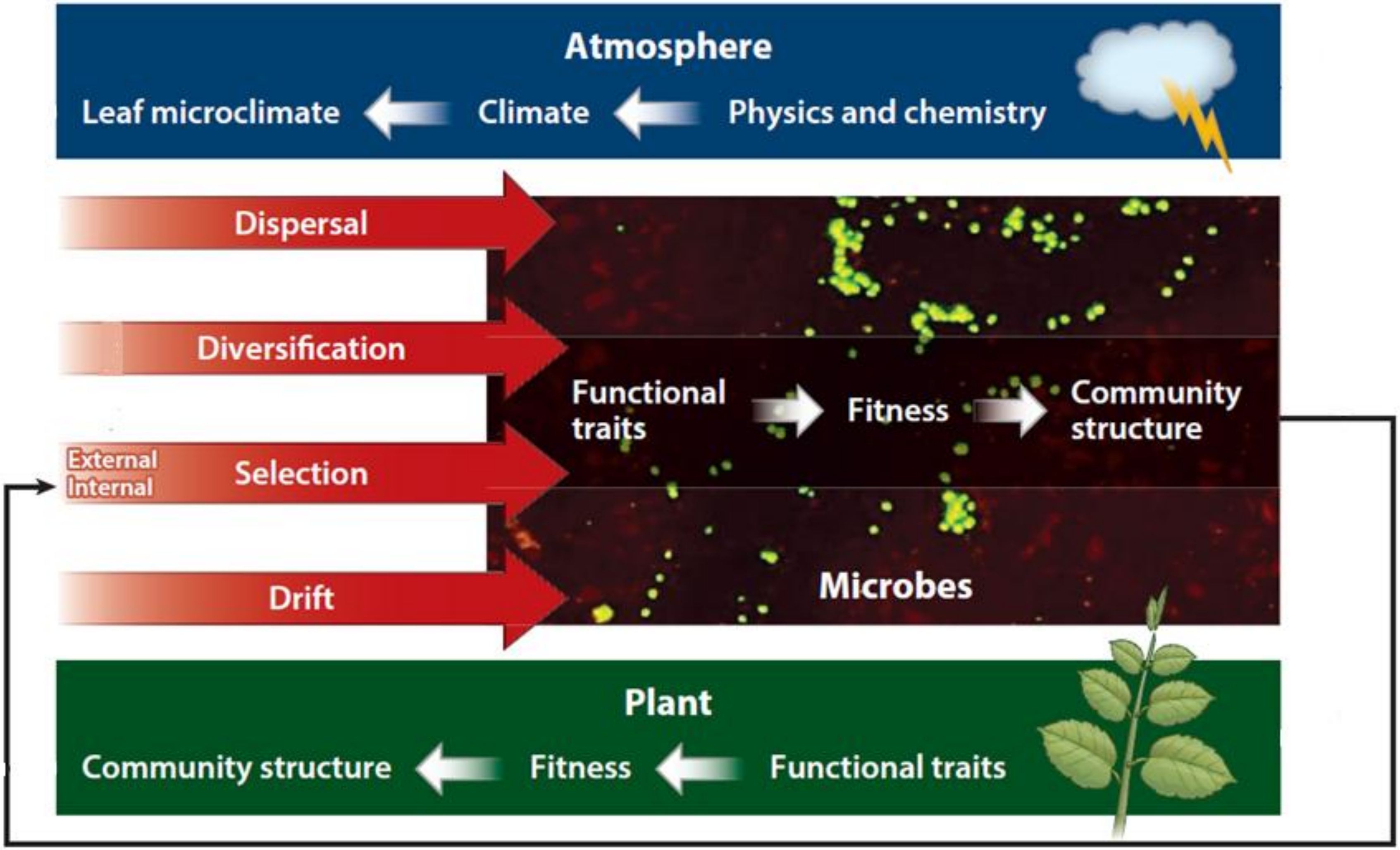
Eco-evolutionary processes shaping microbial communities



Vellend 2010

Nemergut et al. 2013

Internal selection



Questions

1 **How to reconstruct microbial interaction networks?**

2 Can the plant select for beneficial network properties?

How to collect interaction data?

« Easy » when interactions can be observed


Host-parasite


Plant-ant


Plant-herbivore


Plant-pollinator


Plant-seed disperser

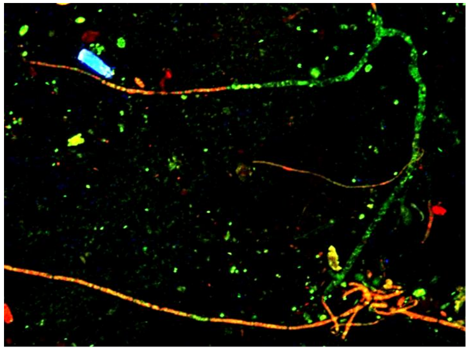

Predator-prey


Anemone-fish

Interaction Web Database, NCEAS

How to collect interaction data?

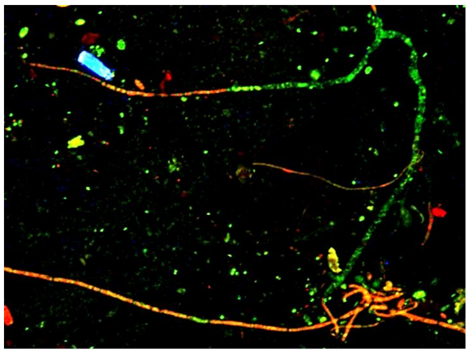
« More difficult » for microorganisms



- Interactions rarely observed *in situ*
- Cultures impossible for most microorganisms

How to collect interaction data?

« More difficult » for microorganisms



- Interactions rarely observed *in situ*
- Cultures impossible for most microorganisms

Learning Ecological Networks from Next-Generation Sequencing Data

Vacher et al. 2016

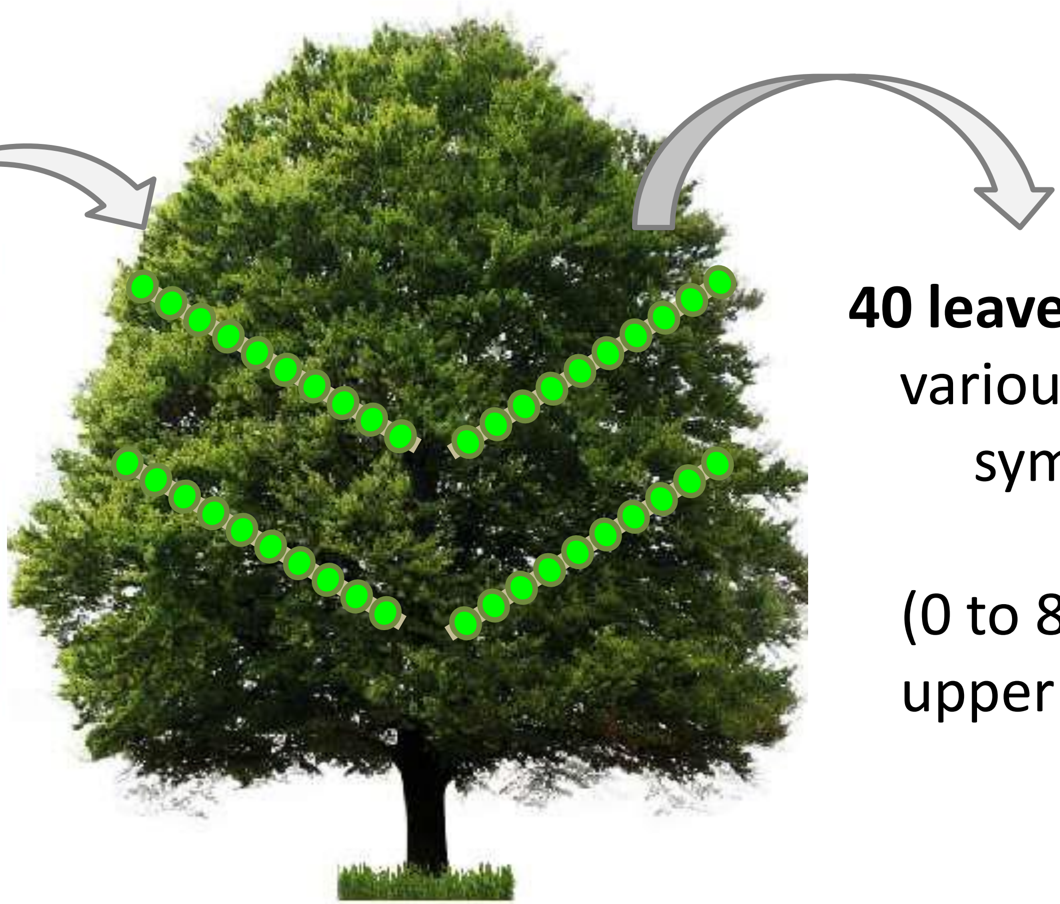
Case study #1

Who's interacting with *Erysiphe alphitoides*, the causal agent of oak powdery mildew?

Sampling design

1 oak tree susceptible to powdery mildew

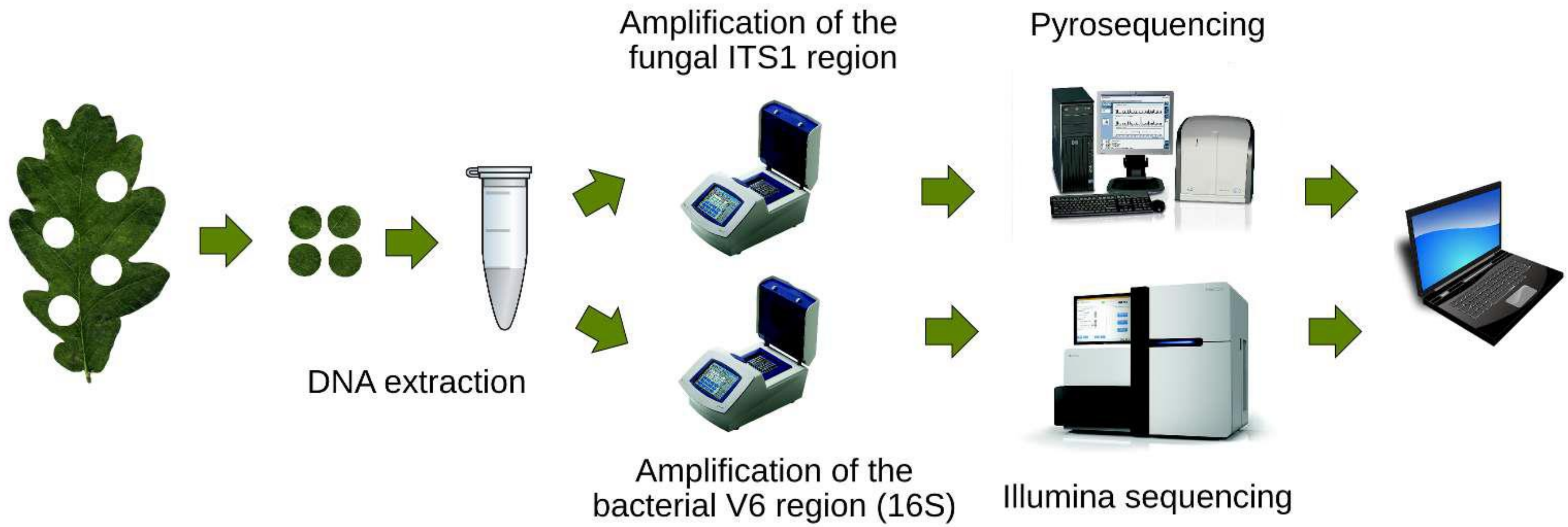
Quercus robur full-sib family (Bourran, FR)



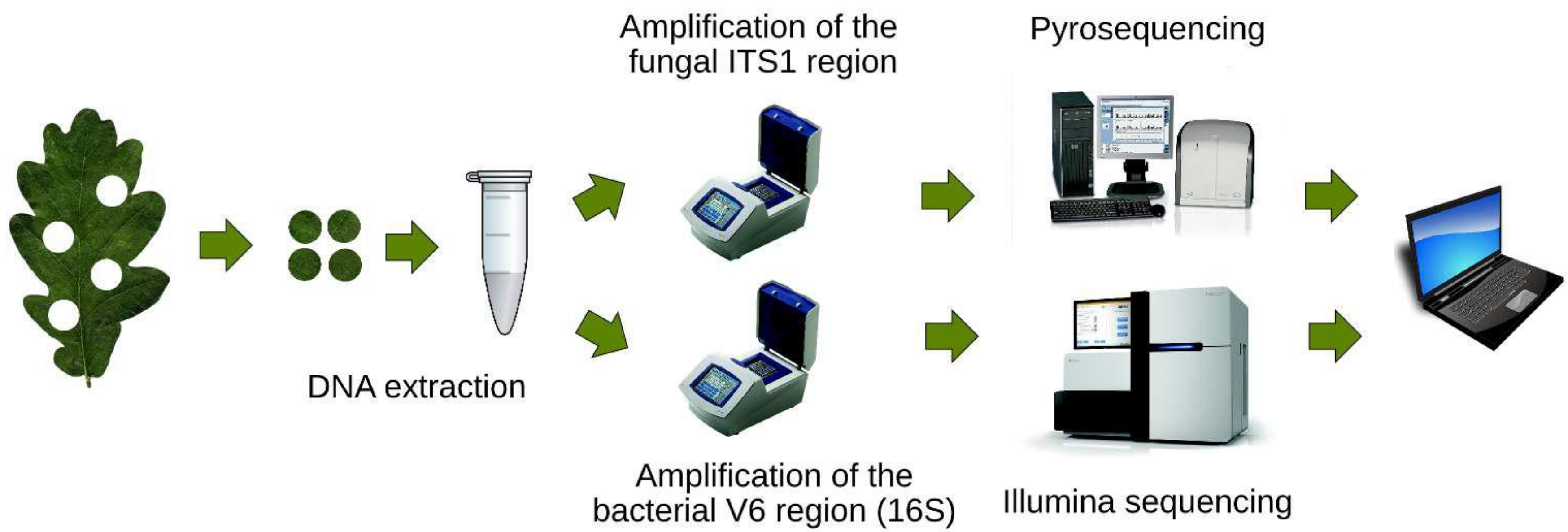
40 leaves displaying various levels of symptoms

(0 to 80% of the upper leaf area)

