



SCIENCE AND
EDUCATION **FOR**
SUSTAINABLE
LIFE

High Technology Methods in Forest Pathology: DNA sequencing and Phenomics

Carmen Romeralo, SLU
14th Sep 2021

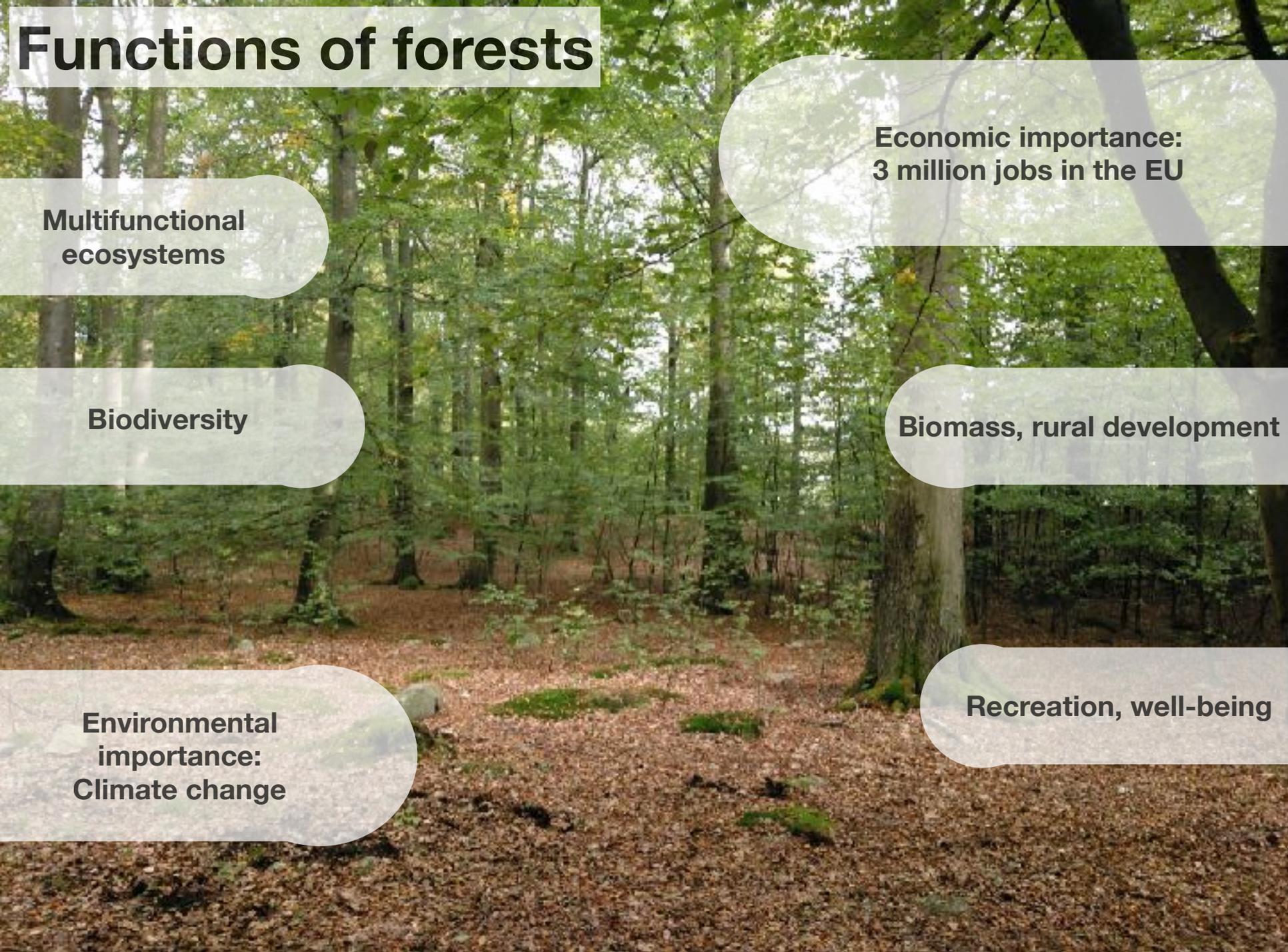
ABOUT ME



I studied forestry (BSc and MSc)
PhD on Forest Pathology

Interested in studying tree fungal diseases, host-pathogen interactions and control of plant diseases, especially biological control using fungal endophytes.

Functions of forests



Multifunctional ecosystems

Biodiversity

**Environmental importance:
Climate change**

**Economic importance:
3 million jobs in the EU**

Biomass, rural development

Recreation, well-being





OF OR RELATED TO LIVING THINGS. FROM GREEK BIŌTIKOS, FROM BIOS 'LIFE'.

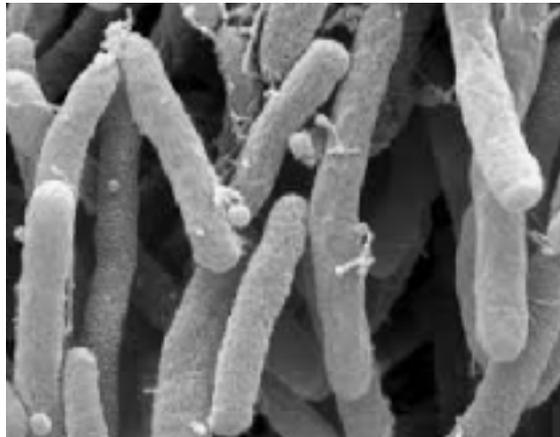
Biotic,

Oxford Advanced Learner's Dictionary

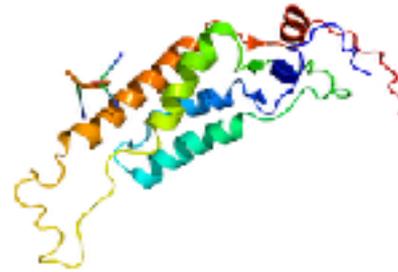


Fungi

Oomycetes (Protists)



Bacteria



Virus



Parasites



Nematodes



Insects







Impact on the ecosystem

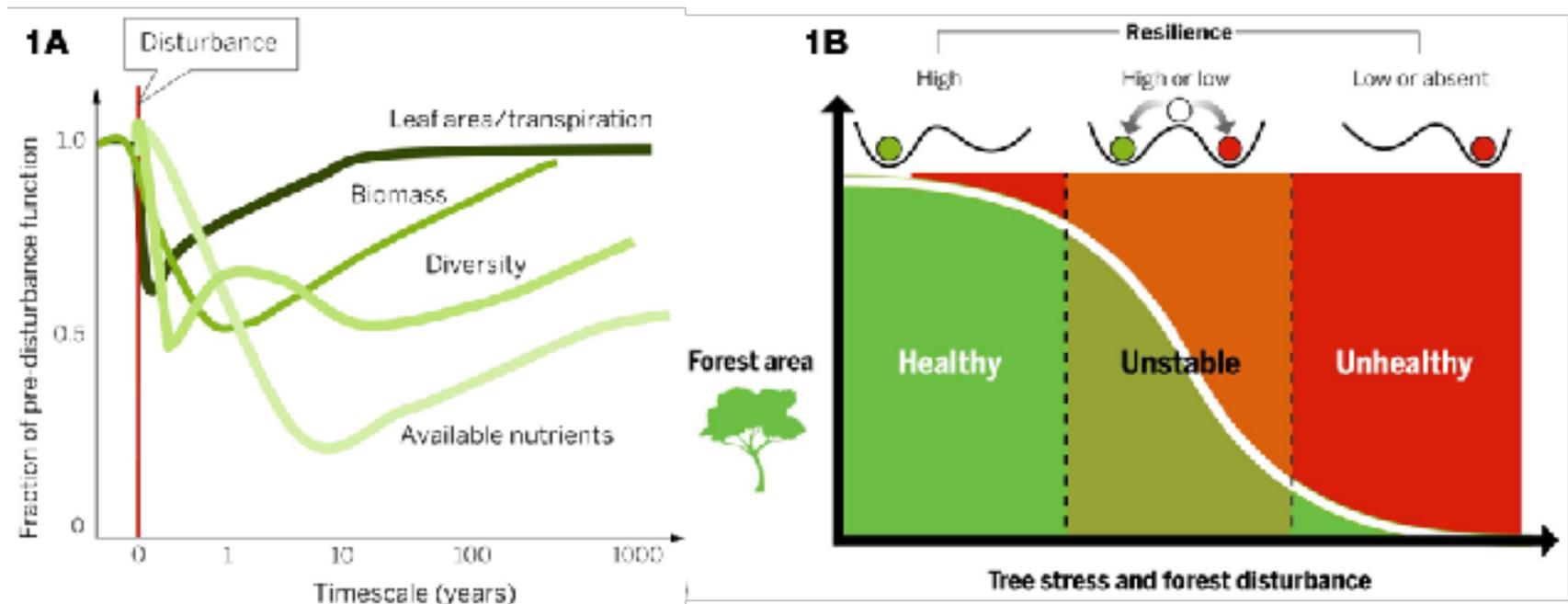
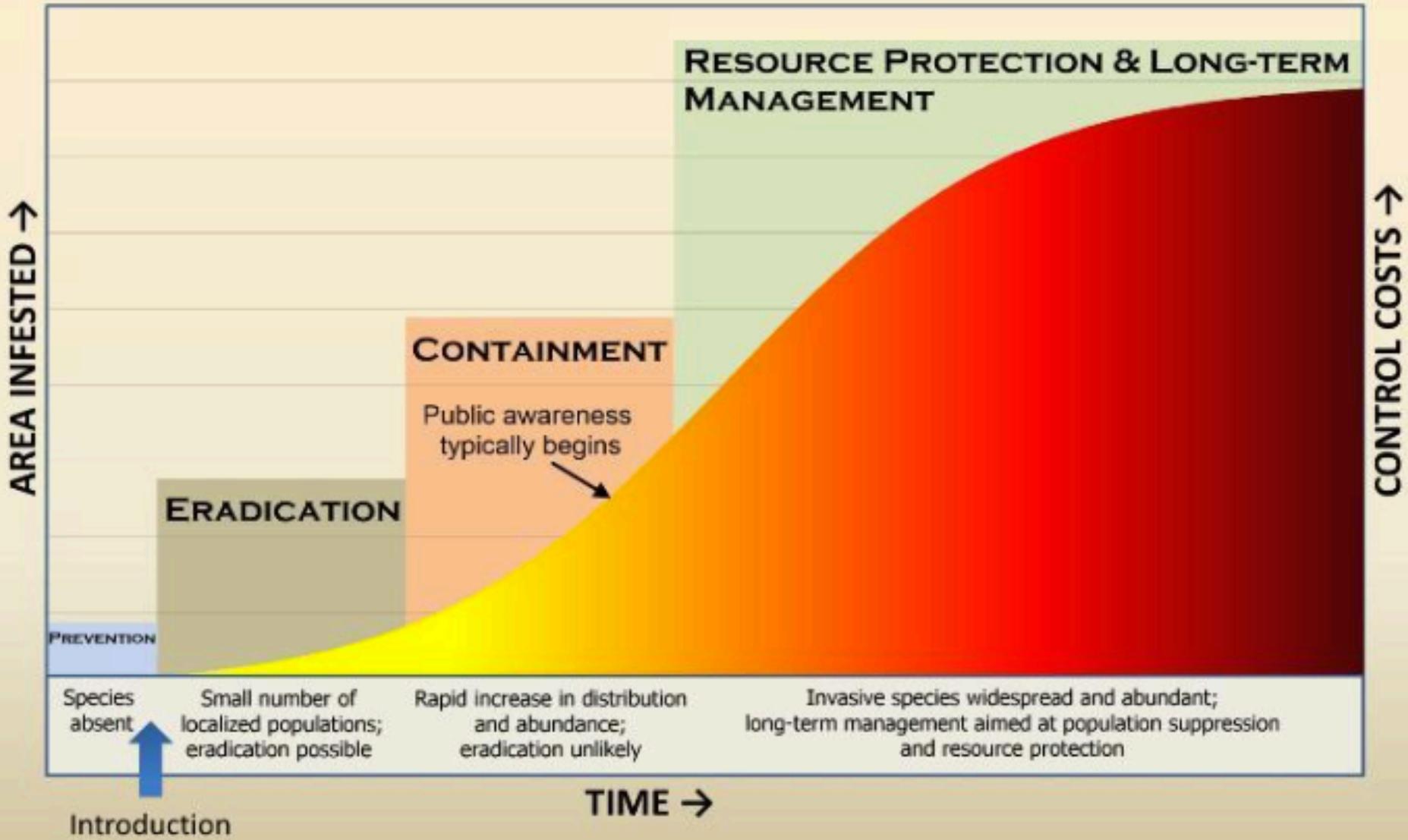


Fig 1a: Escalas de tiempo de recuperación para diferentes funciones forestales después de una perturbación. **Fig 1b:** Representación esquemática de los cambios en la salud de los bosques en función del estrés de los árboles y los disturbios. (De Trumbore et al., 2015, *Science*).



What On
EARTH
Can We Do?

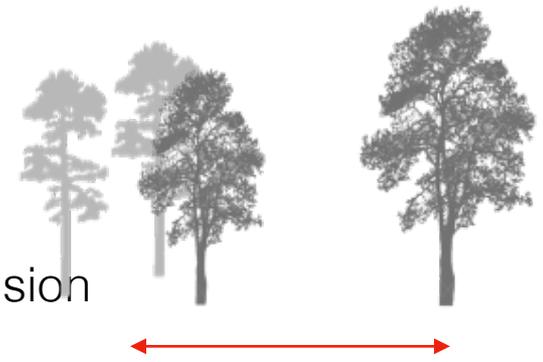


Management of forest diseases

- **Disease management:**
 - Environment, host and pathogen interaction.
 - Pathogen biology and ecology.
- The classical **methods** can be summarized in 4 categories:
 1. Silvicultural
 2. Chemical
 3. Biological
 4. Host resistance

Silvicultural

Aimed at **preventing** diseases and at reducing the expansion of the disease once a stand is infected.



Prevents expansion

Management **guidelines**:

- Avoiding planting susceptible species
- Maintain the health and vigor of the trees
- Protect the genetic resources
- Reduce the amount of inoculum



APPLIED RESEARCH

Vol. 34, 55-64 (2019)
<http://academic.oup.com/forest/advance-article-abstract/doi/10.1093/forest/34/1/55>
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entomology & pathology

Pruning Limber Pine to Reduce Impacts from White Pine Blister Rust in the Southern Rocky Mountains

William R. Jacobi, Phyllis Kredo Davis, Kelly S. Barns, Amanda Crump, and Betsy A. Goodrich

Traitement réussi d'une plantation de pins rouges affectée par le *Gremmeniella abietina*, race européenne

Gaston Laflamme¹

Reçu 1999-01-29; accepté 1999-07-30

PHYTOPROTECTION 80: 55-64

Chemical

“The term fungicide is used in a broad sense for any compound that kills or inactivates fungi”

Fungicides

- Very **limited application in the forests**
- Could be a solution for nurseries.



Directive 2009/128/EC: preference non-chemical methods

Host resistance

Resistance Ability of an individual to prevent infection

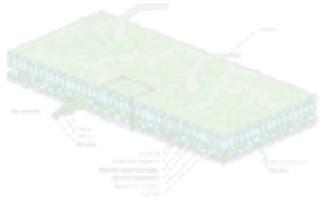
Tolerance Plant become infected but the damaged is limited

Susceptibility Plant can not afford the development of any mechanism to prevent infection

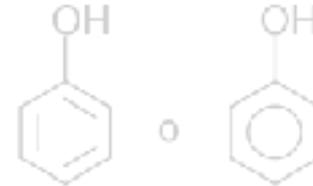
How plants defend themselves?

1. **Preformed** defenses (already in the plant before the attack).

1.1. Physical or structural (cuticle, cell walls, trichomes).



1.2. Chemical preformed (secondary metabolites).



2. **Induced** (activated once the infection has happened).

2.1. Physical (changes on the structure and function of cell wall)

2.2. Chemical (higher concentration of compounds and new compounds)

Host resistance

Resistance Ability of an individual to prevent infection

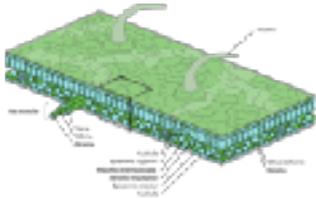
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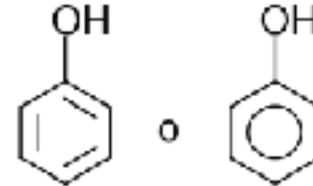
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Biological control

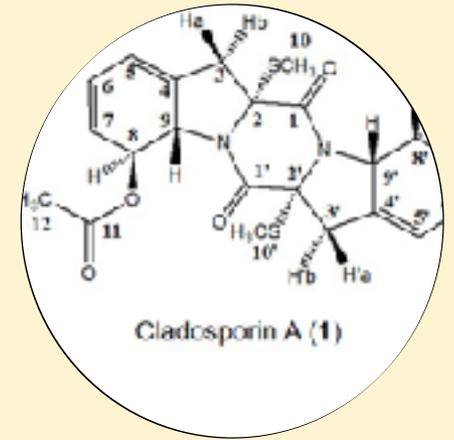
“The use of living organisms or naturally derived bioactive compounds to fight against a disease”

- Alternative method to fight diseases because of the growing public concern over the potentially harmful effects of pesticides (Ownley and Windham, 2007).
- Biological control agents (BCAs) -> other fungi, bacteria, viruses.
- The viability of biological control confirmed only in some cases (e.g. *Heterobasidion* or *Cryphonectria parasitica*).

1. Parasitism



2. Antibiosis

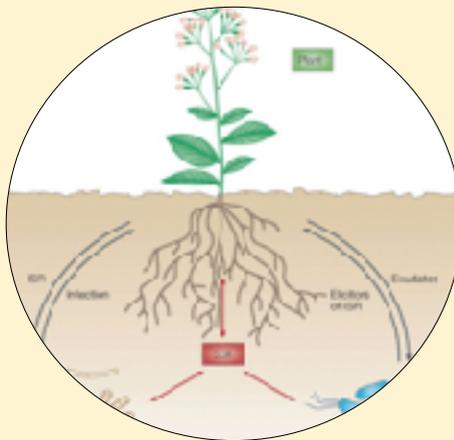


5. Barrier effect

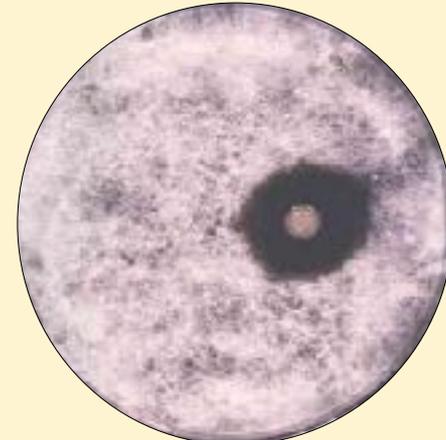


Modes of action
of the BCAs

4. Induction Plant Resistance



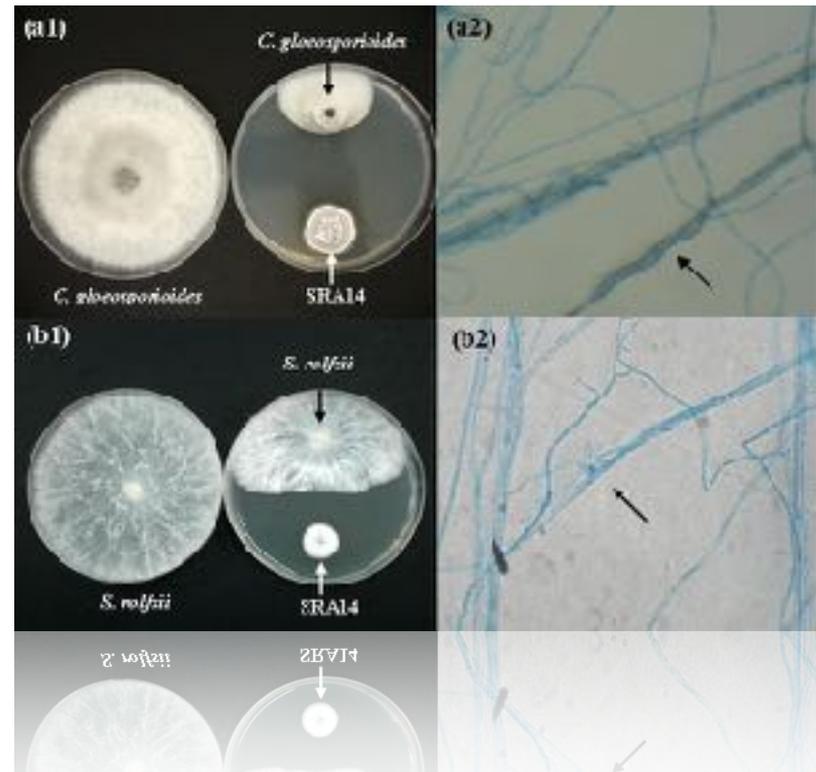
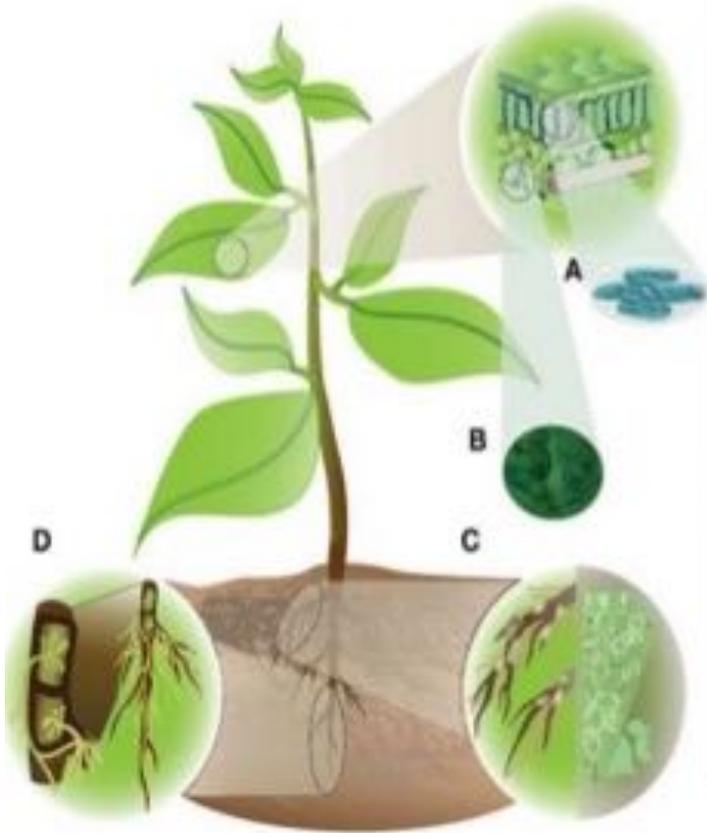
3. Competition



Fungal endophytes

Live inside the plant tissue
Neutral, detrimental or
beneficial

- Becoming a potential biological tool to fight against forest diseases.
- Combine several modes of action



Integrated Pest Management

What is IPM?