Next-Generation Sequencing



Next-Generation Sequencing

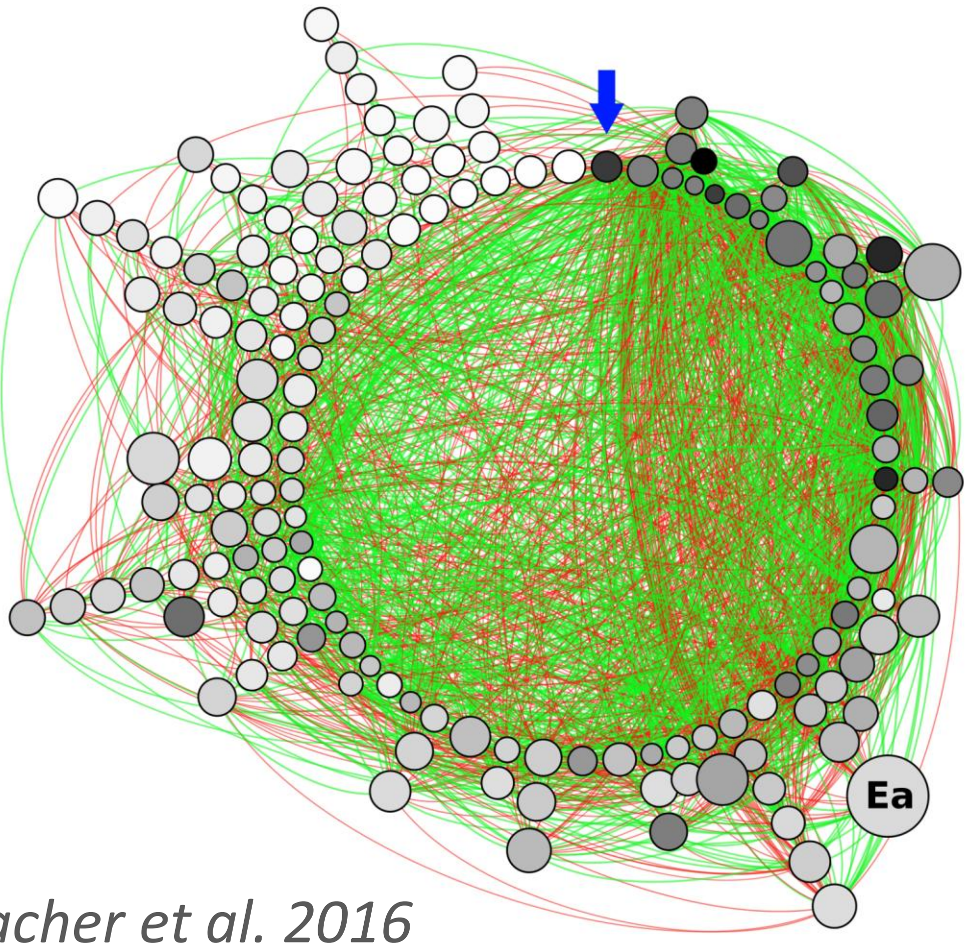


Inferring Correlation Networks from Genomic Survey Data

Jonathan Friedman[1], Eric J. Alm[1,2,3*]

PLOS | COMPUTATIONAL BIOLOGY

Microbial correlation network



Vacher et al. 2016

162 nodes, 2053 edges
51% positive correlations
49% negative correlations

Erysiphe alphitoides is predominantly connected through strong negative links (co-exclusions)

Correlations are not interactions

Significant **correlation** between the abundance of species A and B

Pairwise **interaction** between A and B (e.g. competition, mutualism)

Shared interaction partner (indirect interaction)

Shared environmental requirements (e.g. temperature, light)

Compositional bias (in the case of relative abundances)

Correlations are not interactions

Significant **correlation** between the abundance of species A and B

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Compositional bias (in the case of relative abundances)

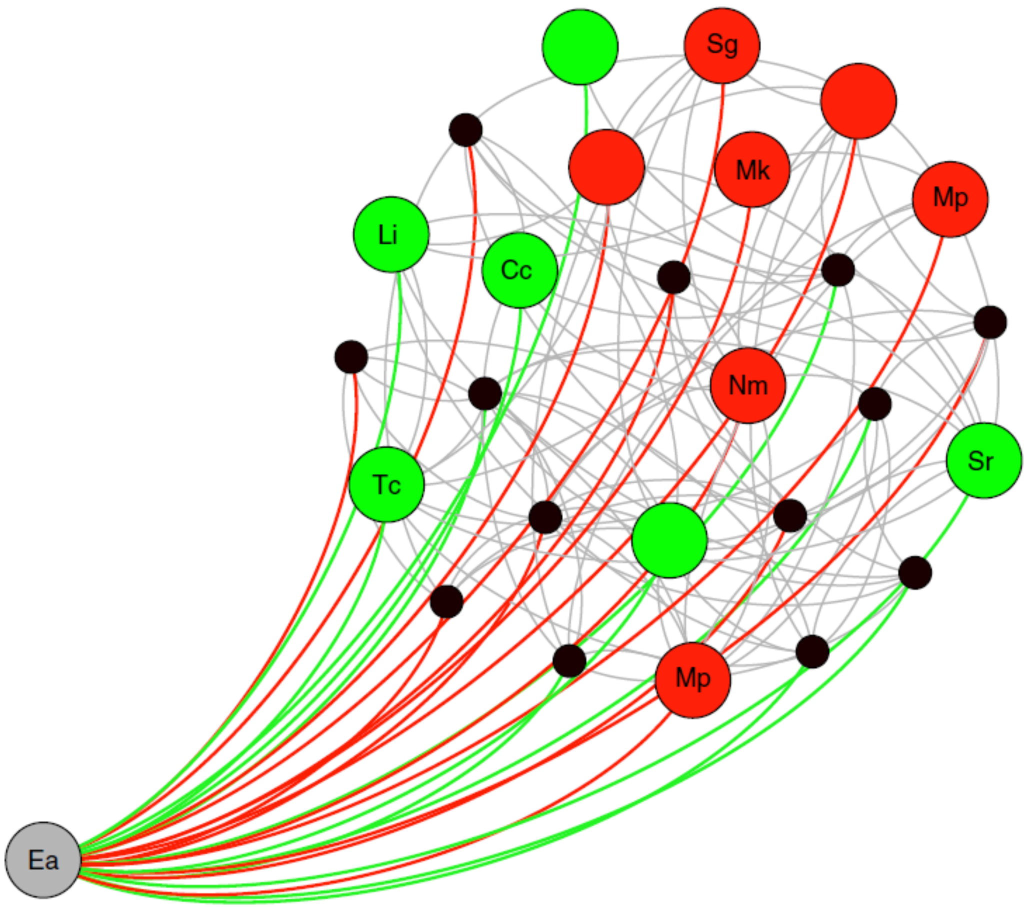
From correlations to putative interactions

1. Position of the leaf in the crown taken as a proxy of its abiotic environment (temperature, UV)
2. Effect of leaf position and sequencing depth on the number of reads per OTU assessed using NBGLMs
3. Inference of direct associations between OTUs based on the residuals of the model

Schwaller et al. 2015. Bayesian inference of graphical model structures using trees.

Jakuschkin et al. 2016

Network of putative interactions



13 bacterial OTUs

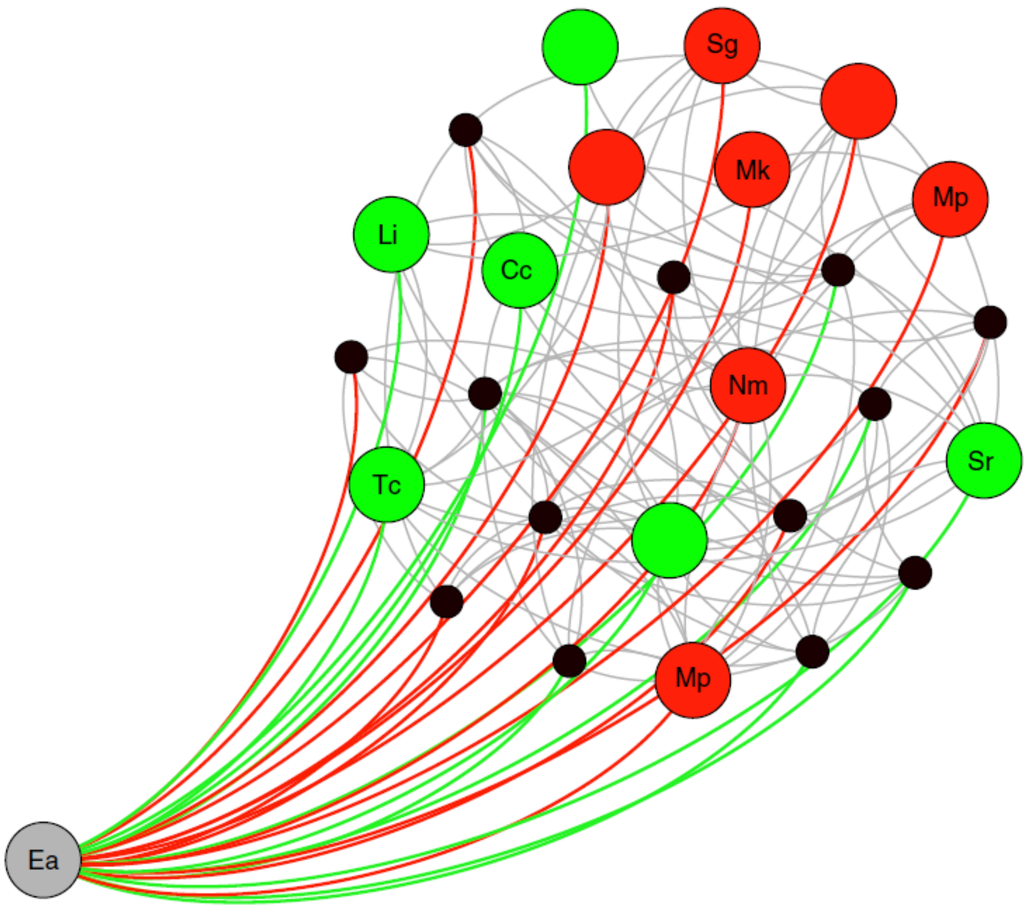
13 fungal species

6 putative facilitators (or facilitated species)

7 putative antagonists

Jakuschkin et al. 2016

Network of putative interactions



Major limitations

✓ **No experimental validation**

✓ **No replication**

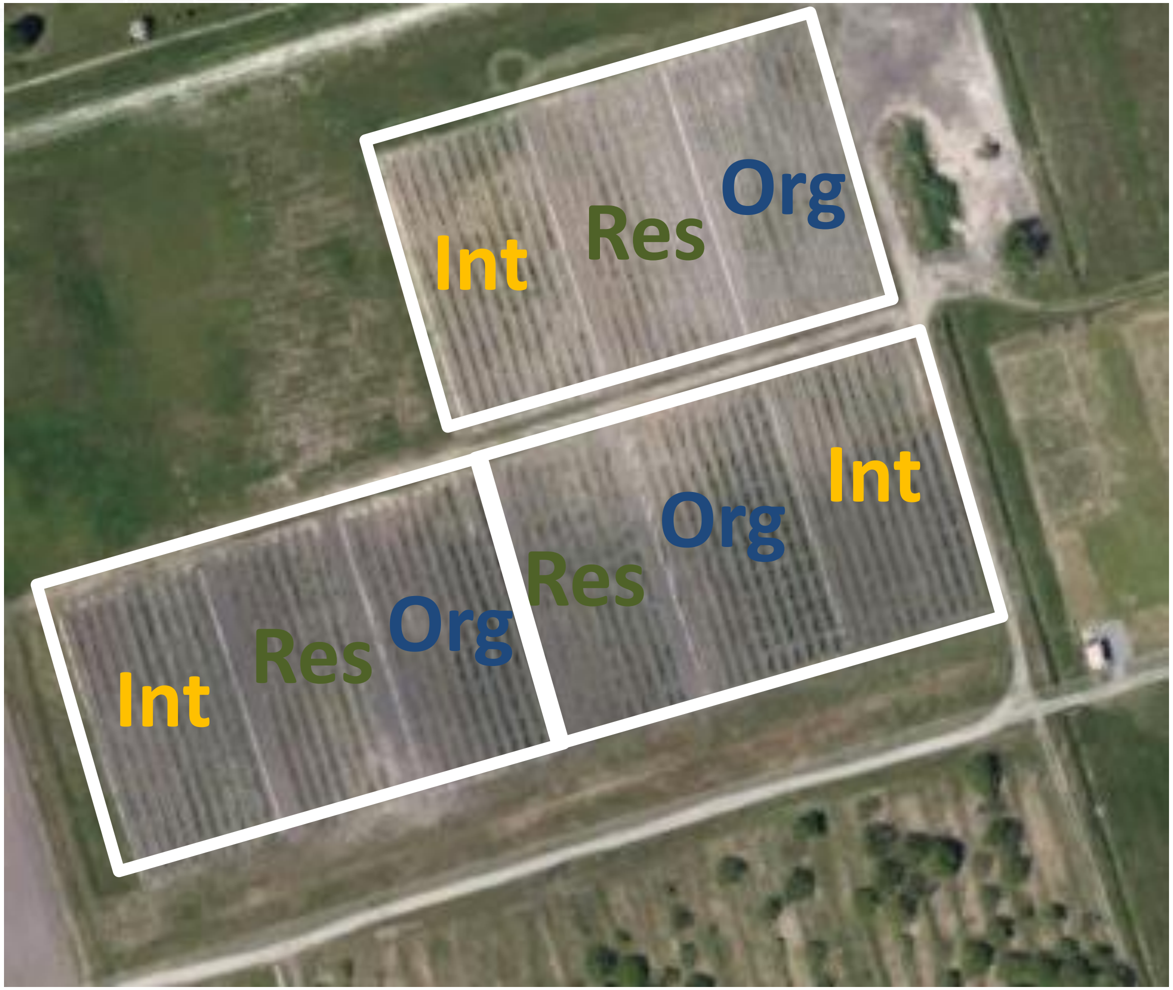
Case study #2

Who's interacting with *Erysiphe necator*, the causal agent of grapevine powdery mildew?