Integrated Pest Management is a science-based approach that combines a variety of techniques. By studying their life cycles and how pests interact with the environment, IPM professionals can manage pests with the most current methods to improve management, lower costs, and reduce risks to people and the environment.

IPM tools include:

- Alter surroundings
- Add beneficial insects/organisms
- Grow plants that resist pests
- Disrupt development of pest
- Prevention of pest problem developing
- Disrupt insect behaviors
- Use pesticides

1 IDENTIFY/MONITOR

Determine the causal agent and its abundance (contact your local extension agent for help).

2 EVALUATE

The results from monitoring will help to answer the questions: Is the pest causing damage? Do we need to act? As pest numbers increase toward the economic threshold further treatments may be necessary.

3 PREVENT

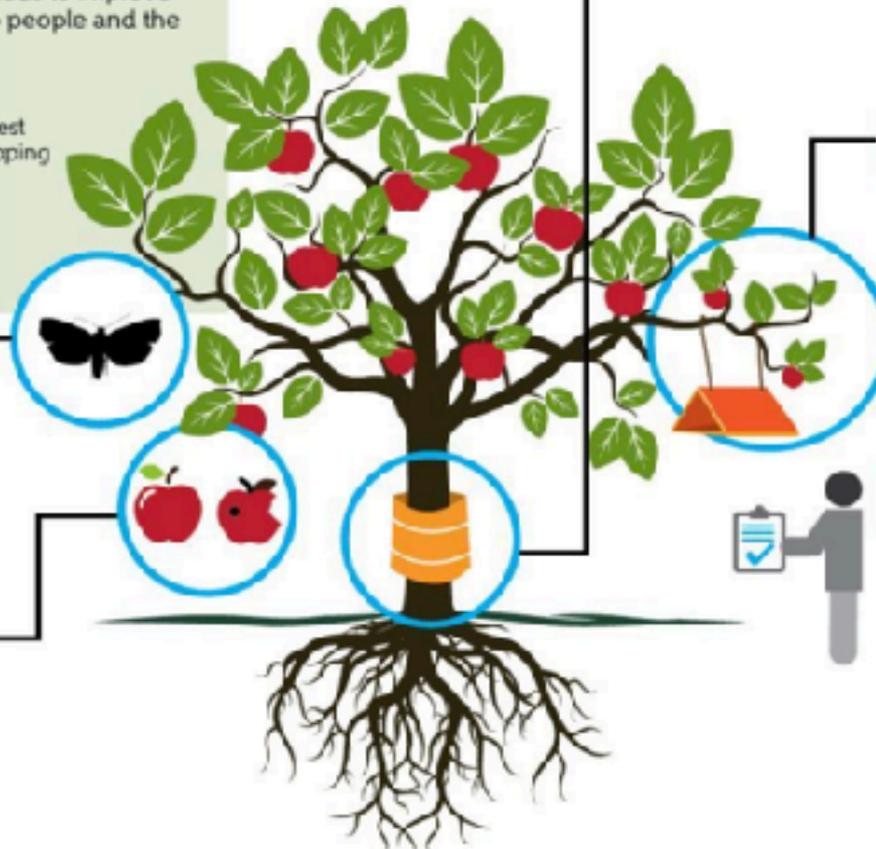
Some pest problems can be prevented by using resistant plants, planting early, rotating crops, using barriers against climbing pests, sanitation, and sealing cracks in buildings.

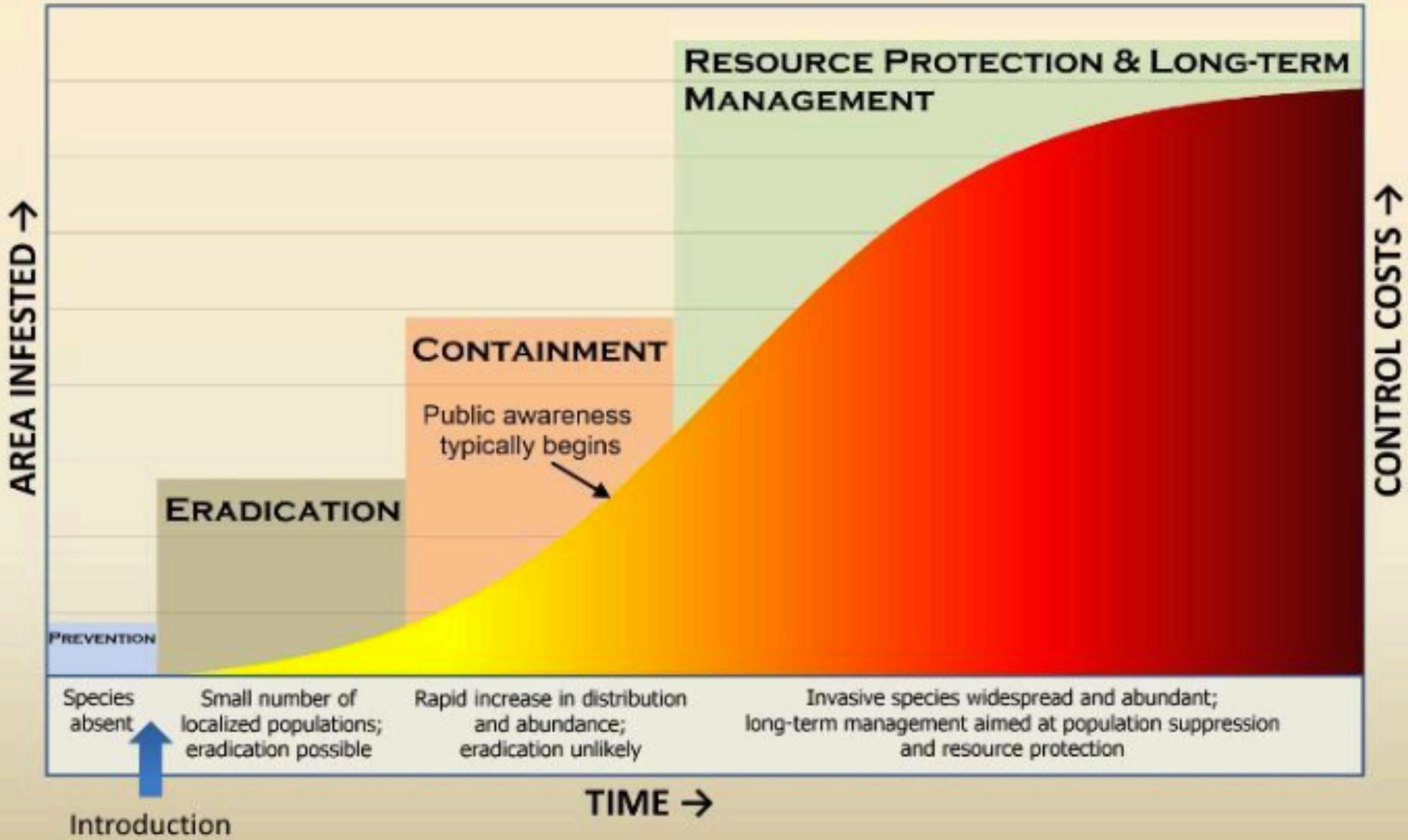
4 ACTION

IPM uses multiple tools to reduce pests below an economically damaging level. A careful selection of preventive and curative treatments will reduce reliance on any one tactic and increase likelihood of success.

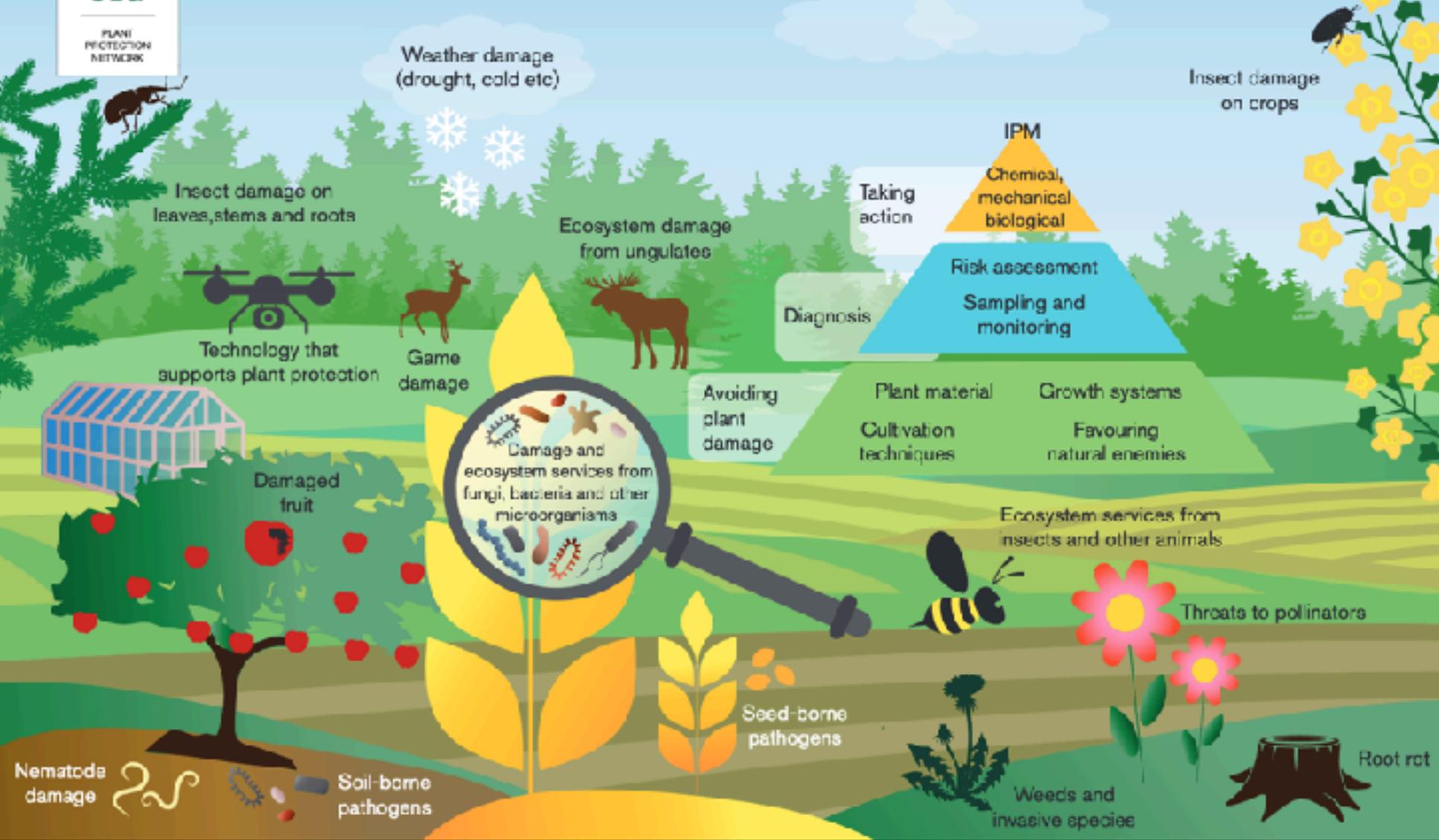
5 MONITOR

Continue to monitor the pest population. If it remains low or decreases, further treatments may not be necessary, but if it increases and exceeds the action threshold, another IPM tool should be used.