- Replicated networks
- Explicit covariates
- Experimental validation
- Statistics & Machine-Learning

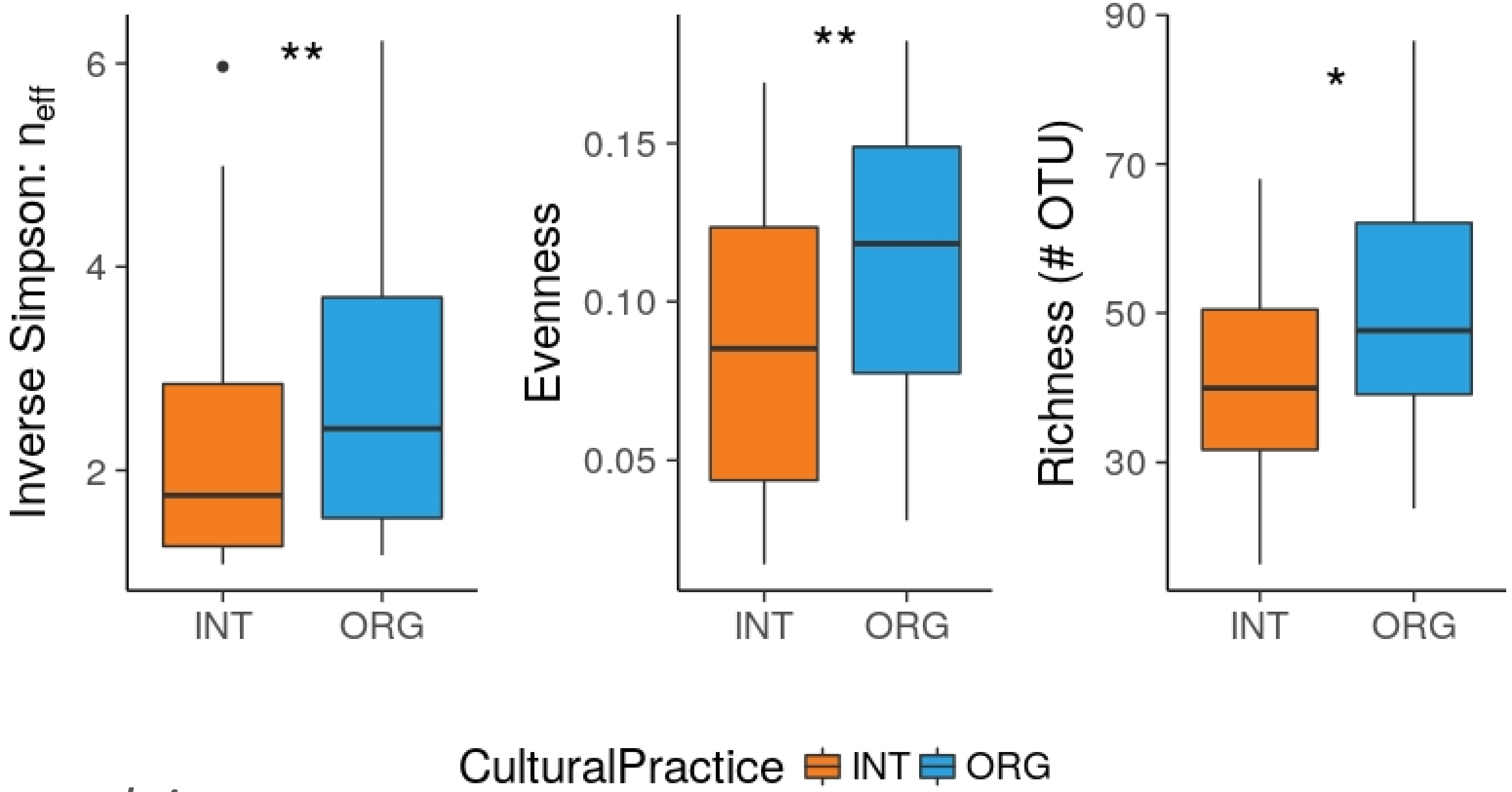
Charlie Pauvert's PhD thesis

Experimental design



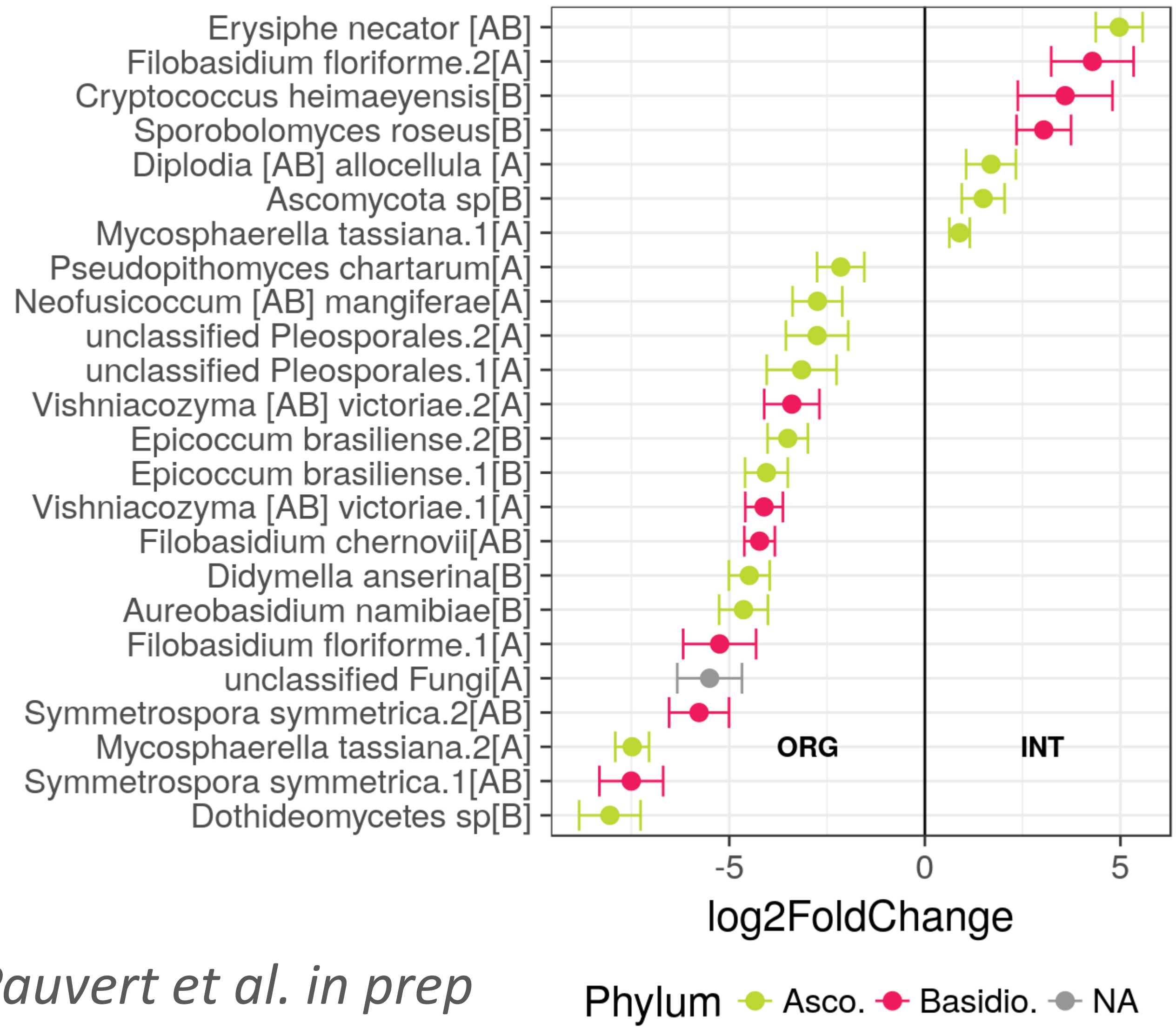
ResIntBio experiment, INRA-Bordeaux (PI: Laurent Delière & Jessica Vallance)

Influence of the farming system on microbial diversity



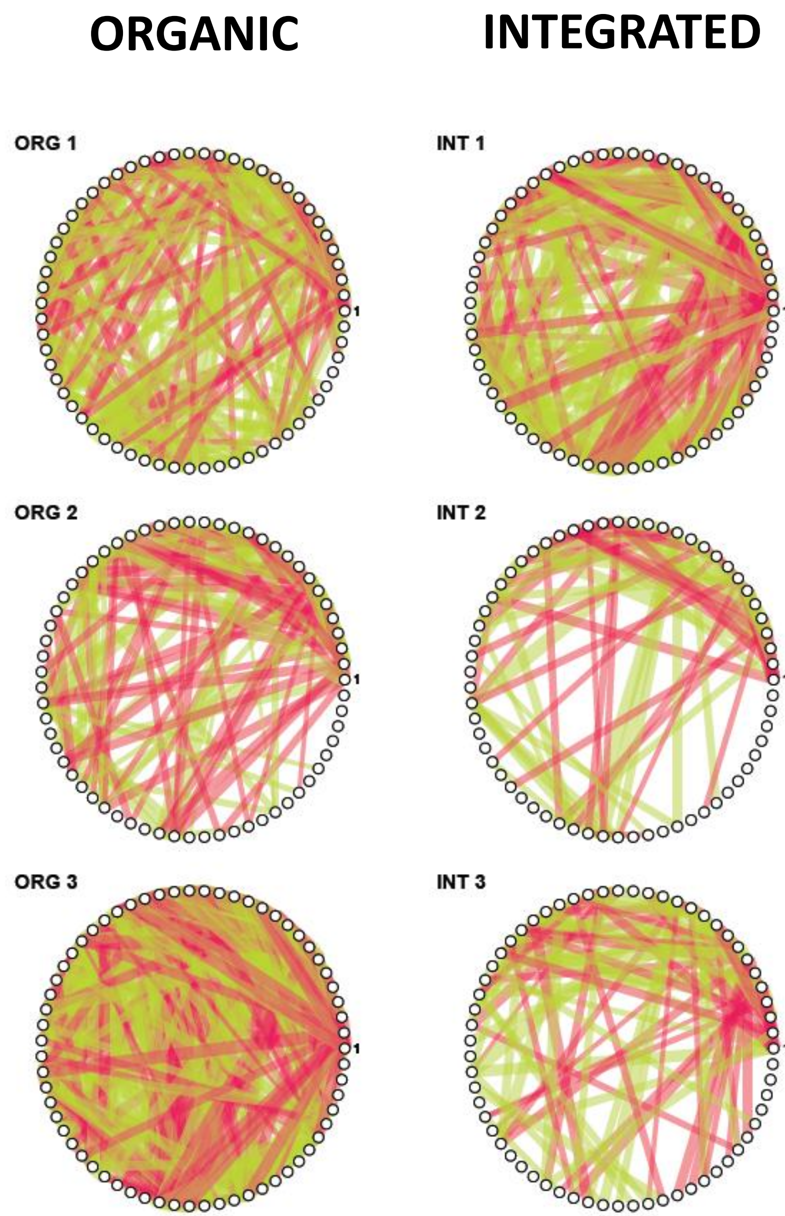
Pauvert et al. in prep

Influence of the farming system on microbial composition



Pauvert et al. in prep

Influence of the farming system on microbial associations



Microbial association network inferred using SparCC

No effect of the farming system on network properties

Pauvert et al. in prep

Questions

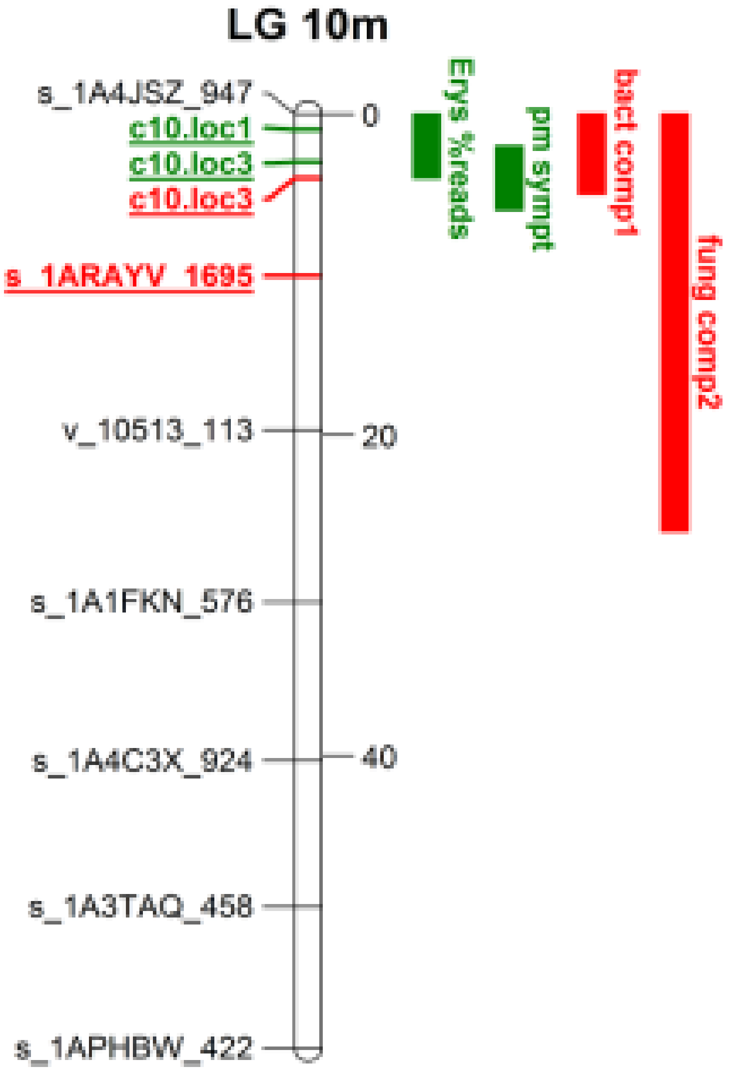
1 How to reconstruct microbial interaction networks?