Research areas within plant protection at SLU



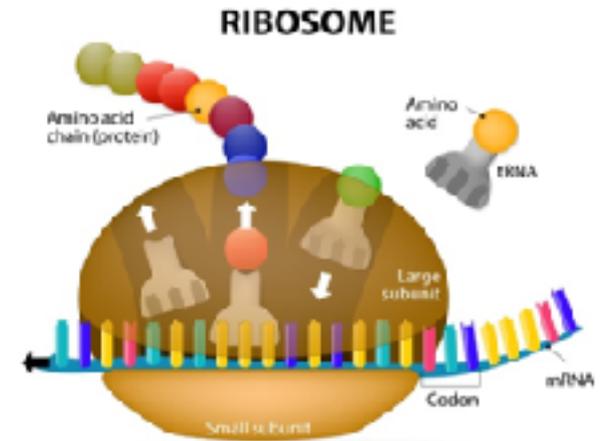
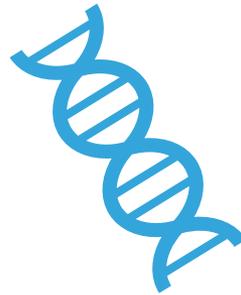
What is the technology that supports plant protection?

Technology

1. Detection of the **pathogen and microorganisms**
 - A. DNA barcoding
 - B. Metabarcoding/Metagenomics
2. Detection of the **early symptoms in the host**
 - C. Phenomics

Identification of Fungi

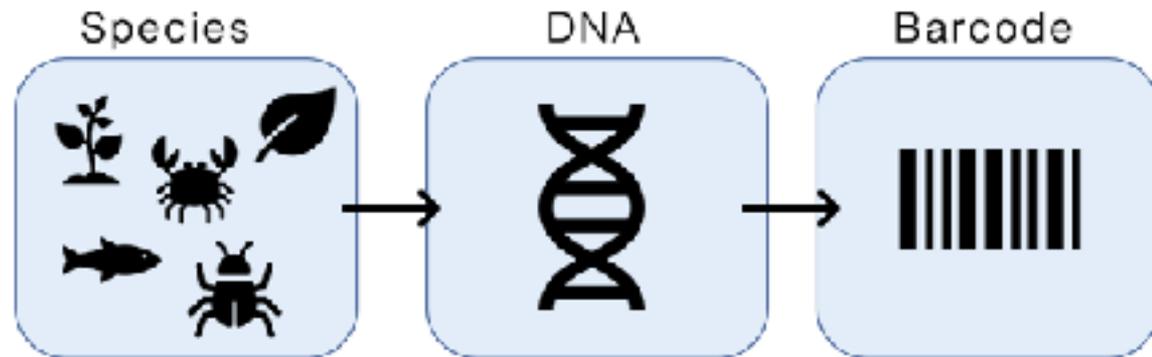
DNA barcode



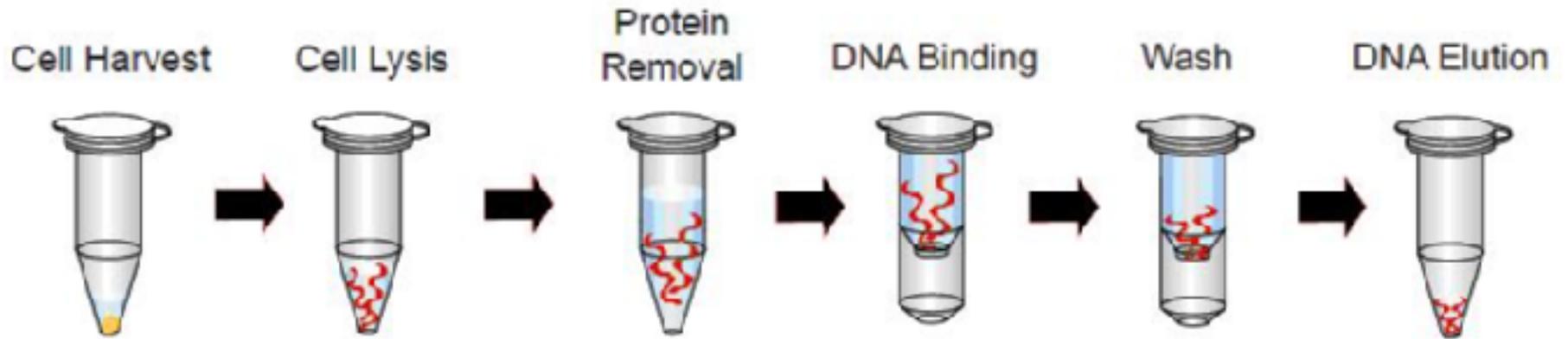
©Dcigna / Shutterstock.com

International Barcode of Life

<https://ibol.org>



DNA extraction



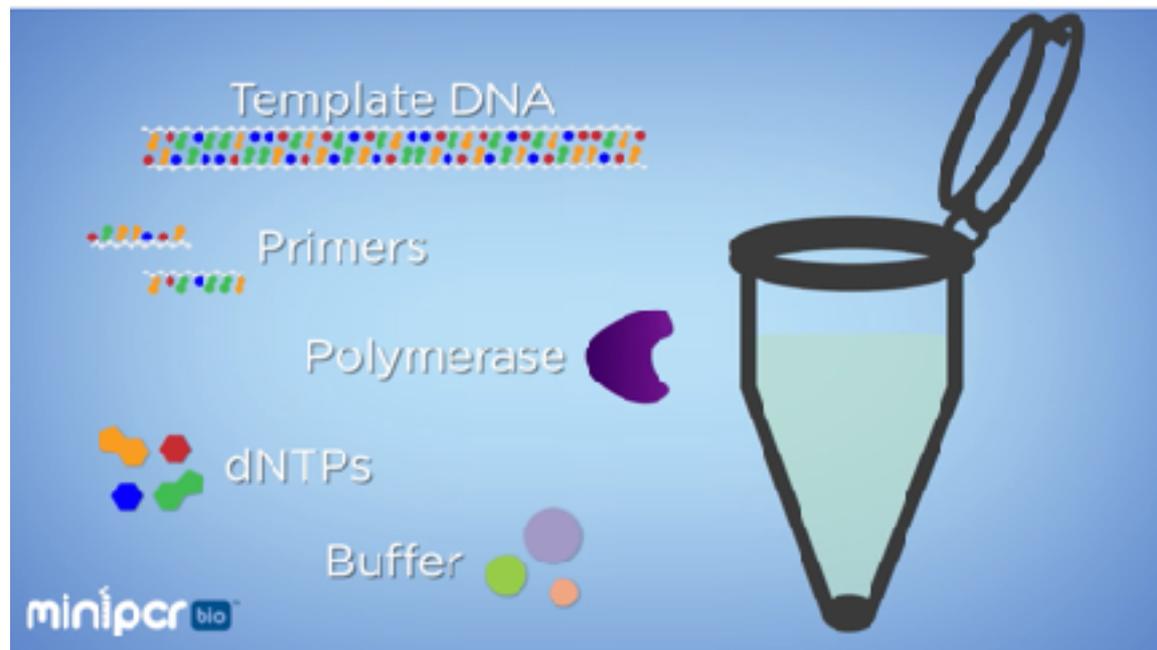
PCR steps

Denaturation

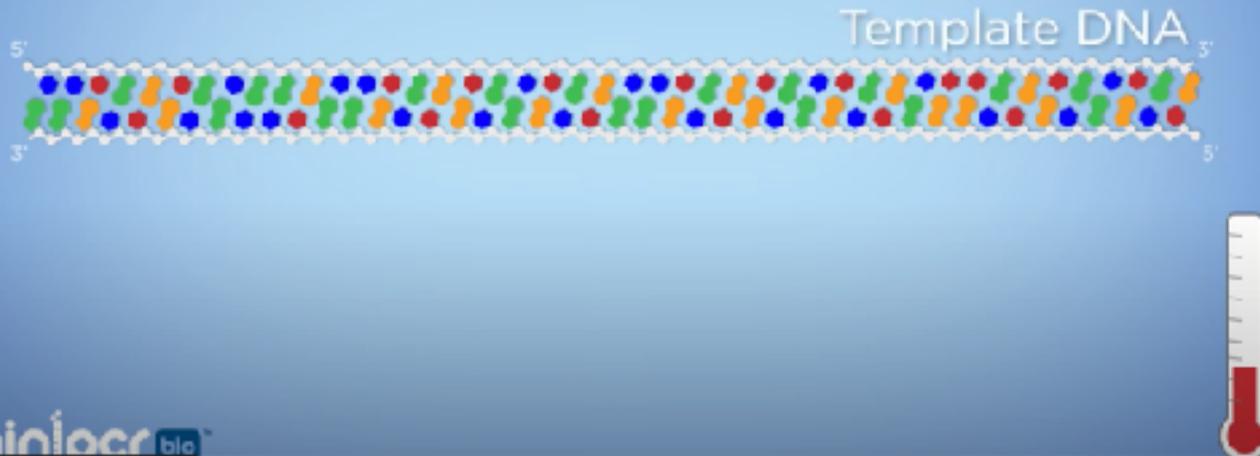
Annealing

Extension

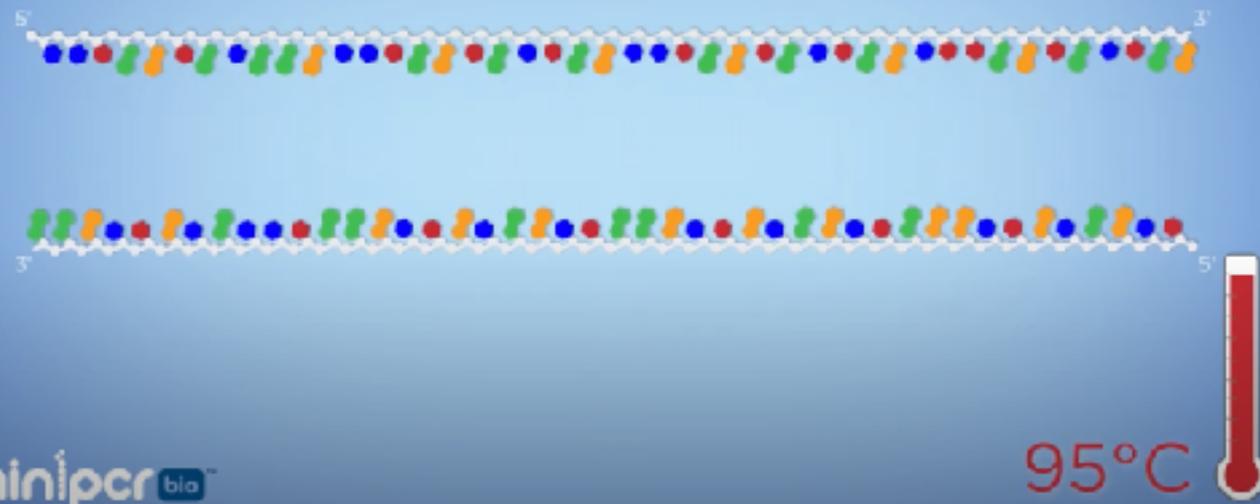
Video: (https://www.youtube.com/watch?v=c07_5BfIDTw)