2 **Can the plant select for beneficial network properties?**

Genetic architecture of variation in phyllosphere microbiota

Quercus robur

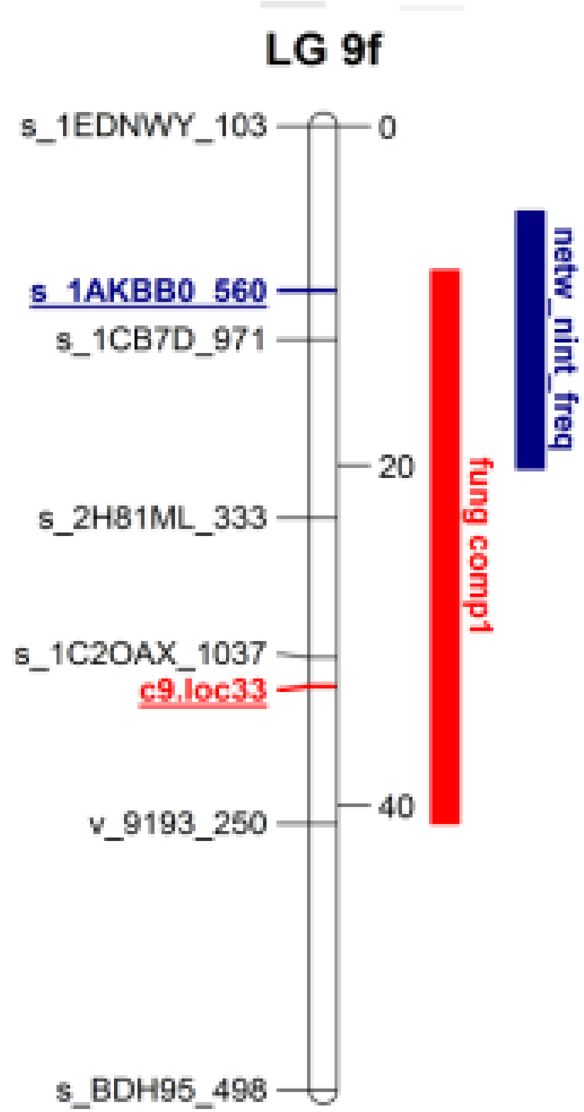
Full-sib family (Bourran, FR)



QTLs for variation in fungal and bacterial community composition

Colocalization between QTLs that control bacterial composition and susceptibility to powdery mildew

Jakuschkin et al. in prep



QTLs for variation in microbial association network properties

Microbial association network inferred using SparCC

Significant QTL for the frequency of negative associations (PEV=16.6%)

Jakuschkin et al. in prep

Research priorities (1)

Assess the relative roles of **dispersal, evolutionary diversification, selection, and drift** in shaping PMCs

Decipher phyllosphere **microbial interaction networks** and assess their role in plant health

Identify of the **plant traits** that shape PMCs and the underlying **genes** in model and non-model plant species

Research priorities (2)

Identify which **whole-community properties** of PMCs mediate plant performance and ecosystem functions

Assess the influence of environmental change on the **evolutionary diversification** of PMCs and the subsequent effects on plant fitness

Analyse the **global biogeography** of PMCs; predict changes in PMCs distribution and function under climate change scenarios.

Applied perspectives

BIOCONTROL

Identify automatically potential antagonists of plant pathogens

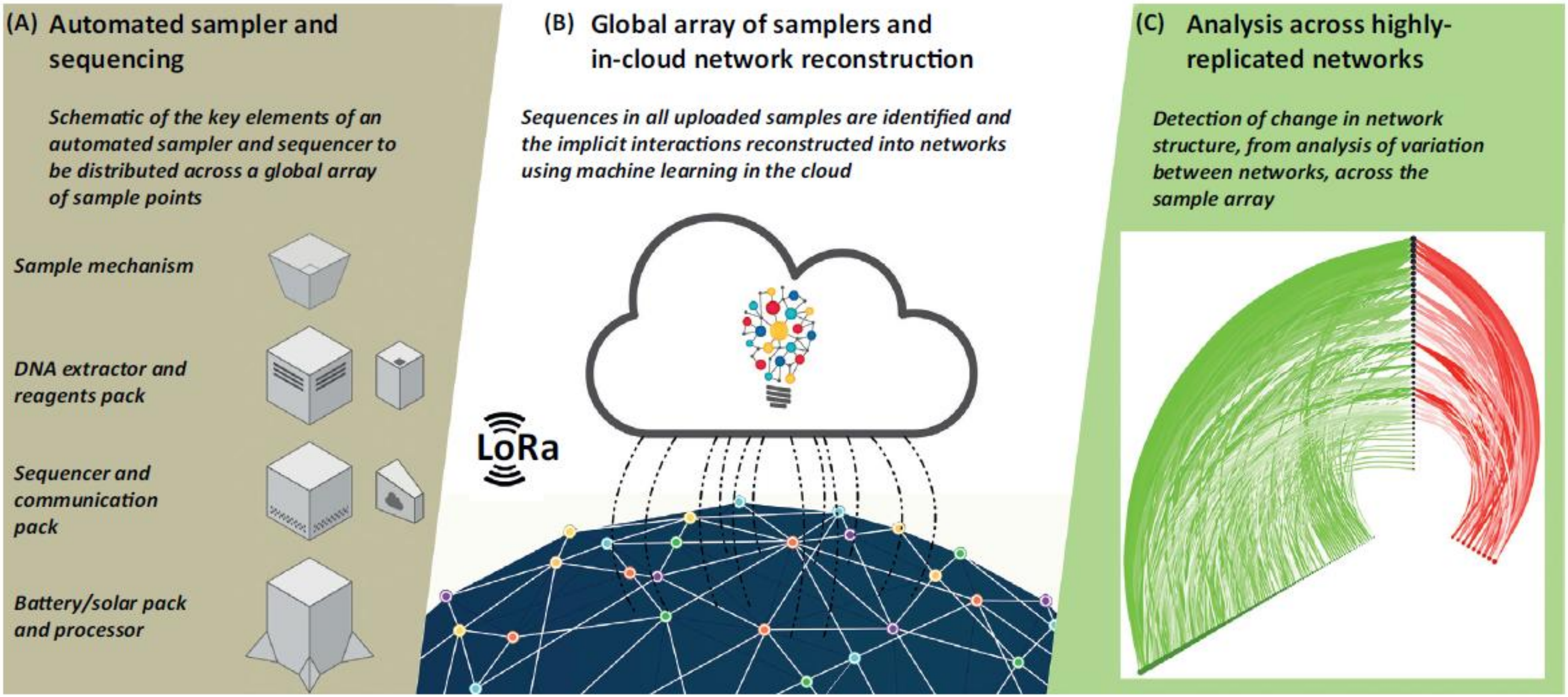
PLANT BREEDING

Create plant varieties that select for beneficial microbial communities

BIOMONITORING

Use NGS networks to monitor changes in ecosystem functioning

Next-Generation Biomonitoring



Bohan et al. 2017

Many thanks to…

The Phyllosphere: Microbial Jungle at the Plant–Climate Interface

Corinne Vacher,[1] Arndt Hampe,[2] Annabel J. Porté,[1] Ursula Sauer,[3] Stéphane Compant,[3] and Cindy E. Morris[4]

Annu. Rev. Ecol. Evol. Syst. 2016. 47:1–24

AIT, Austria

INRA

Many thanks to…

Stéphane Robin, INRA, Paris
- ➢ Statistical Inference of Ecological Networks

Dave Bohan, INRA, Dijon
- ➢ Machine-Learning of Ecological Networks

Trends in Ecology & Evolution

Next-Generation Global Biomonitoring: Large-scale, Automated Reconstruction of Ecological Networks

David A. Bohan,[1,*] Corinne Vacher,[2] Alireza Tamaddoni-Nezhad,[3] Alan Raybould,[4] Alex J. Dumbrell,[5] and Guy Woodward[6]

Many thanks to…

Boris Jakushkin

Deciphering the Pathobiome: Intra- and Interkingdom Interactions Involving the Pathogen *Erysiphe alphitoides*

Boris Jakuschkin[1] · Virgil Fievet[1] · Loïc Schwaller[2,3] · Thomas Fort[1] · Cécile Robin[1] · Corinne Vacher[1]

Thomas Fort

PeerJ

Foliar fungal communities strongly differ between habitat patches in a landscape mosaic

Thomas Fort[1], Cécile Robin[1], Xavier Capdevielle[1], Laurent Delière[2] and Corinne Vacher[3]

Many thanks to…

Charlie Pauvert

Learning microbial networks from NGS data: application to biocontrol

Tania Fort

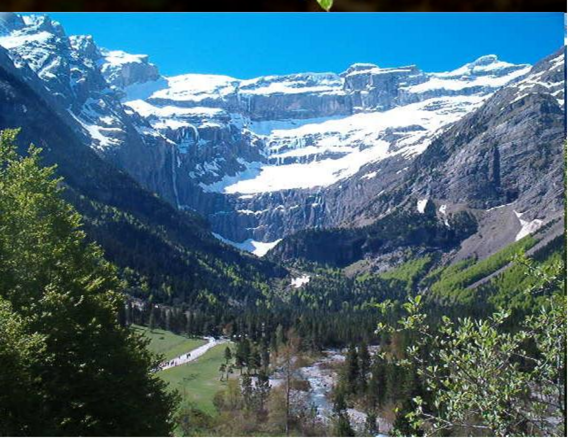
Influence of microclimate on leaf microbiota and feedback effects on leaf physiology and phenology

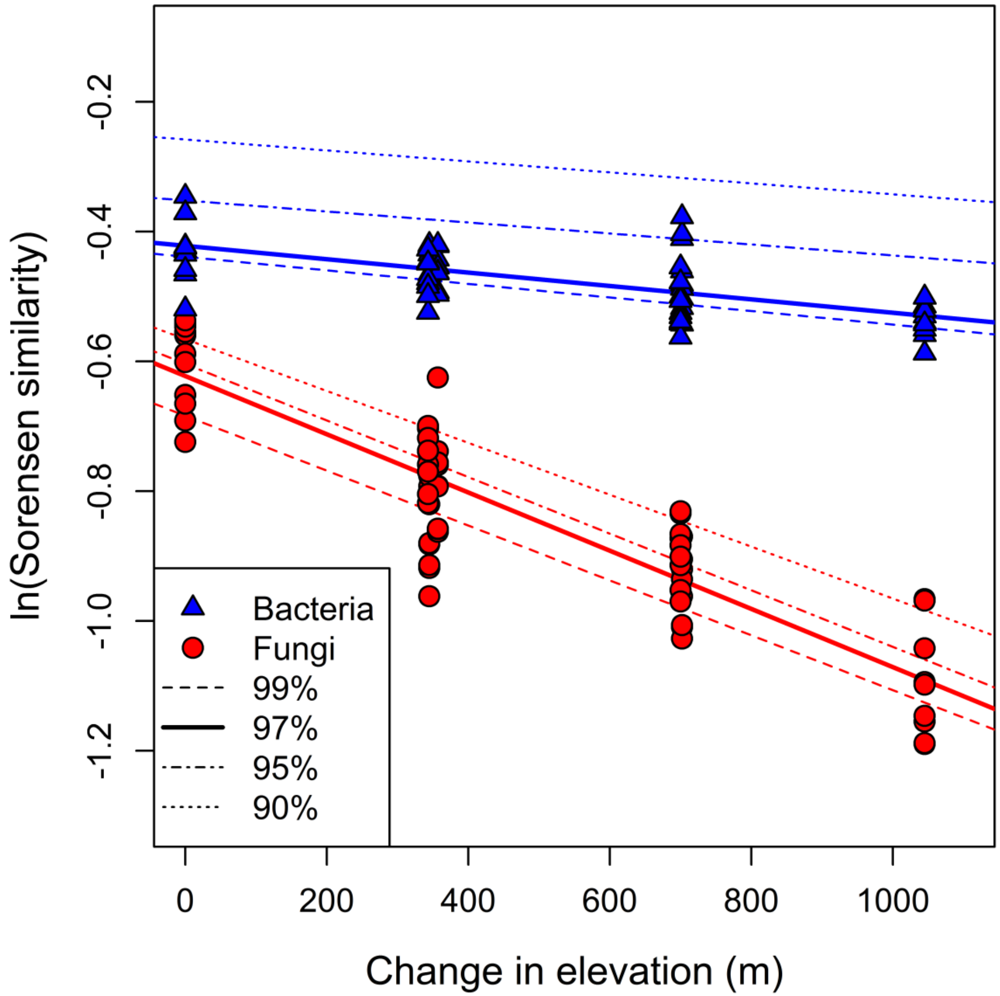
Perspectives

VERTIGE project (PI: Heidy Schimann, UMR EcoFoG)

COPAS, Nouragues, French Guyana

Distance-decay of similarity in PMCs





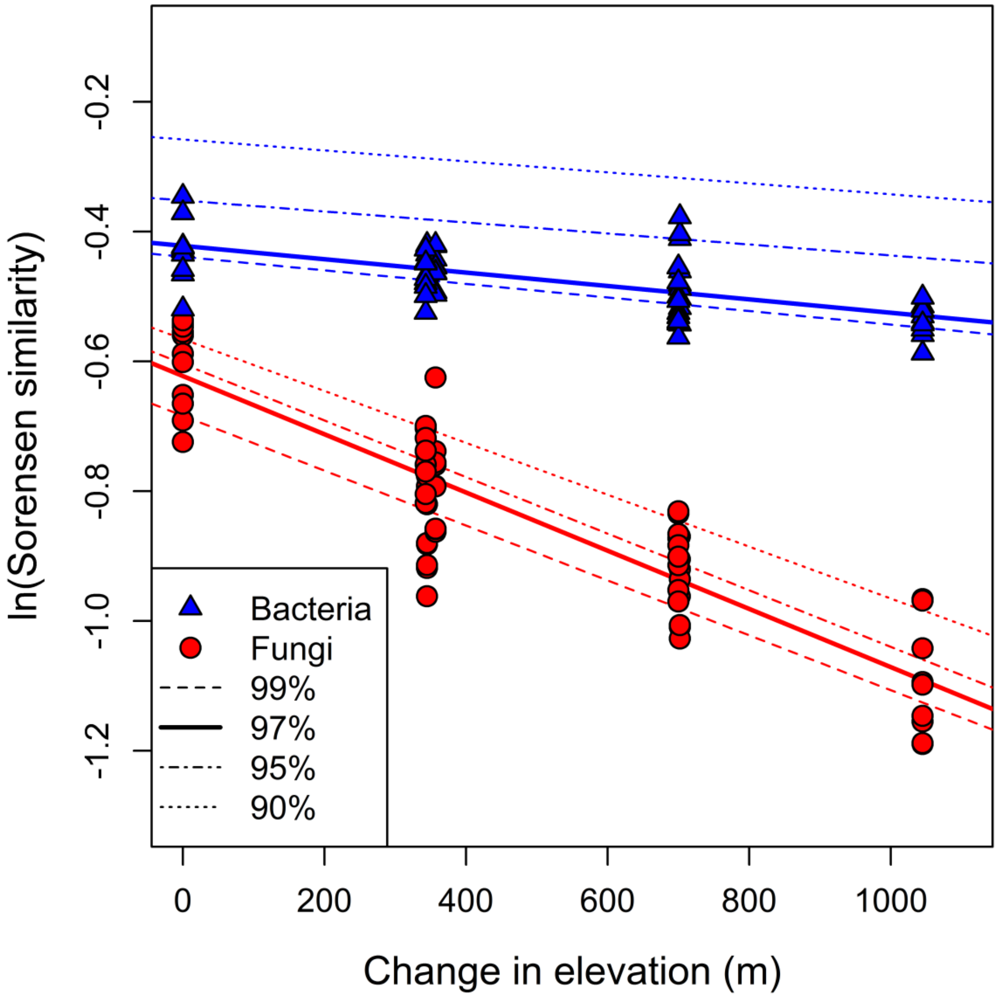
Vacher et al. 2016

Distance-decay of similarity in PMCs

Higher dispersal abilities of bacteria

Higher sensitivity of fungi to temperature variations

Methodological bias (different barcodes)