Denaturation



Denaturation



Annealing



minipcr blo™



Annealing

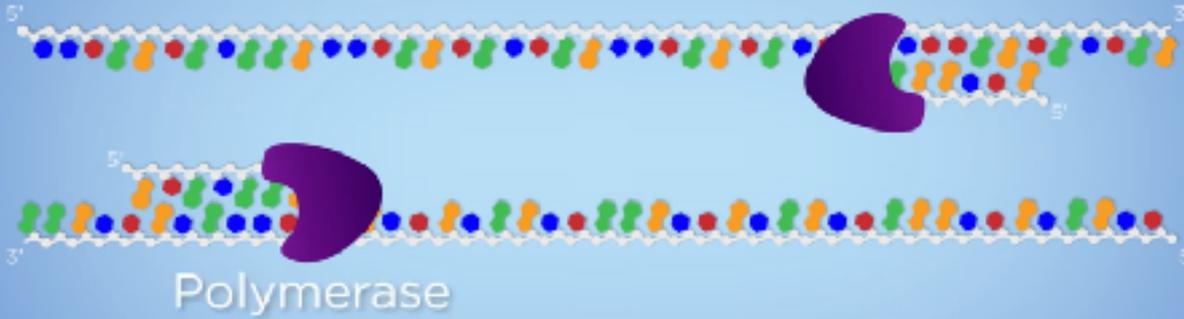


minipcr blo™

50-65°C



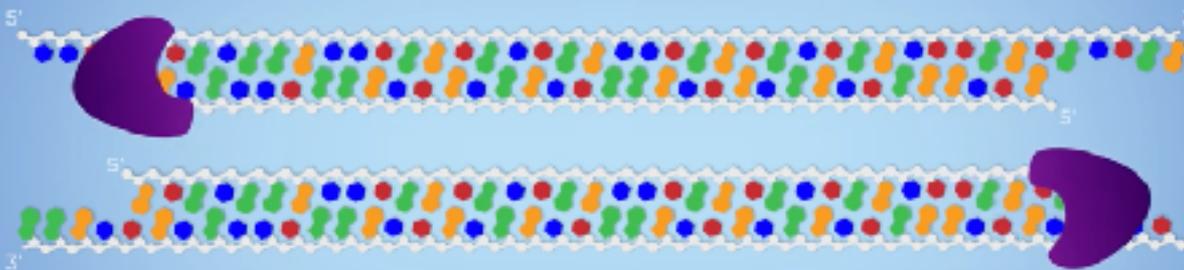
Extension



72 °C

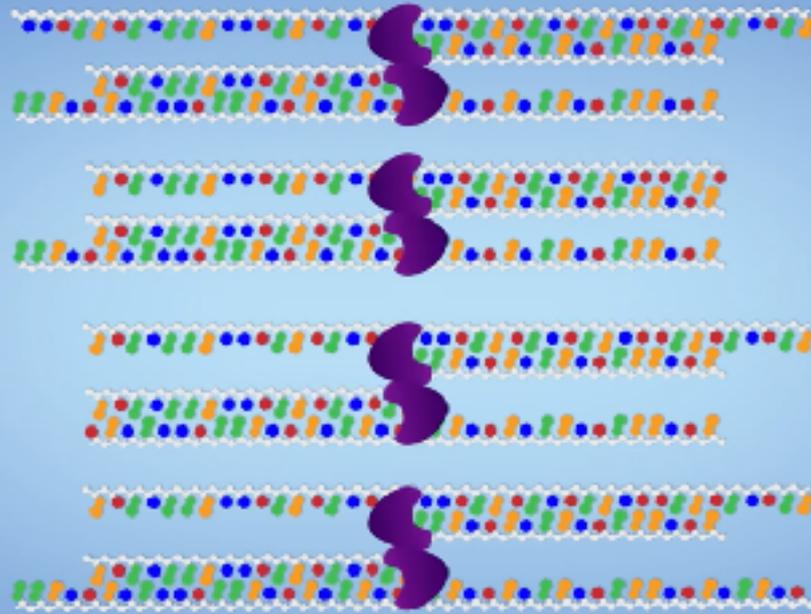


Extension

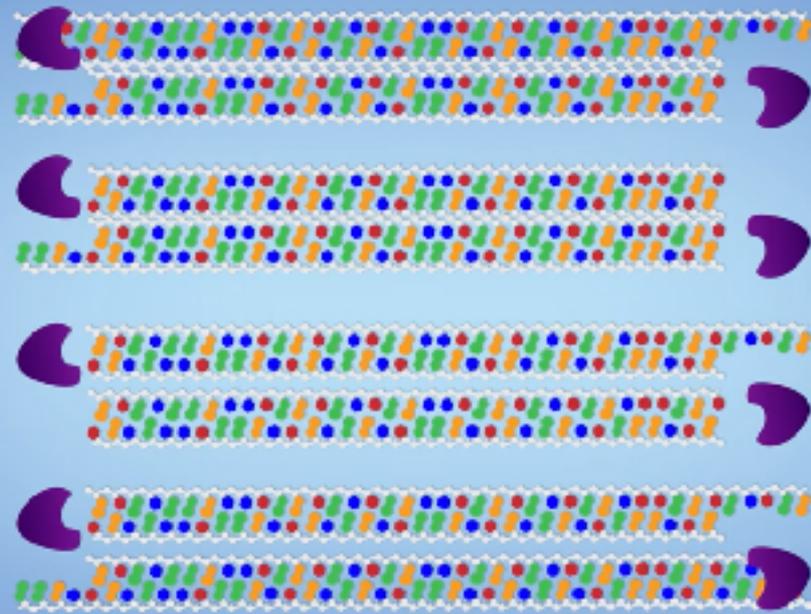


72 °C





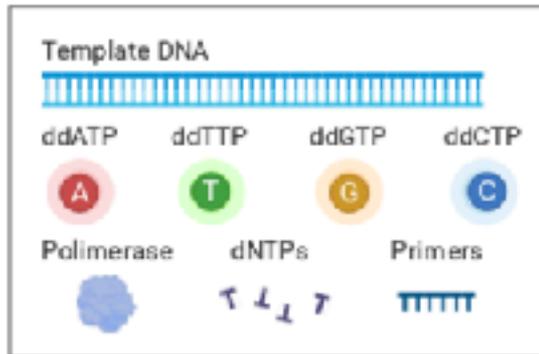
minipcr^{blo}



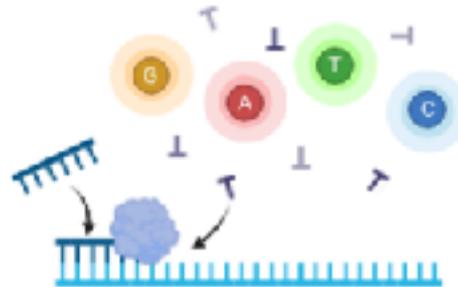
minipcr^{blo}

Sanger Sequence

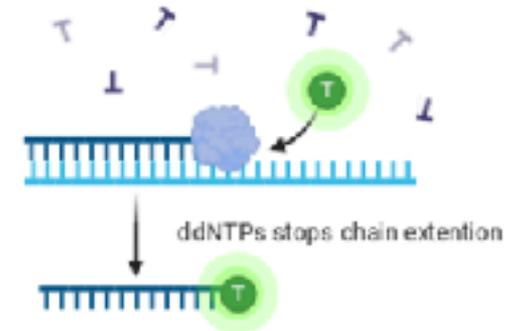
Reagents



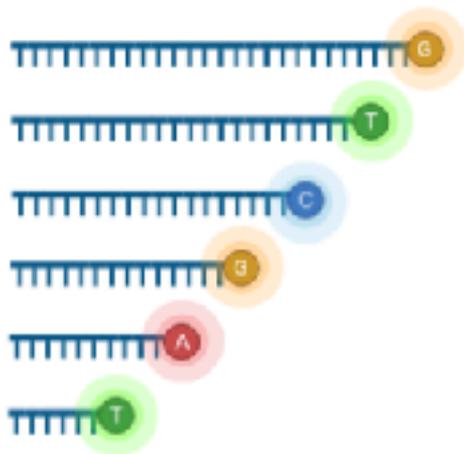
① Primer annealing and chain extension



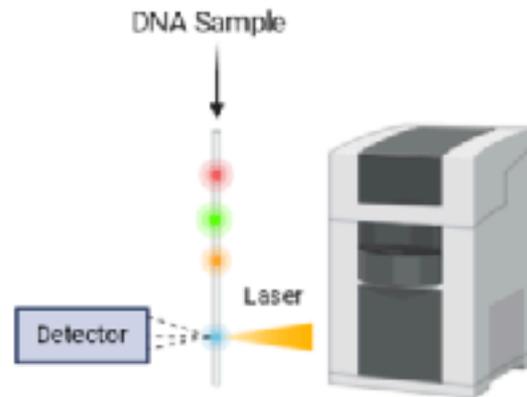
② ddNTP binding and chain termination



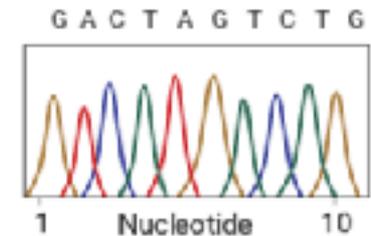
③ Fluorescently labelled DNA sample



④ Capillary gel electrophoresis and fluorescence detection

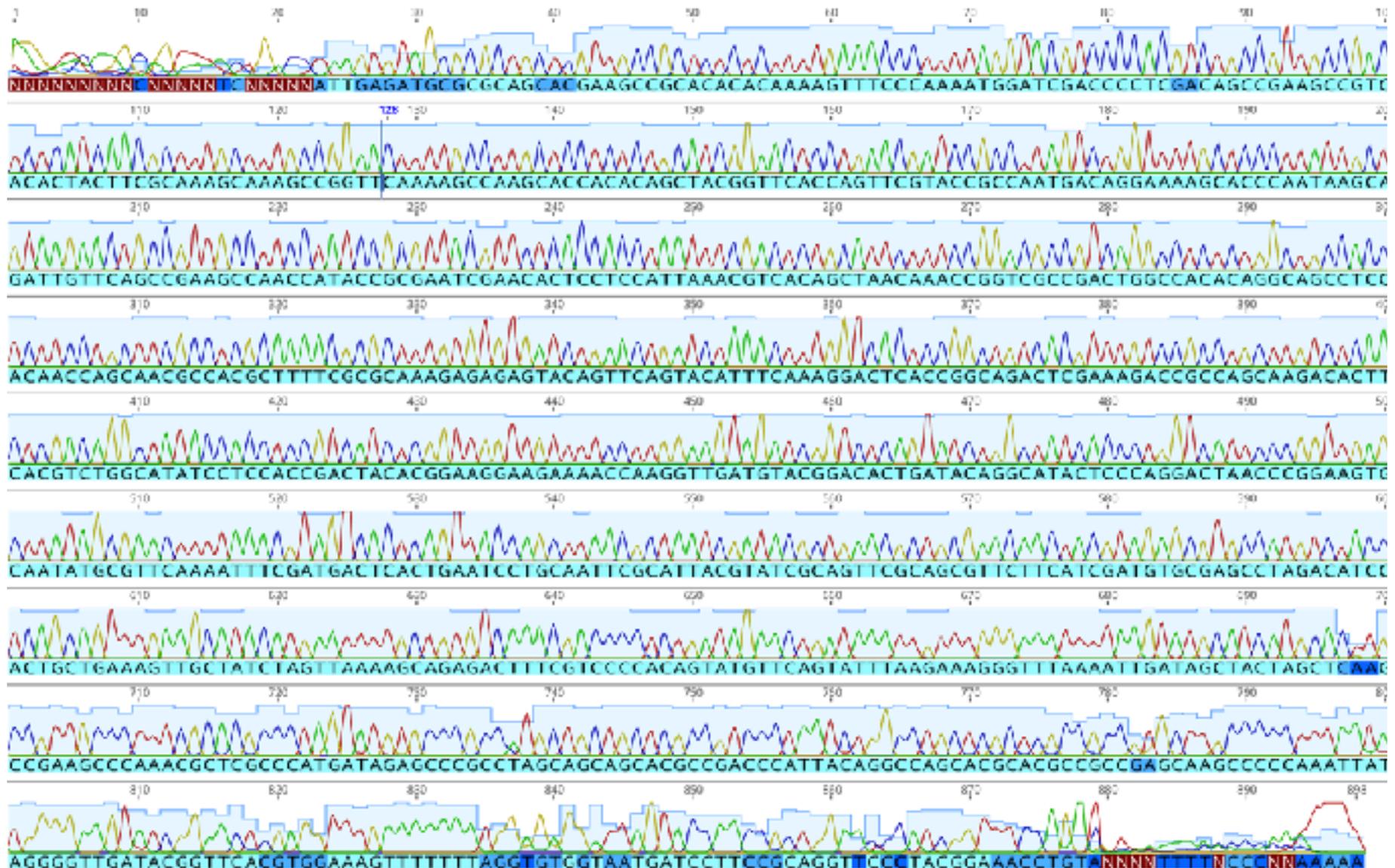


⑤ Sequence analysis and reconstruction





Nucleotides





BLAST (Basic Local Alignment Search Tool)



U.S. National Library of Medicine
National Center for Biotechnology Information

carmenromeralo@gm...