Vacher et al. 2016

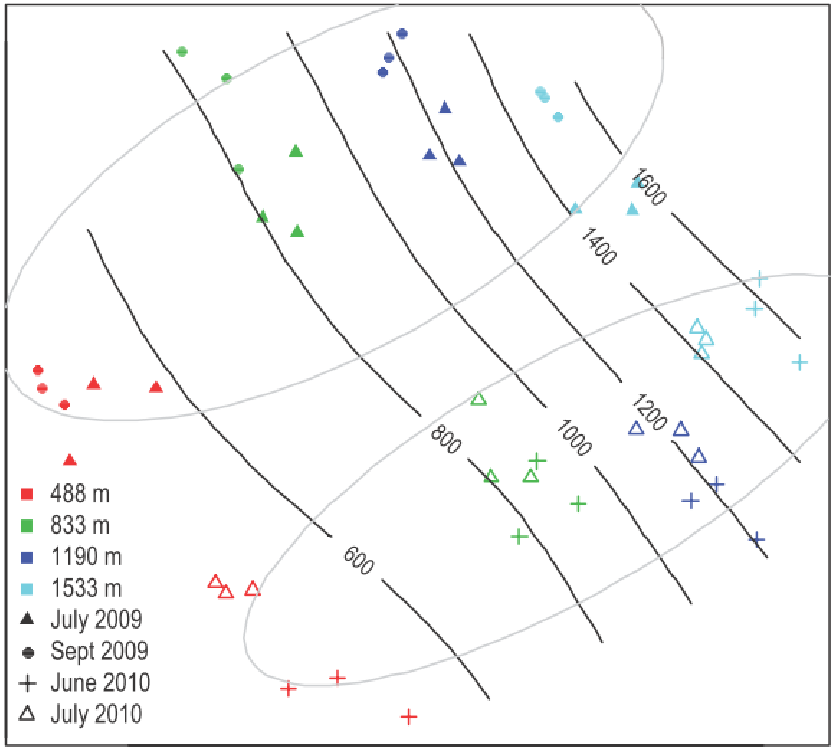
Measurement of bacterial emissions above plant canopies

Microflux project (PI: Yves Brunet)

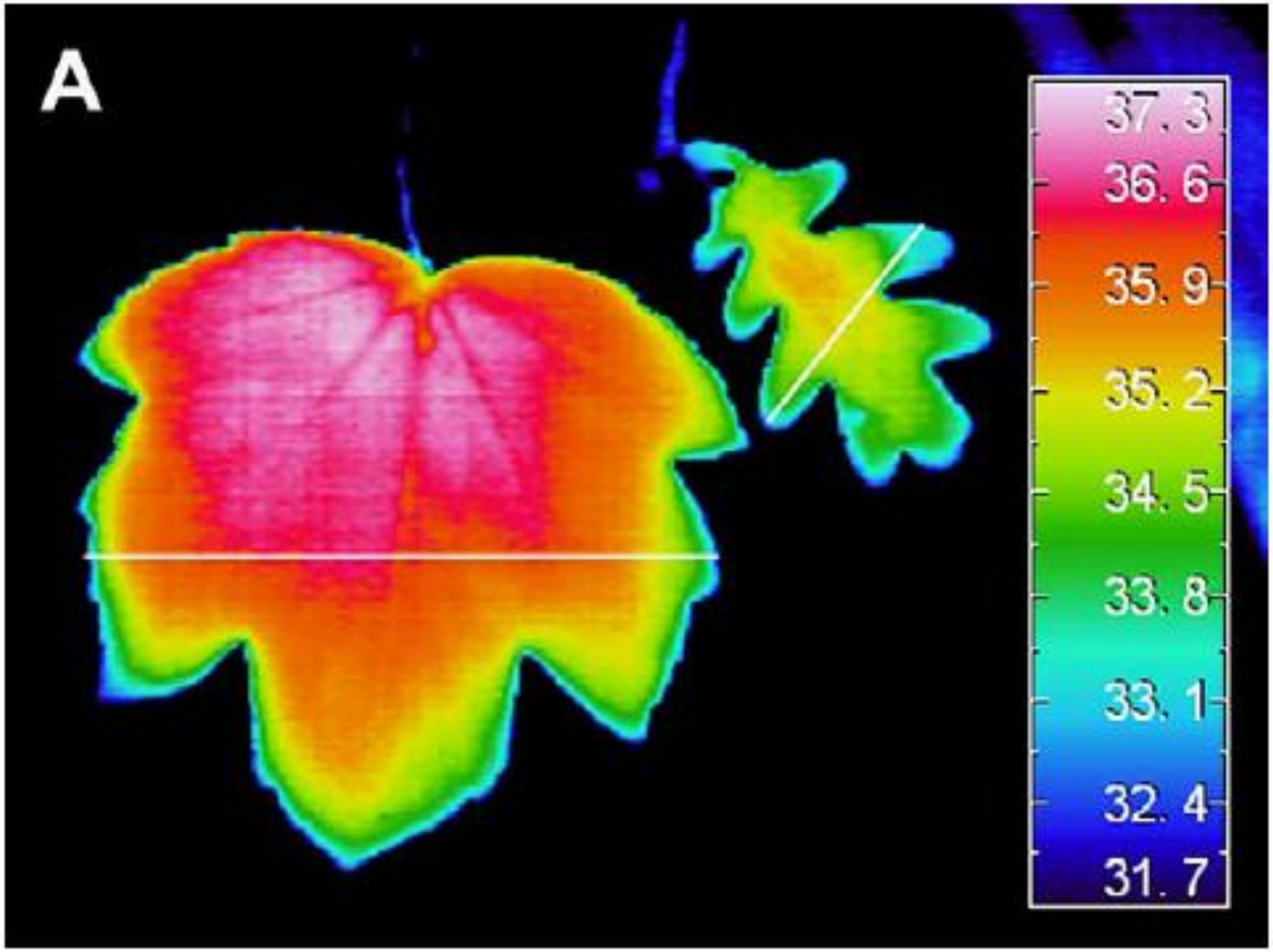
New Phytologist

The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient

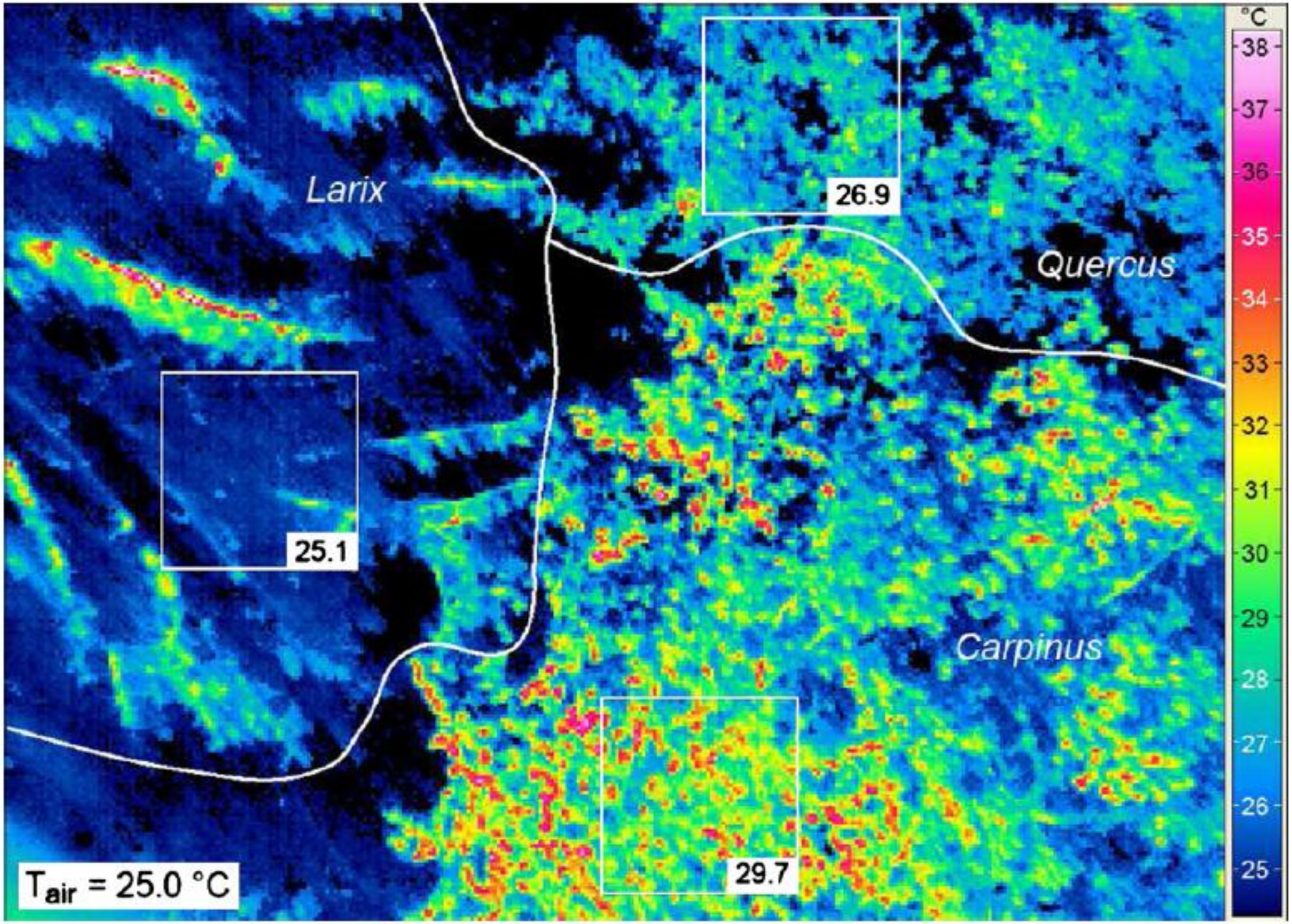
Tristan Cordier[1,2], Cécile Robin[1,2], Xavier Capdevielle[1,2], Olivier Fabreguettes[1,2], Marie-Laure Desprez-Loustau[1,2] and Corinne Vacher[1,2]



Phyllosphere: a heterogeneous habitat



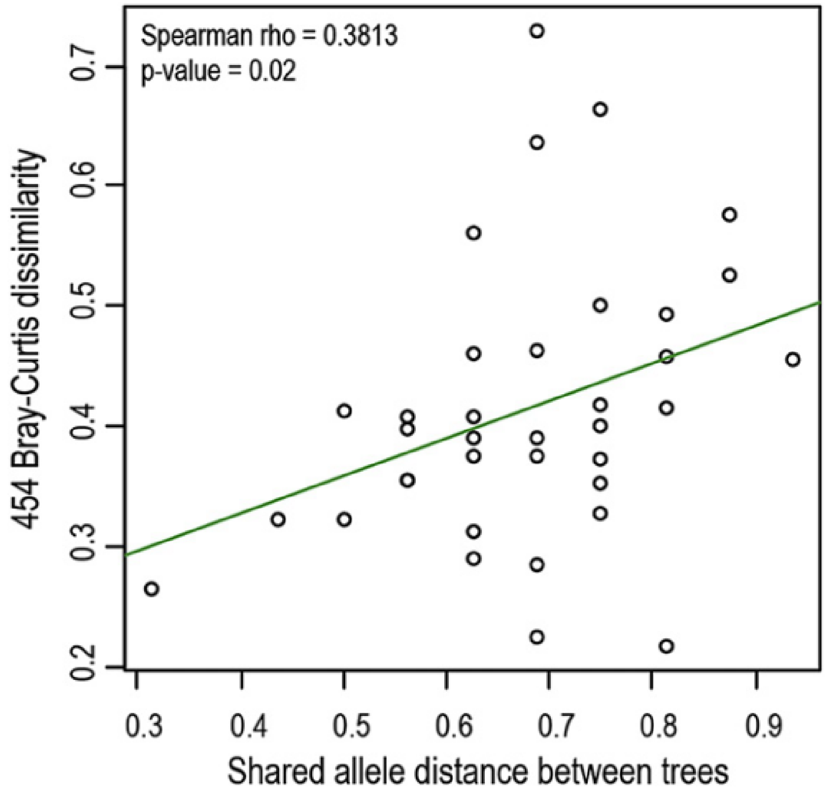
Stokes et al. 2006

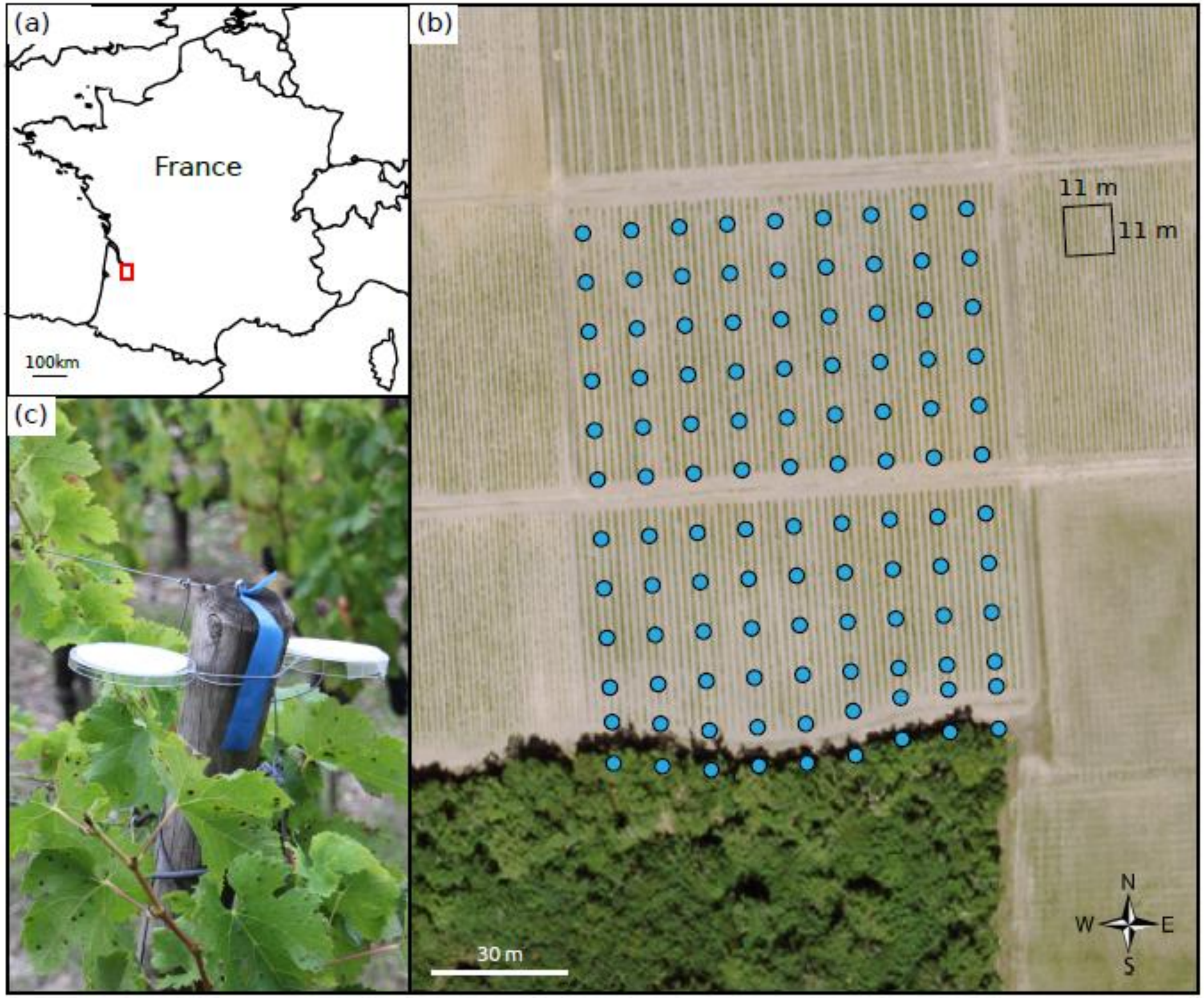


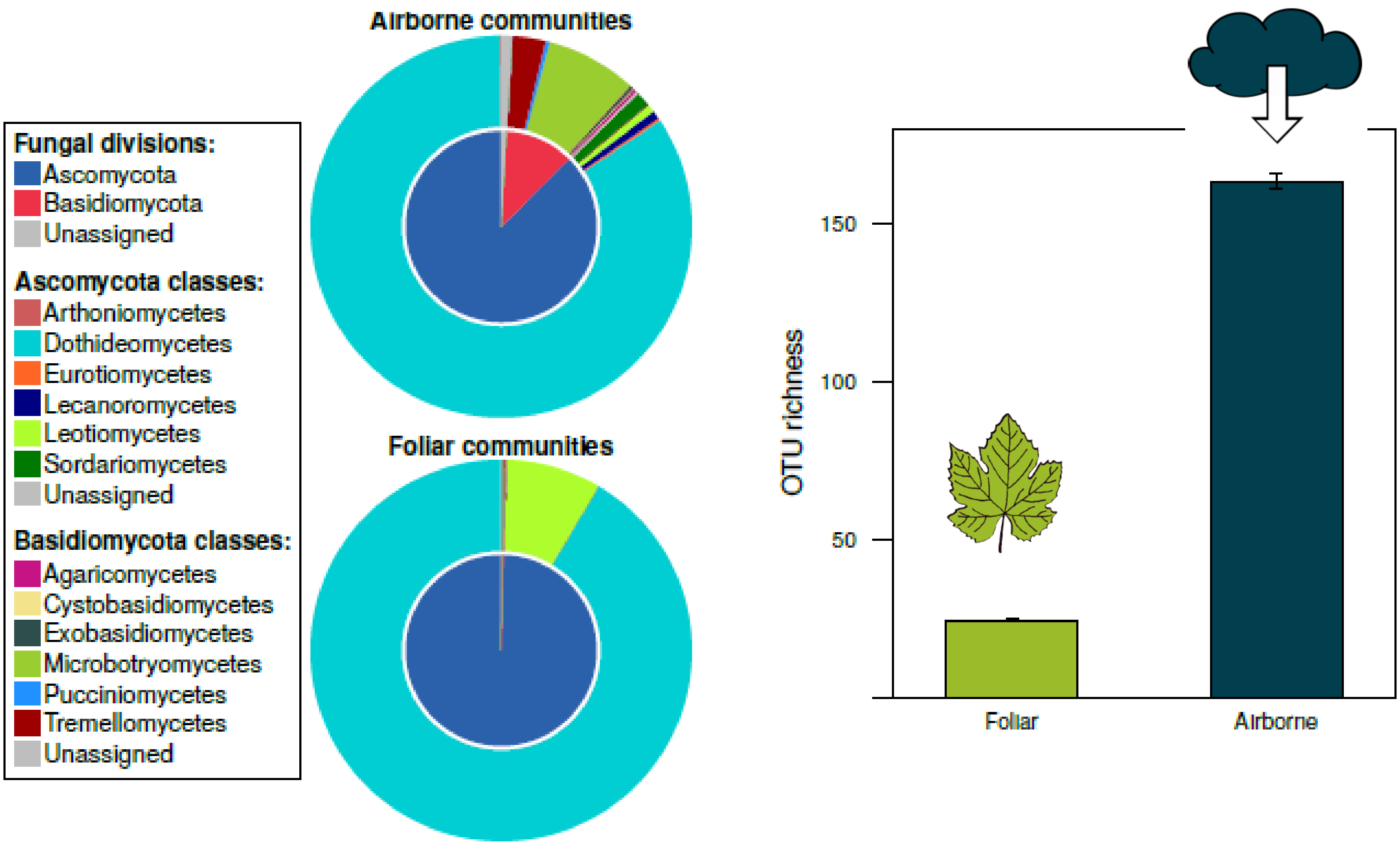
Leuzinger & Korner 2007

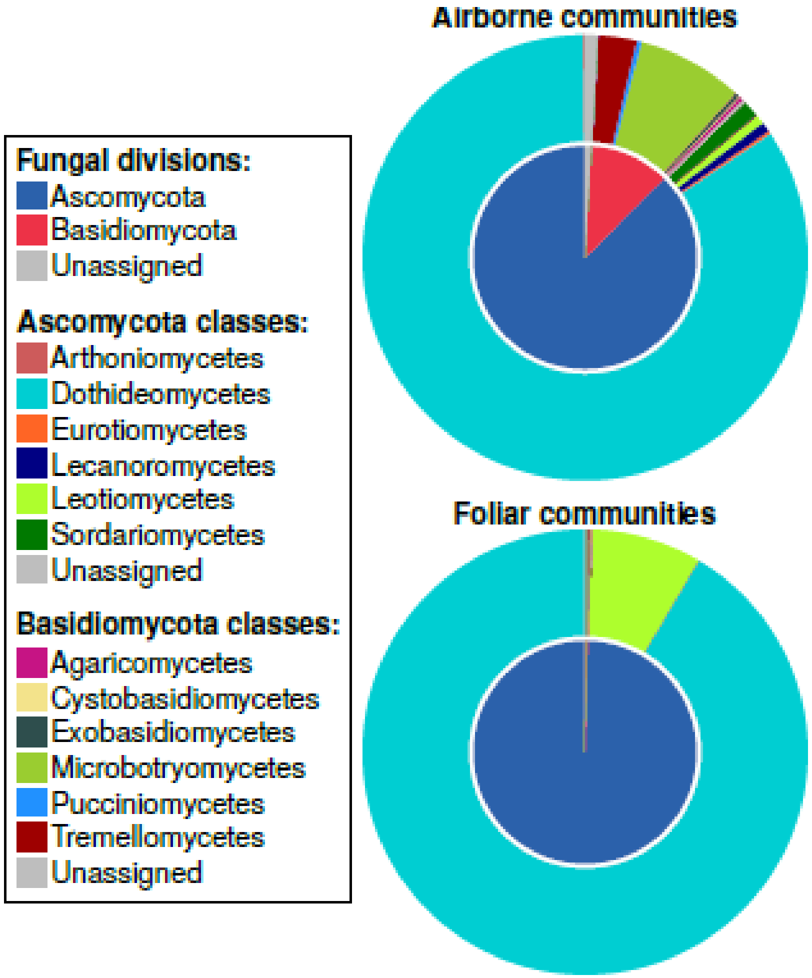
Spatial variability of phyllosphere fungal assemblages: genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*)

Tristan CORDIER[a,b,*], *Cécile ROBIN*[a,b], *Xavier CAPDEVIELLE*[a,b], *Marie-Laure DESPREZ-LOUSTAU*[a,b], *Corinne VACHER*[a,b]




(a)
France
100km
(c)
(b)
11 m
11 m
30 m
N
W
E
S


Airborne communities
Foliar communities
Fungal divisions:
Ascomycota
Basidiomycota
Unassigned
Ascomycota classes:
Arthoniomycetes
Dothideomycetes
Eurotiomycetes
Lecanoromycetes
Leotiomycetes
Sordariomycetes
Unassigned
Basidiomycota classes:
Agaricomycetes
Cystobasidiomycetes
Exobasidiomycetes
Microbotryomycetes
Pucciniomycetes
Tremellomycetes
Unassigned
OTU richness
150
100
50
Foliar
Airborne



Airborne communities
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Eurotiomycetes
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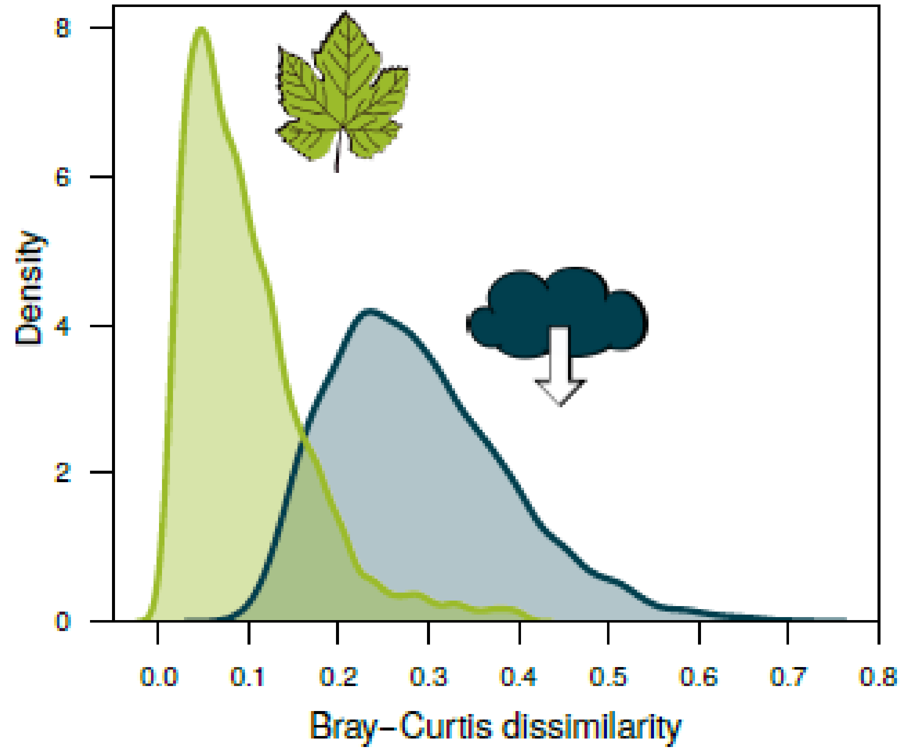
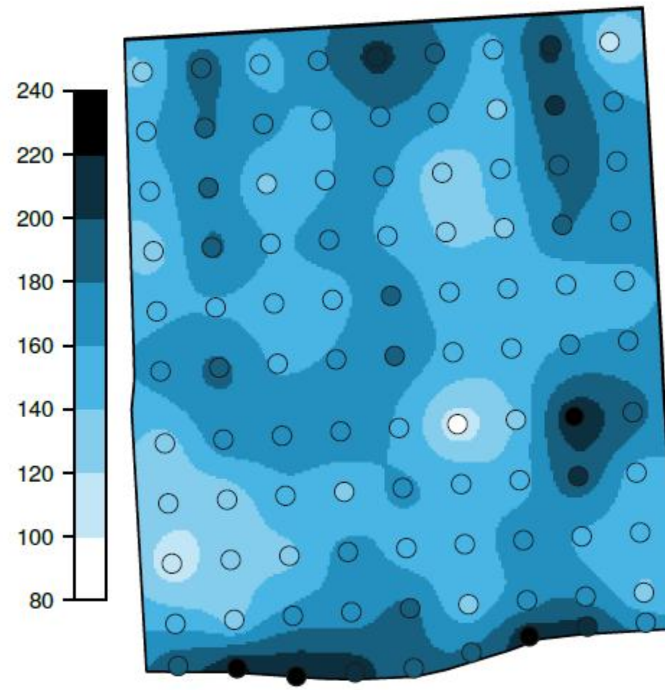
Leotiomycetes
Sordariomycetes
Unassigned
Basidiomycota classes:
Agaricomycetes
Cystobasidiomycetes
Exobasidiomycetes
Microbotryomycetes
Pucciniomycetes
Tremellomycetes
Unassigned