COVID-19 Information

[Public health information \(CDC\)](#) | [Research information \(NIH\)](#) | [SARS-CoV-2 data \(NCBI\)](#) | [Prevention and treatment information \(HHS\)](#) | [Español](#)



BLAST®

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Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

NEWS

BLAST+ 2.12.0 is here!

We have made some improvements to how BLAST multi-threads and the amount of memory required by makeblastdb.

Tue, 13 Jul 2021 12:00:00 EST

[More BLAST news...](#)

Web BLAST

Nucleotide BLAST
nucleotide ► nucleotide

blastx
translated nucleotide ► protein

tblastn
protein ► translated nucleotide

Protein BLAST
protein ► protein



BLAST (Basic Local Alignment Search Tool)

BLAST® » blastn suite » results for RID-KZ5T72A3013

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[← Edit Search](#)

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[Search Summary](#) ▾

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Job Title	Nucleotide Sequence
RID	KZ5T72A3013 Search expires on 09-16 02:23 am Download All ▾
Program	BLASTN ? Citation ▾
Database	rRNA_typestrains/ITS_RefSeq_Fungi See details ▾
Query ID	Id Query_3437
Description	None
Molecule type	dna
Query Length	862
Other reports	Distance tree of results MSA viewer ?

Filter Results

Organism exclude only top 20 will appear

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity

to

E value

to

Query Coverage

to

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[Descriptions](#)

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[Alignments](#)

[Taxonomy](#)

Sequences producing significant alignments

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Show



select all 55 sequences selected

[GenBank](#)

[Graphics](#)

[Distance tree of results](#)

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	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
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<input checked="" type="checkbox"/>	Diaporthe betulae CFCC 60469 ITS region: from TYPE material	Diaporthe betulae	165	165	13%	1e-40	92.31%	462	NR_147576.1
<input checked="" type="checkbox"/>	Diaporthe mshothecarpus OGMCC 3.15.181 ITS region: from TYPE material	Diaporthe mshothecarpus	219	219	18%	7e-57	91.77%	530	NR_147522.1

Metagenomics: Technological Revolution

Open Access
PLANT SCIENCE

REVIEW ARTICLE
published: 23 May 2014
doi: 10.3389/fpls.2014.00218



Analysis of plant microbe interactions in the era of next generation sequencing technologies

Claudia Kniel*

Faculty of Gene Science and Bioprocess Engineering—Molecular Biology of the Rhizosphere, Faculty of Agriculture, University of Bonn, Germany



NIH Public Access

Author Manuscript

Unlabeled Author Manuscript; available in PMC 2014 September 26.

Published in final edited form as:

Cell. 2013 September 26; 155(1): 27–38. doi: 10.1016/j.cell.2013.08.006.

The Next-Generation Sequencing Revolution and its Impact on Genomics

Daniel C. Koboldt¹, Karyn Meltz Steinberg¹, David E. Larson¹, Richard K. Wilson¹, and Elaine Mardis^{1,*}

¹The Genome Institute, School of Medicine, Washington University, St. Louis, MO 63103, USA.

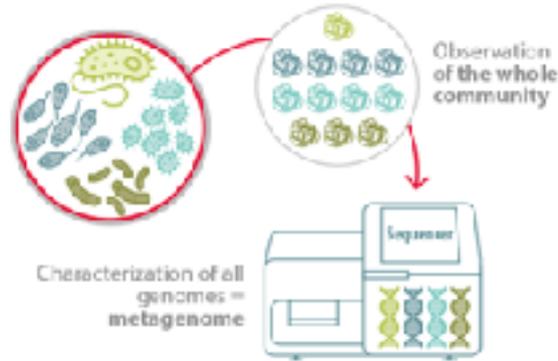


They allow to identify multiple organisms; pathogens and other microorganisms (e.g. endophytes).

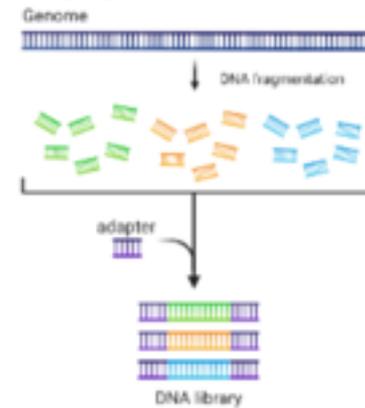
- **Introduction to NGS:**
- https://www.youtube.com/watch?v=ToKUGz_YhC4

Metagenomics

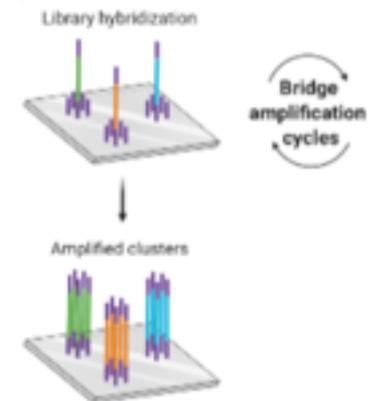
What is metagenomics?



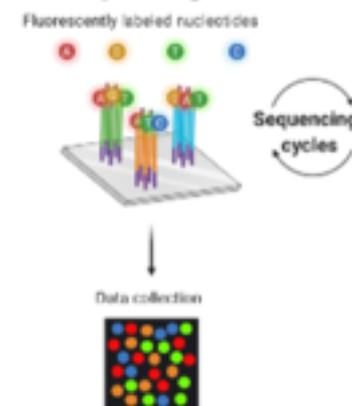
1 Library preparation



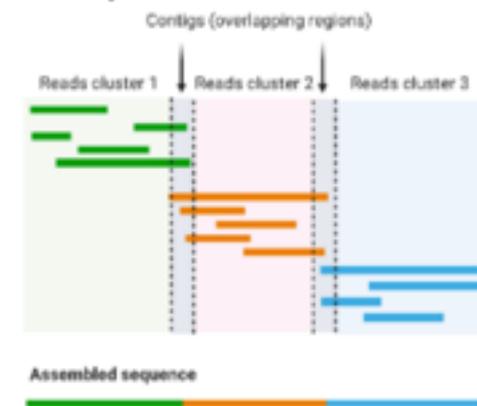
2 DNA library bridge amplification



3 DNA library sequencing



4 Alignment and data analysis



NGS Platform

HOLOBIONT

MICROORGANISMS



HOLOBIONT, IS AN ASSEMBLAGE OF A HOST AND THE MANY OTHER SPECIES LIVING IN OR AROUND IT, WHICH TOGETHER FORM A DISCRETE ECOLOGICAL UNIT.

Lynn Margulis

TERMS & DEFINITIONS

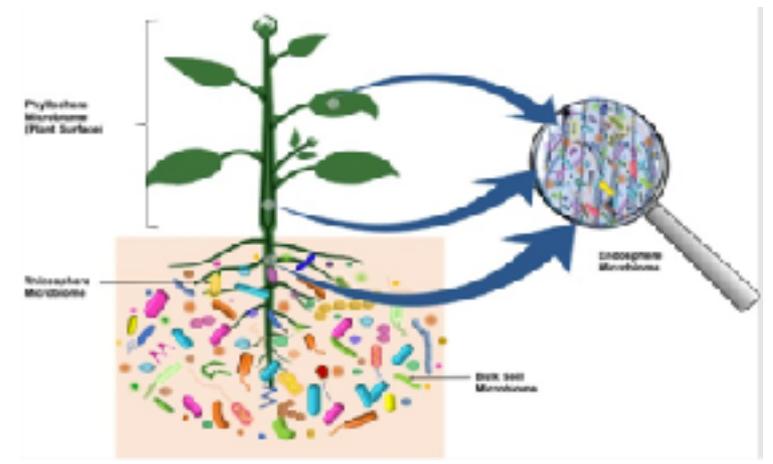
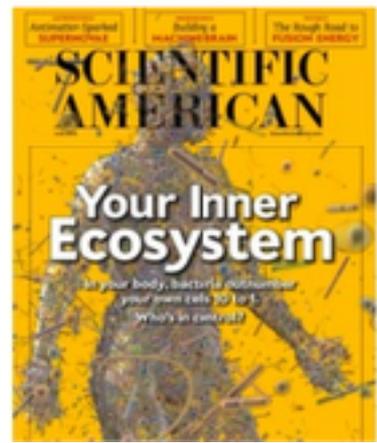
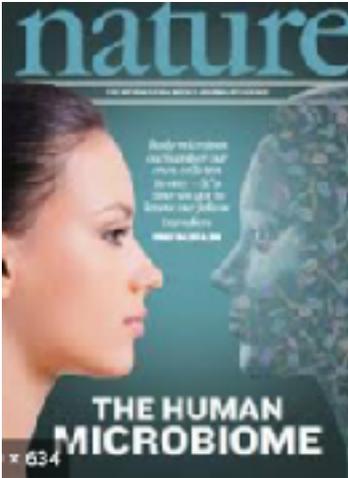
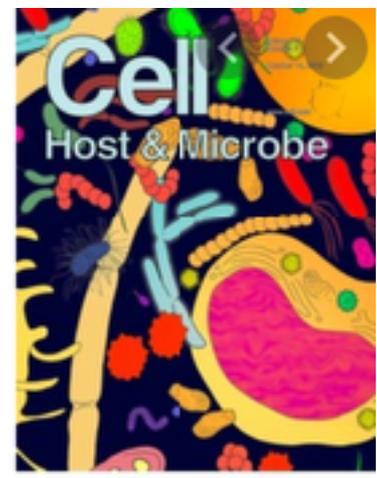
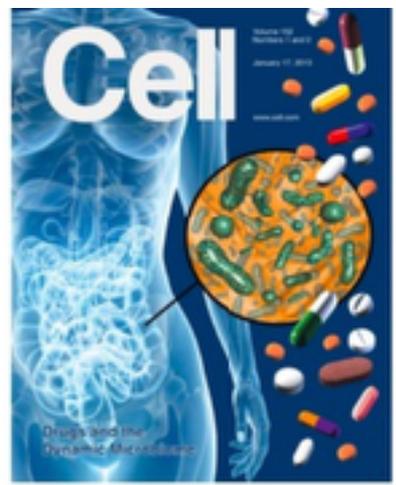
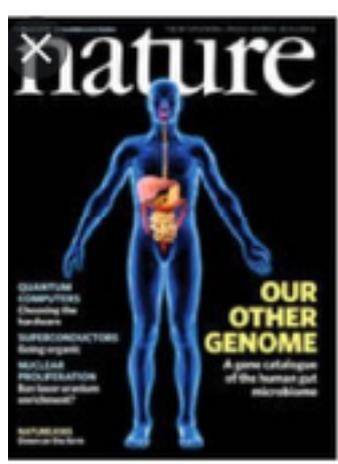
WHILE COLONIZING A HEALTHY PLANT, **ENDOPHYTES** DO NOT CREATE SYMPTOMS DURING THEIR LIFE CYCLE.