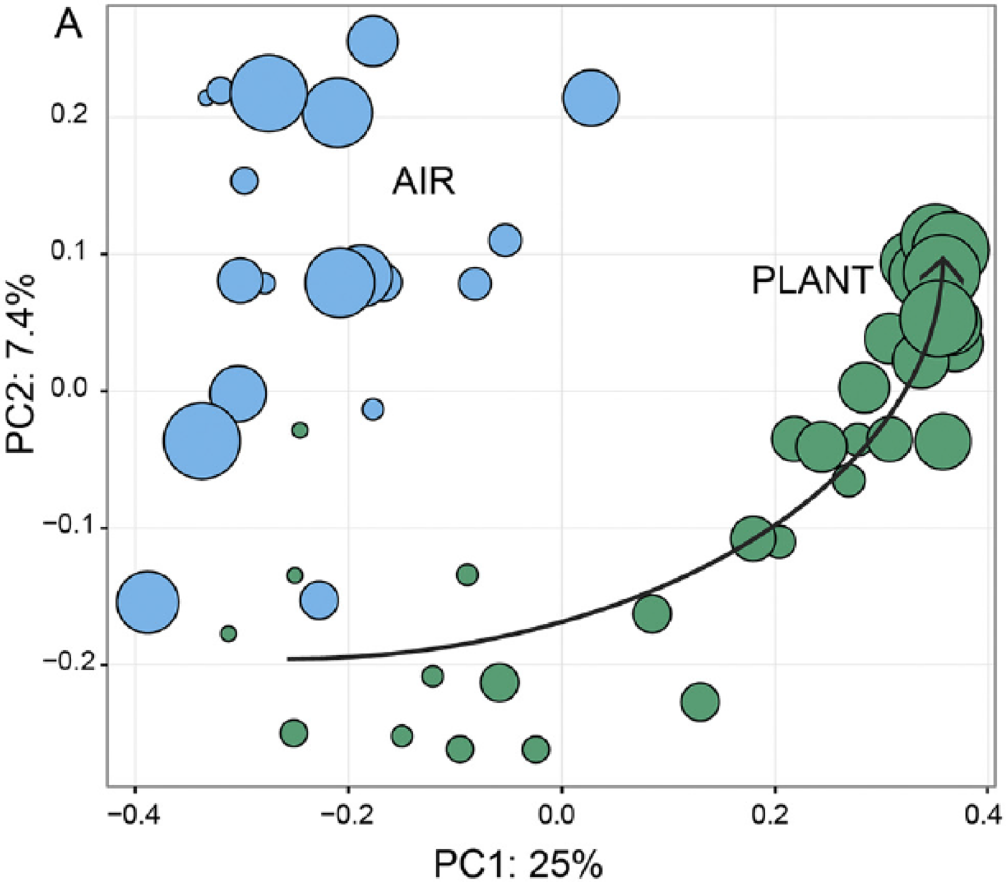
Density
0
2
4
6
8
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Bray–Curtis dissimilarity


11 m
11 m
30 m
N
W
E
S


240
220
200
180
160
140
120
100
80

Demonstration of external selection in controlled conditions

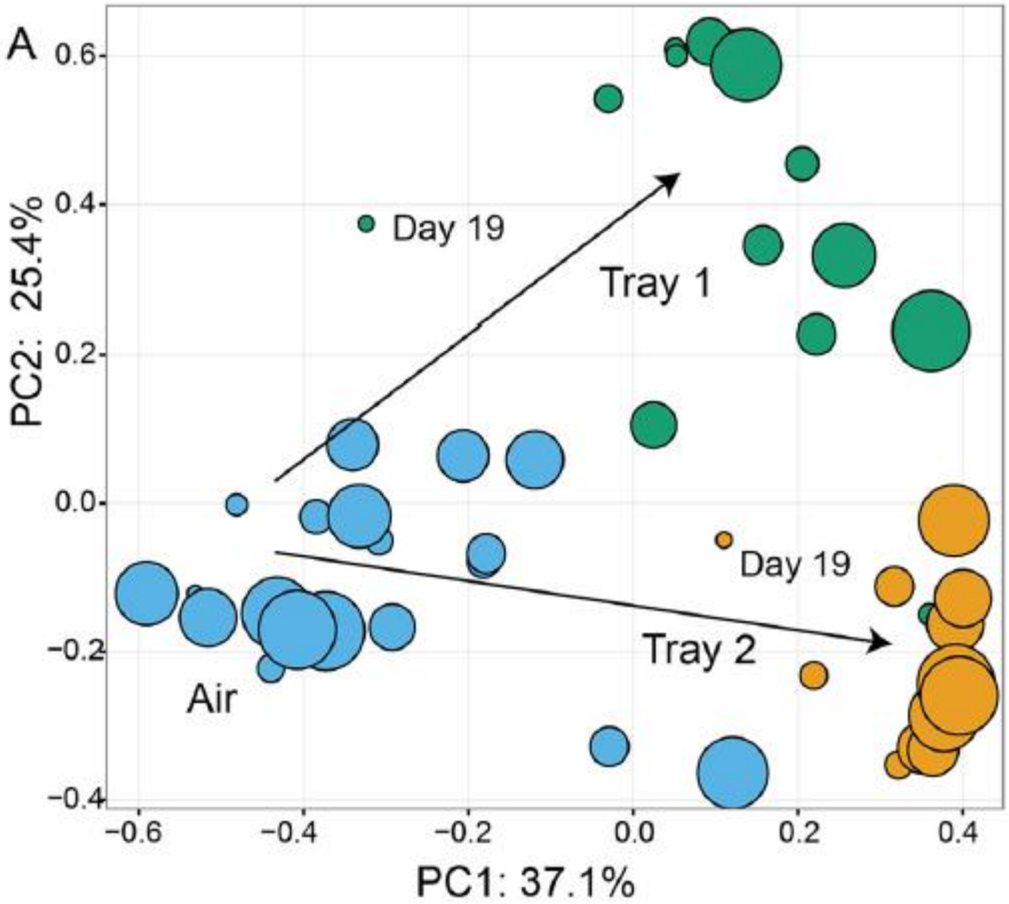
Sterilized seeds and soil



Maignien et al. 2014

Evidence for ecological drift

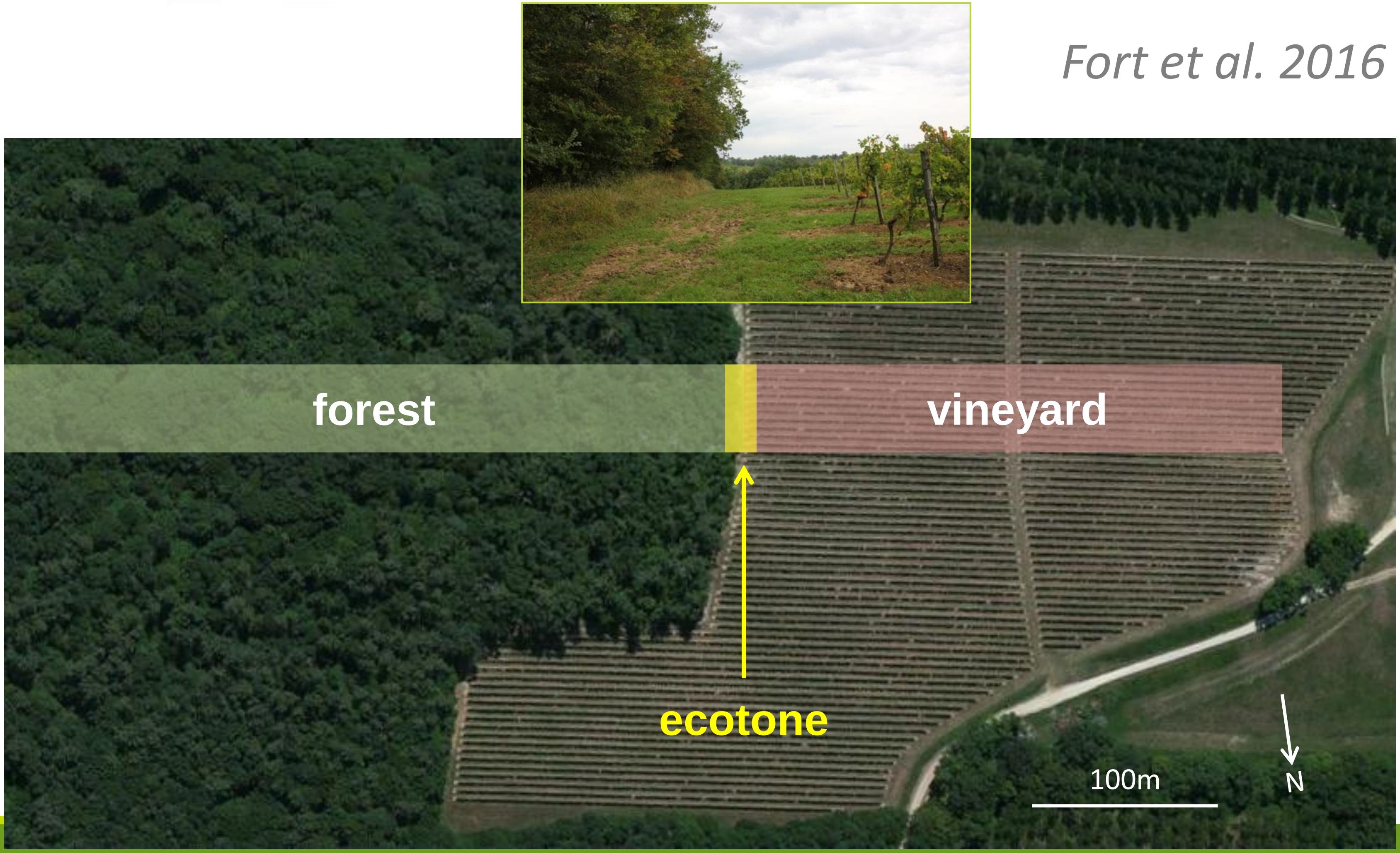
Stochasticity in colonization order and priority effects



Maignien et al. 2014

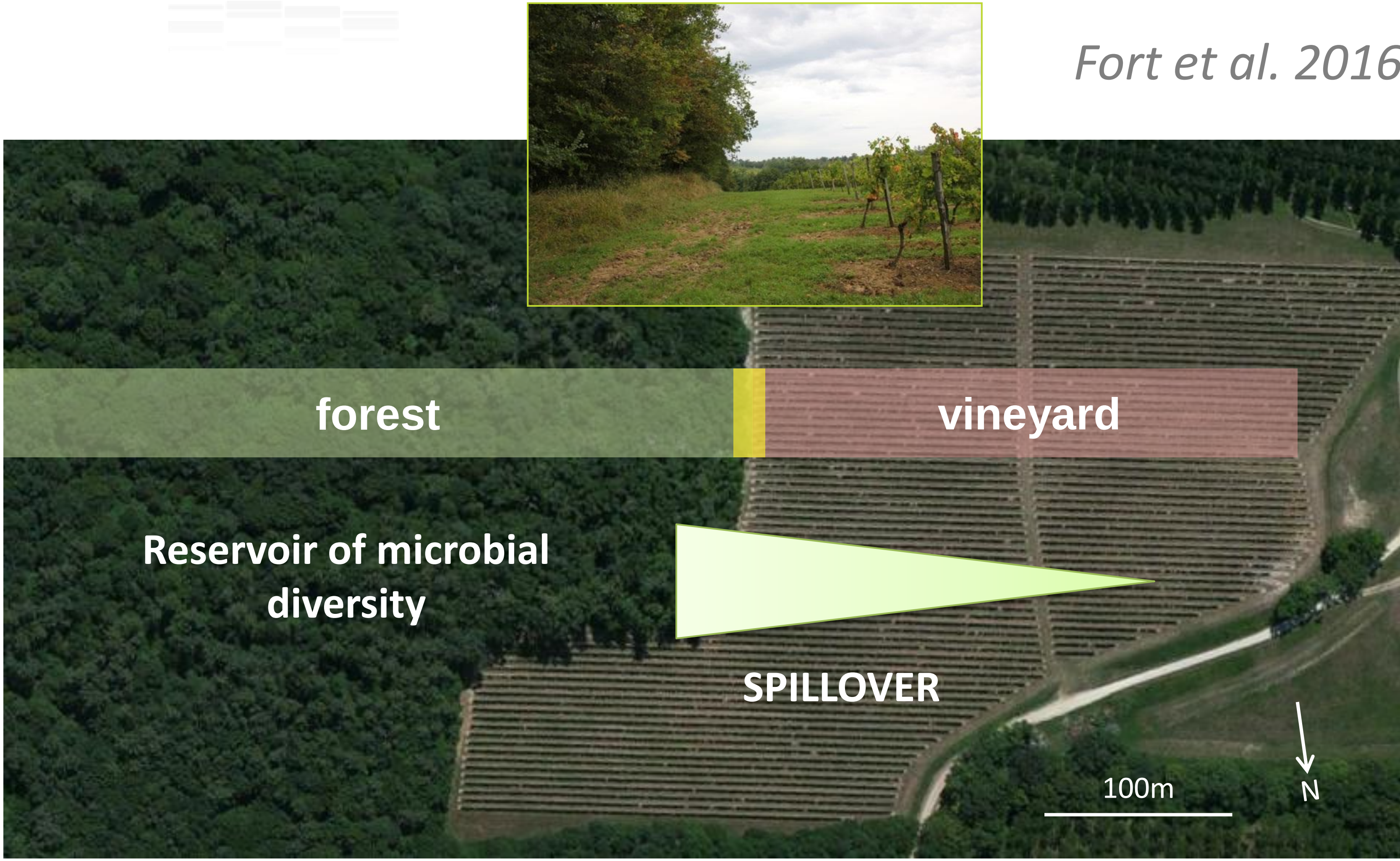
Demonstration of external selection in natural conditions

Fort et al. 2016

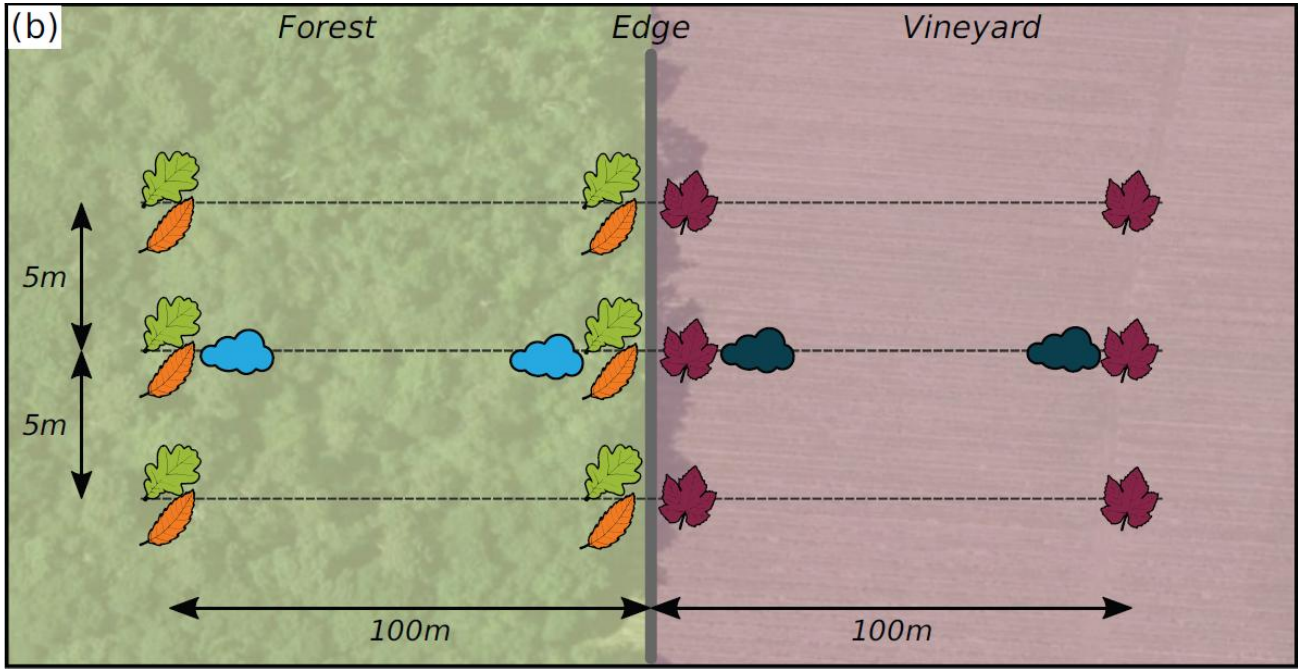


Hypothesis

Fort et al. 2016

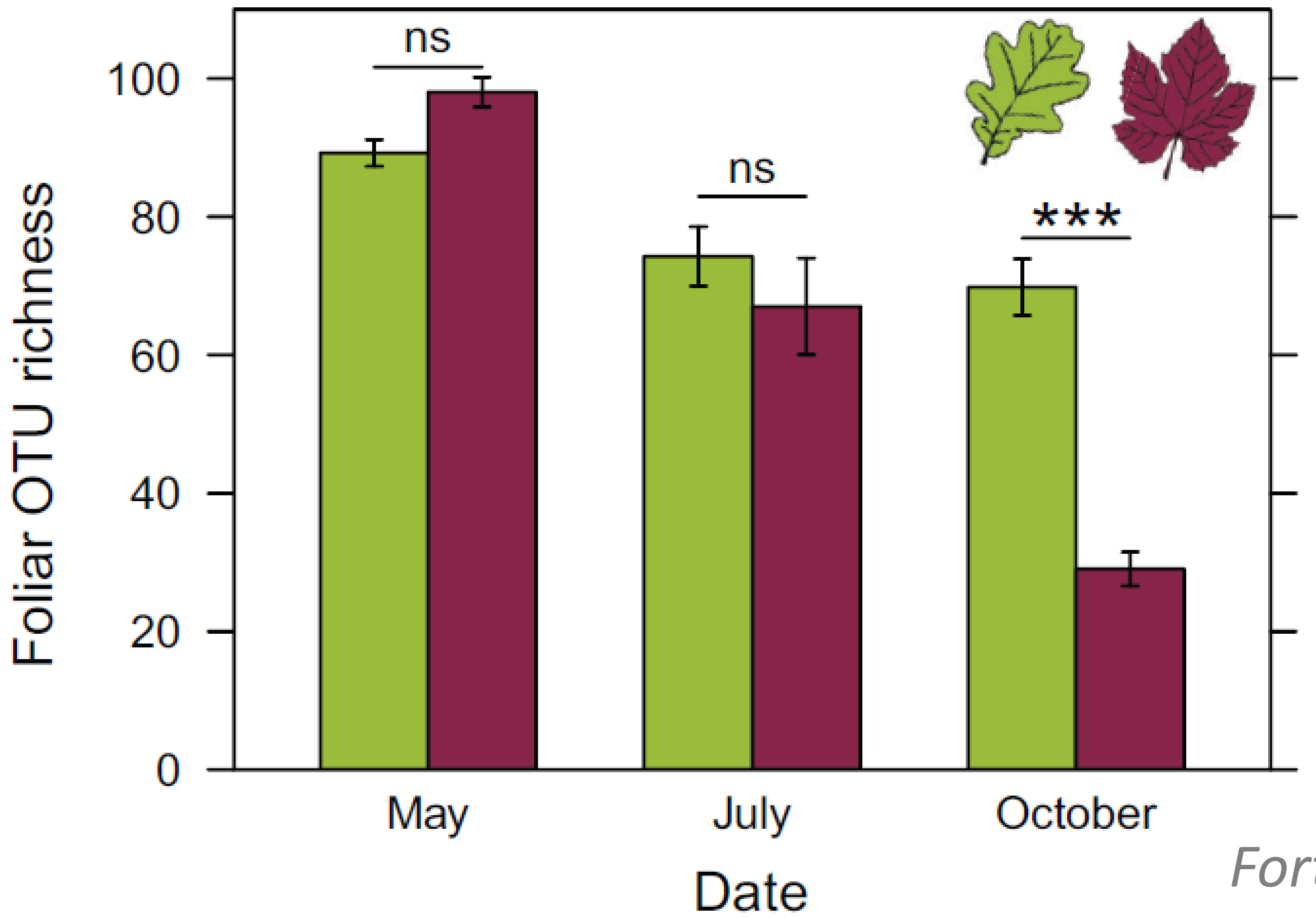


Sampling of airborne and phyllosphere microbial communities



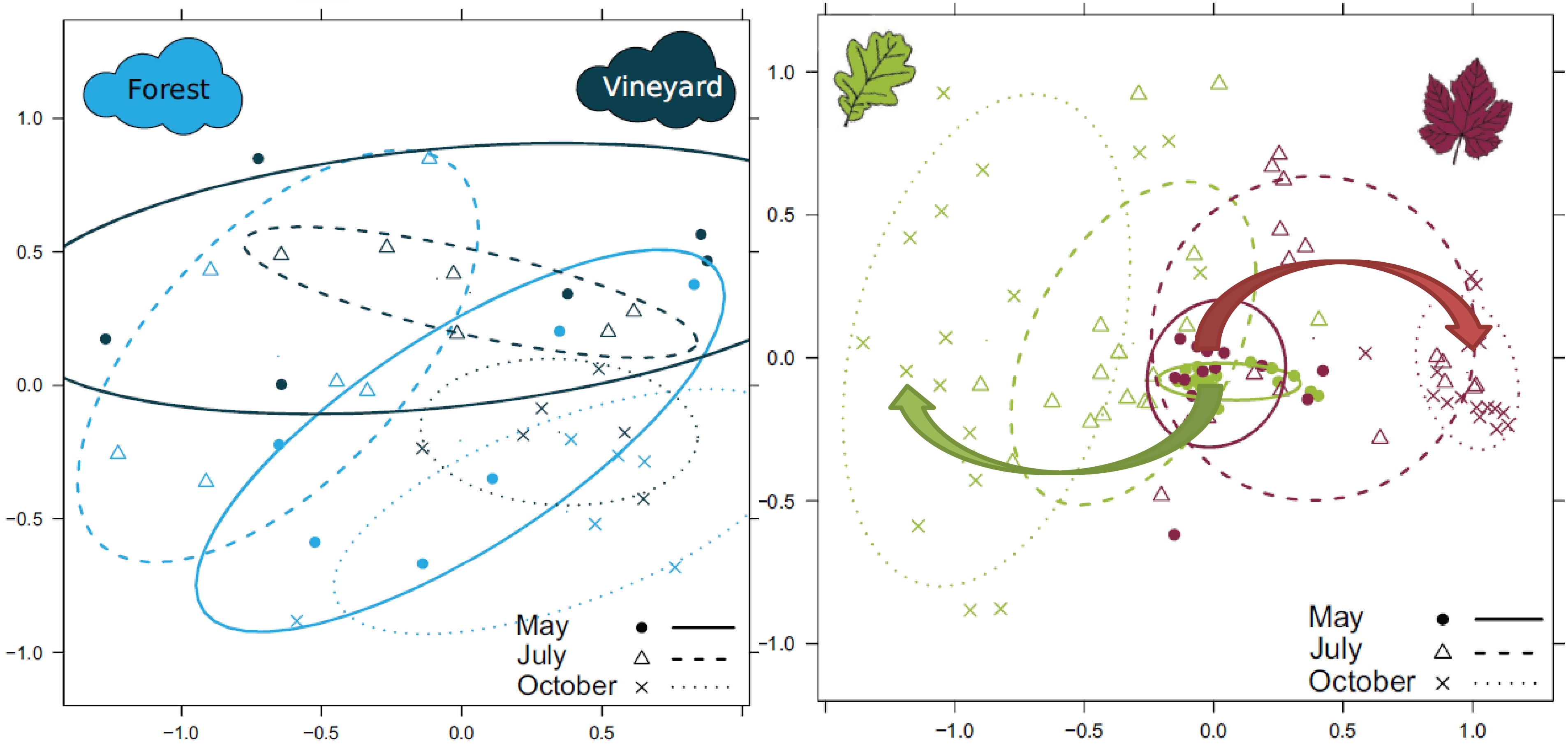
Fort et al. 2016

Forest patches are not a reservoir of microbial diversity



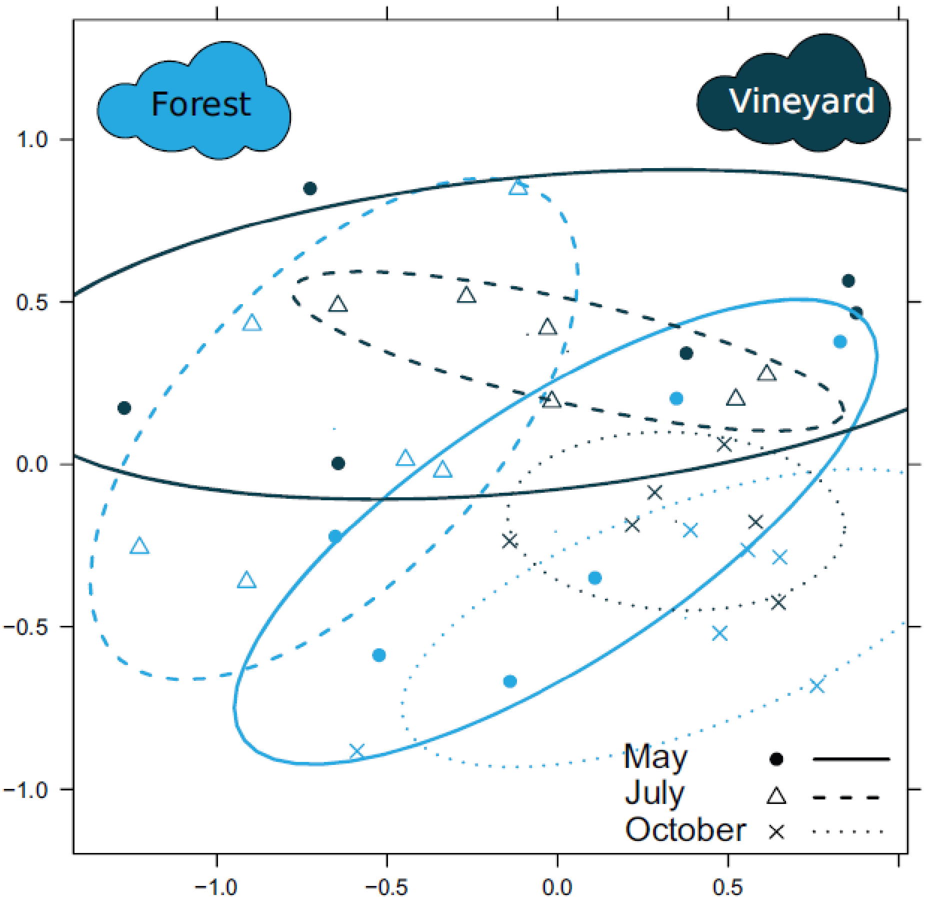
Fort et al. 2016

Airborne and phyllosphere communities have different dynamics



Fort et al. 2016

Airborne and phyllosphere communities have different dynamics

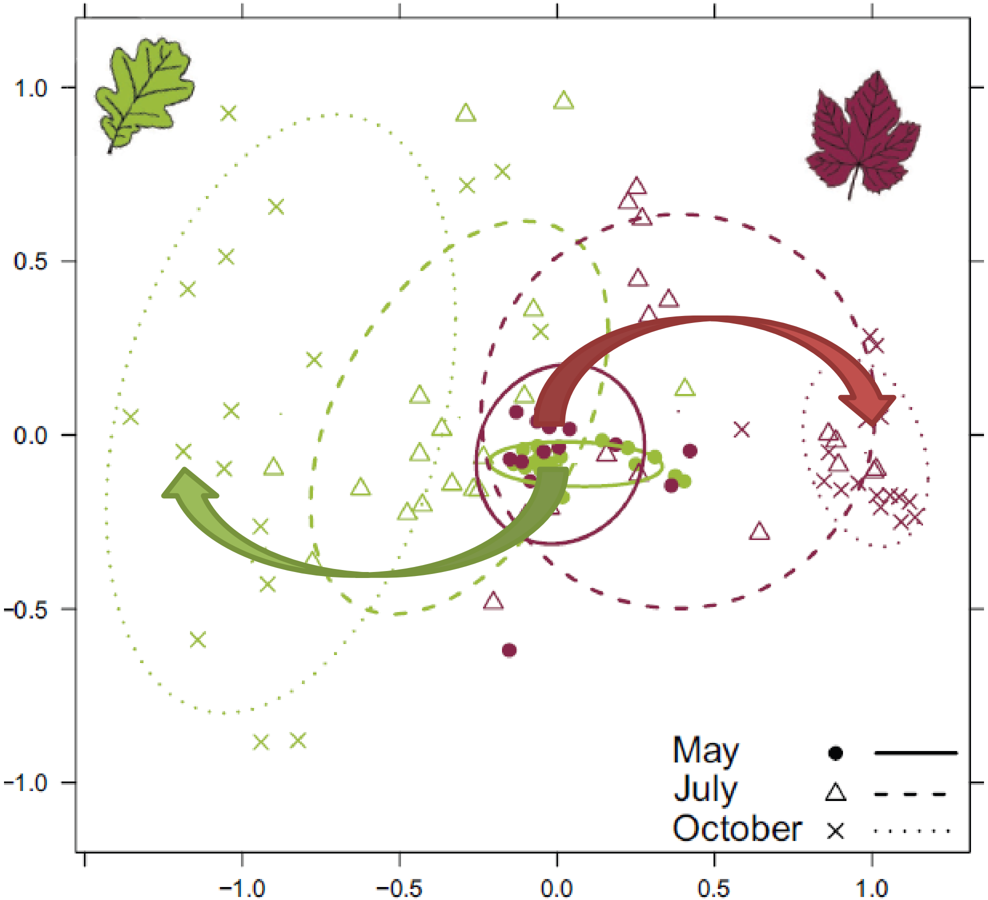


Airborne communities do not differ between habitats and are quite stable through time.

Fort et al. 2016

Airborne and phyllosphere communities have different dynamics

Phyllosphere communities of oak and grapevine have divergent trajectories



Fort et al. 2016