Anna Maria Pirttilä · A. Carolin Frank
Eds. of Endophytes of Forest Trees

MICROBIOME

1 THE MICROORGANISMS IN A PARTICULAR ENVIRONMENT (=MICROBIOTA)
1.1. THE COMBINED GENETIC MATERIAL OF THE MICROORGANISMS IN A PARTICULAR ENVIRONMENT.

Lexico Diccionario by Oxford



**BUT NOT FOR BEING SMALL THEY
ARE LESS IMPORTANT!!**

If you think you are **too small**
to make a difference,
try sleeping with a **mosquito.**

– Dalai Lama



WHY MICROBIOME IS IMPORTANT?

- ▶ Development, growth, and health of macroorganisms are influenced by the complex microbial communities they host.
- ▶ Plants rely on their microbiome for a number of life-support functions (e.g. nutrient acquisition and protection against stress factors).
- ▶ The structure and diversity of these communities depend on the host, the geographical location and abiotic and biotic factors.

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- ▶ The structure and diversity of these communities depend on the host, the geographical location and abiotic and biotic factors.

RESEARCH

Open Access

Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem

Hui Yao^{1,2}, Xiang Sun¹, Chao He¹, Pulak Maltra¹, Xing-Chun Li¹ and Liang-Dong Guo^{1,2*}



Review

Forest Tree Microbiomes and Associated Fungal Endophytes: Functional Roles and Impact on Forest Health

Eeva Terhonen¹, Kathrin Blumenschein¹, Andriy Kovalchuk² and Fred O. Asiegbu^{2,3*}

Fungal endophytes limit pathogen damage in a tropical tree

A. Elizabeth Arnold^{1*}, Luis Carlos Mejía¹, Damsodh Kalle¹, Erik I. Rojas¹, Zelayda Maynard¹, Nancy Robbins¹, and Edward Allen Herre¹

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Fungal endophytes: modifiers of plant disease

Peggy E. Busby^{1,2} · Mary Hilout² · George Newcombe²



New Phytologist

Full paper | [Free Access](#)

Common foliar fungi of *Papulus trichocarpa* modify *Melampsora* rust disease severity

Peggy E. Busby¹, Kabir G. Peay¹, George Newcombe²

First published: 13 November 2015 | <https://doi.org/10.1111/nph.13742> | Citations: 70

PROCEEDINGS B

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Research



On this article: Arnold AE, Herre EA, Mejía LC, Guo LD (2017) Exposure to the leaf litter microbiomes of healthy adults protects seedling fern damage damage. *Proc R Soc B*

Exposure to the leaf litter microbiome of healthy adults protects seedlings from pathogen damage

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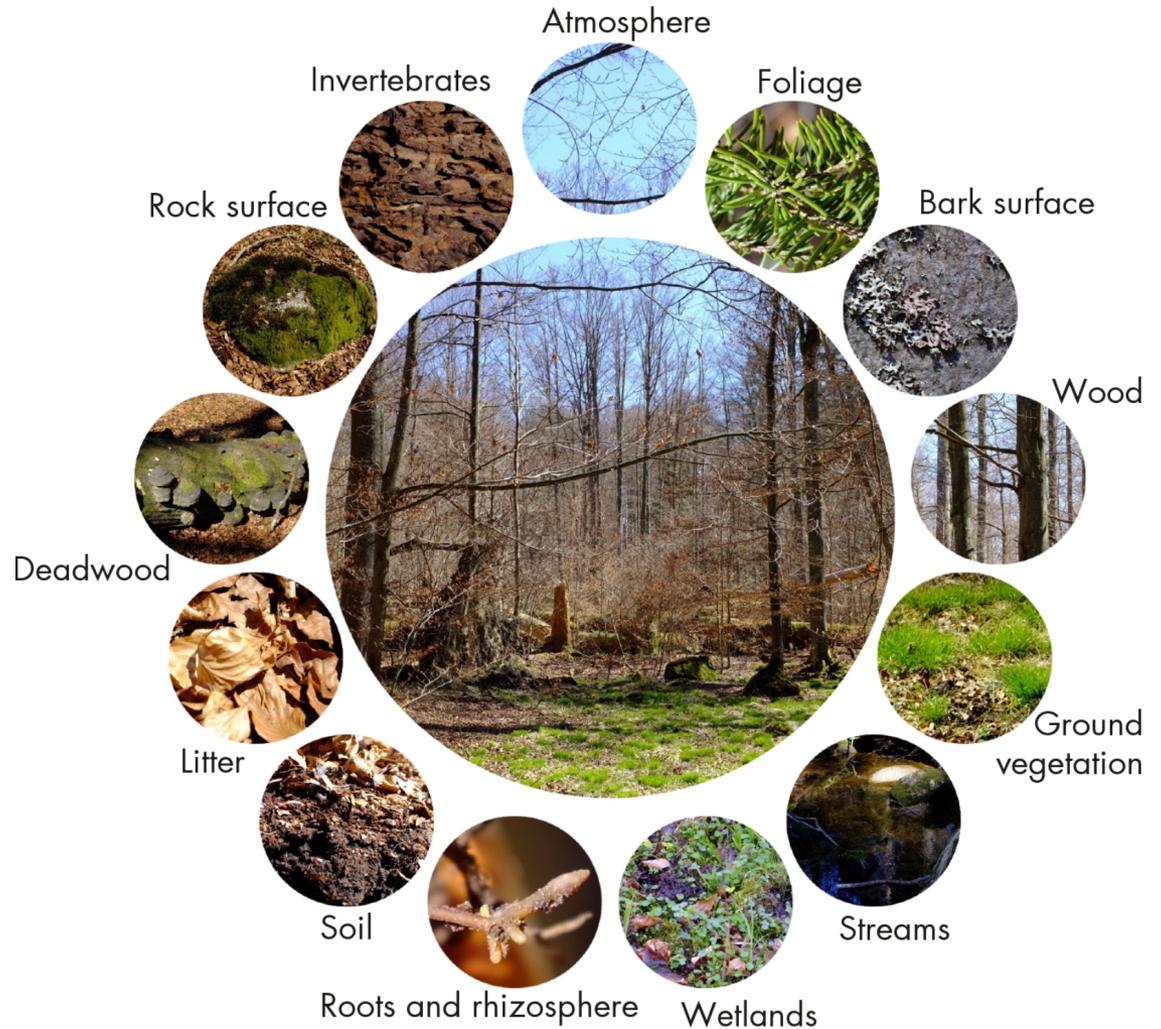
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REVIEW ARTICLE

Forest microbiome: diversity, complexity and dynamics

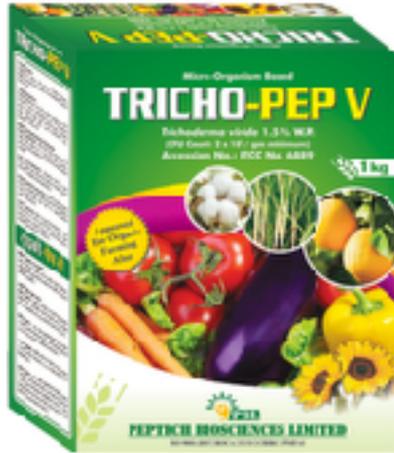
Petr Baldrian¹

Dymanic communitites



Biological fungicides based on endophytes

Verticillium albo-atrum



durably protected with DutchTrig®

Trichoderma viride

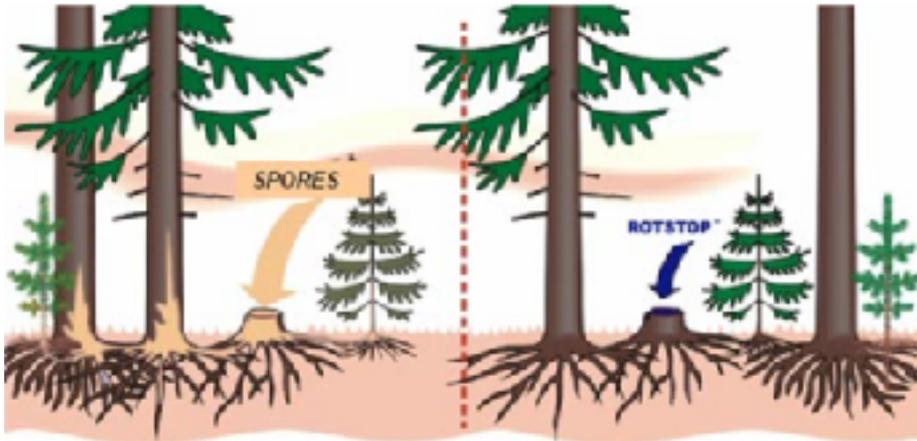


vaccination is efficient and easy



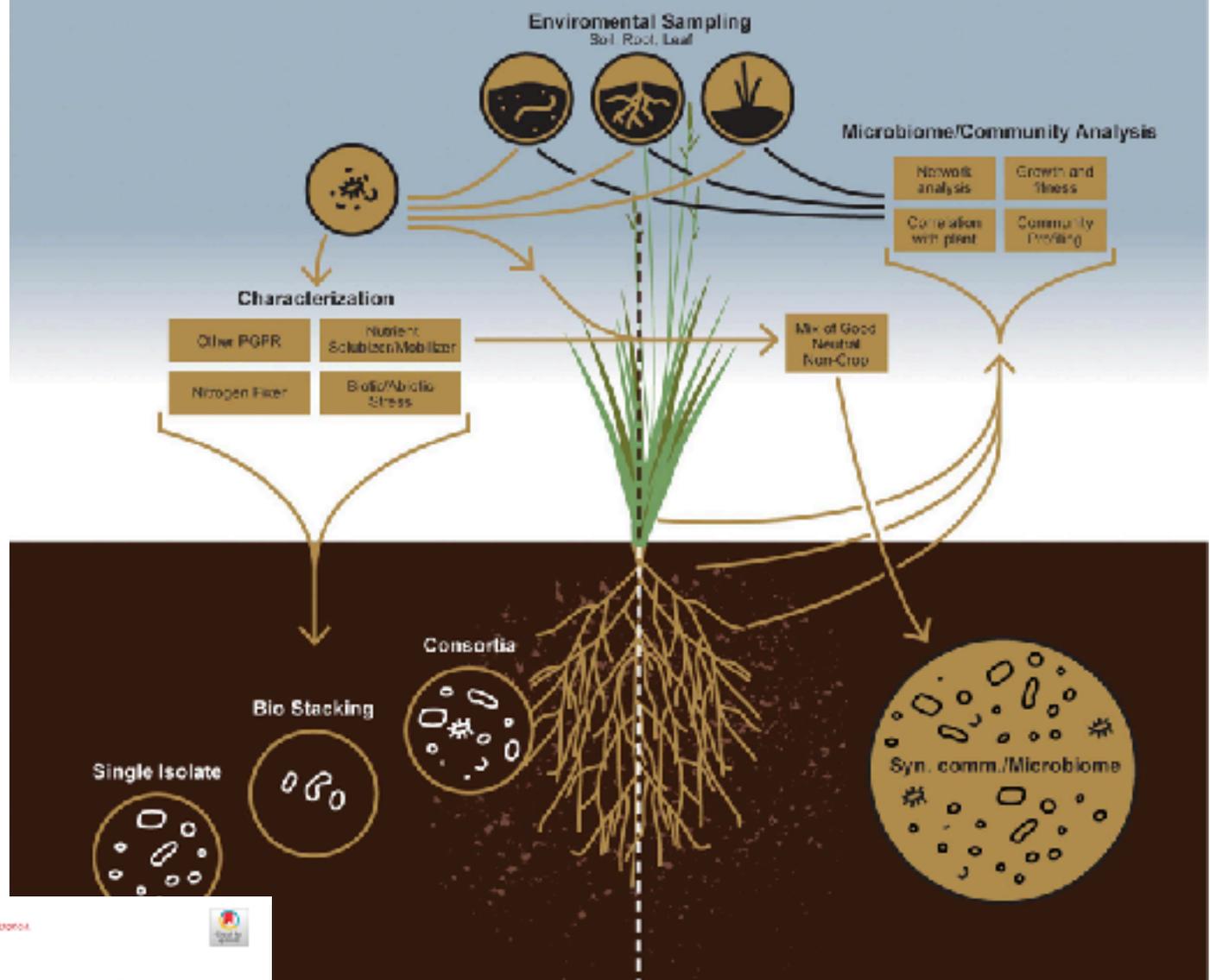
Rhizobium spp

Forest biological fungicides



Reductionist Approach

Holistic Approach



KEY WORDS
 Plant Microbiome, 21 December 2020 | <https://doi.org/10.3389/fpls.2020.587004>



Microbe to Microbiome: A Paradigm Shift in the Application of Microorganisms for Sustainable Agriculture

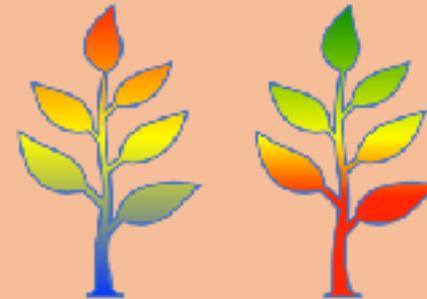
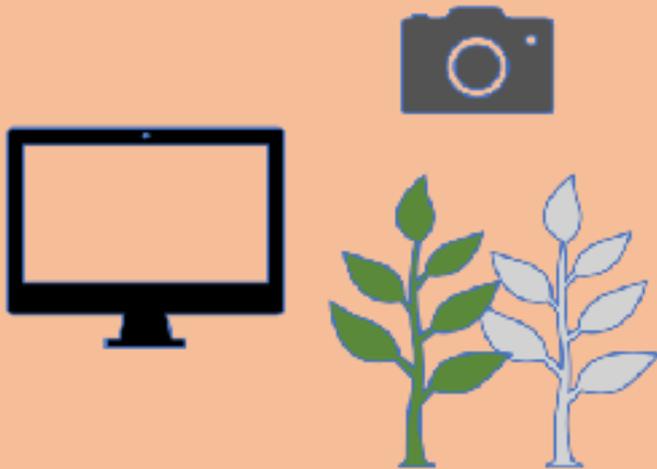
Prasun Ray¹, Yashrajaram Lakshminarayanan¹, Jessy L. Labadie¹ and Selby D. Cooper^{1*}

Monitor disease progression and host responses by the use of phenomics



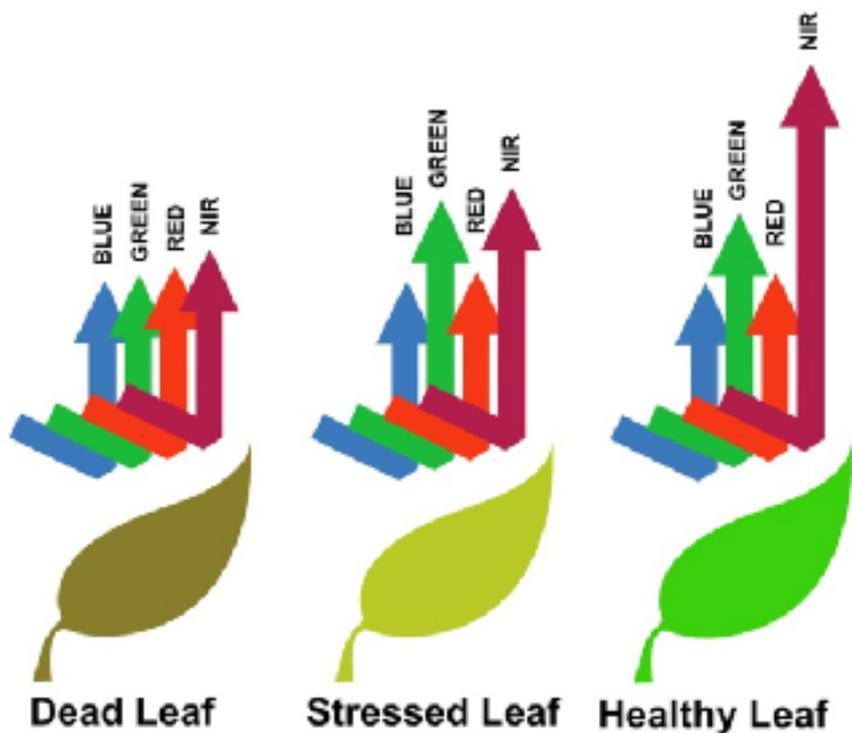
Phenomics

RGB-Thermal



Physiological responses

NDVI: Normalized difference vegetation index



HEALTHY

STRESSED

VEGETATION REFLECTANCE

50% NIR 8% RED

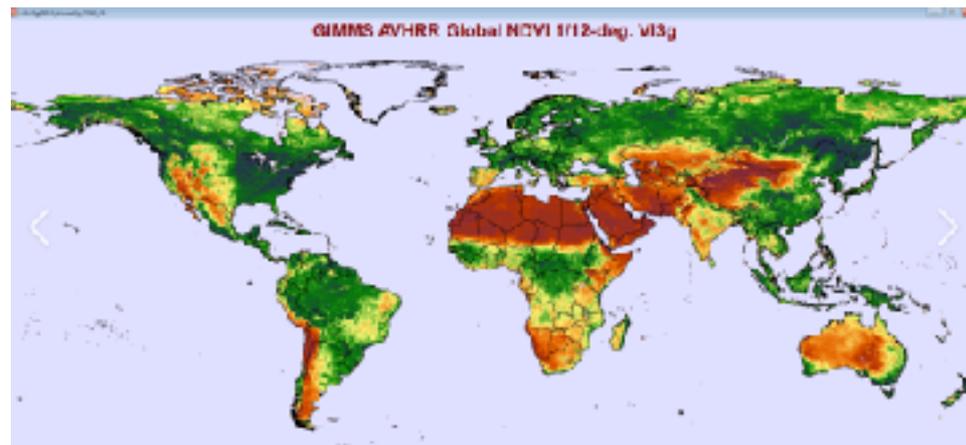
40% NIR 30% RED



NDVI = 0.72

NDVI = 0.14

$$NDVI = \frac{NIR - RED}{NIR + RED}$$



Phenocave

A



B

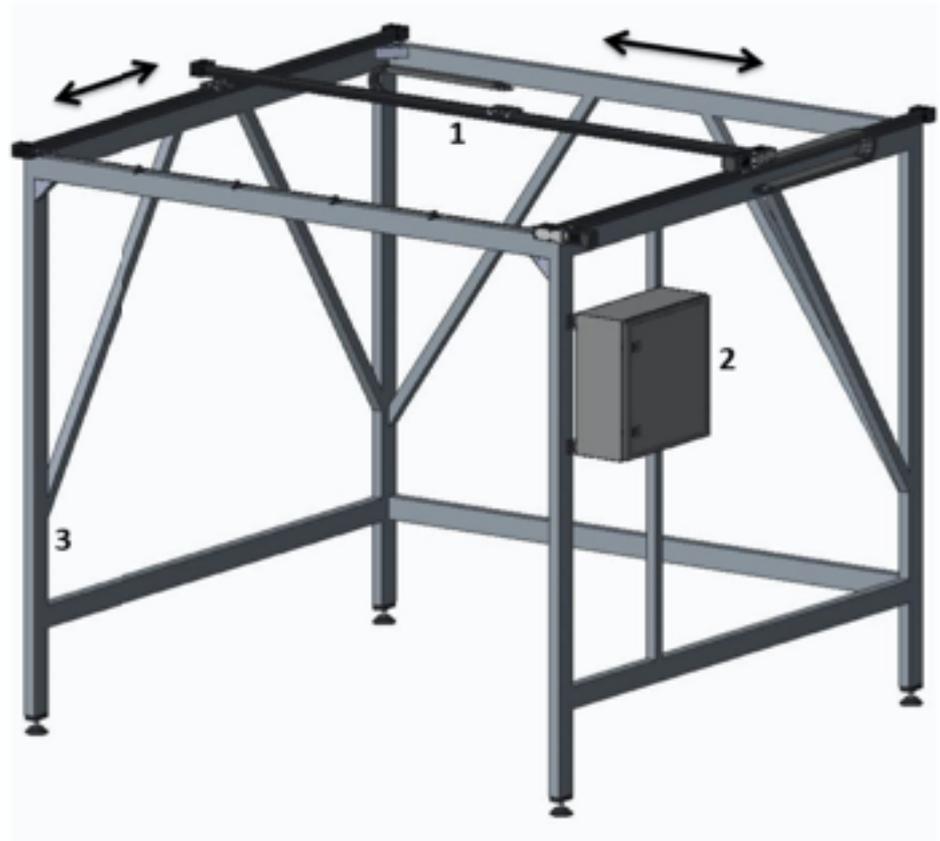


Figure 1. (A) Phenocave installed in the Biotron chamber with two imaging sensors mounted, a DSLR Canon EOS and a multi-spectral MicaSense Altum; (B) schematic Phenocave model. The gray arrows indicate the movement on the XY coordinates.

RESISTREE Project

Identification of *Phytophthora* resistant beech trees by composition of endophyte communities, lesion formation and phenomic approaches.





Phytophthora

APPLIED RESEARCH

For. Sci. 00(00):1-3

doi:10.1093/for/fis013

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entomology & pathology

Phytophthora cinnamomi Involved in the Decline of Holm Oak (*Quercus ilex*) Stands in Southern Italy

Salvatore Frisullo, Giuseppe Lima, Costantino Magnano di San Lio, Ippolito Camele,
Luigi Molisano, Ivana Puglisi, Antonella Pane, Giovanni Enrico Agosteo,
Leonardo Prudente, Santa Olga Cacciola



Plant Pathology An International Journal edited by
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Free Access

Phytophthora root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies

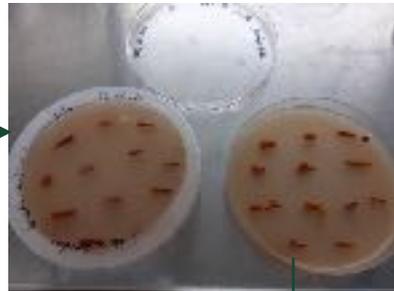
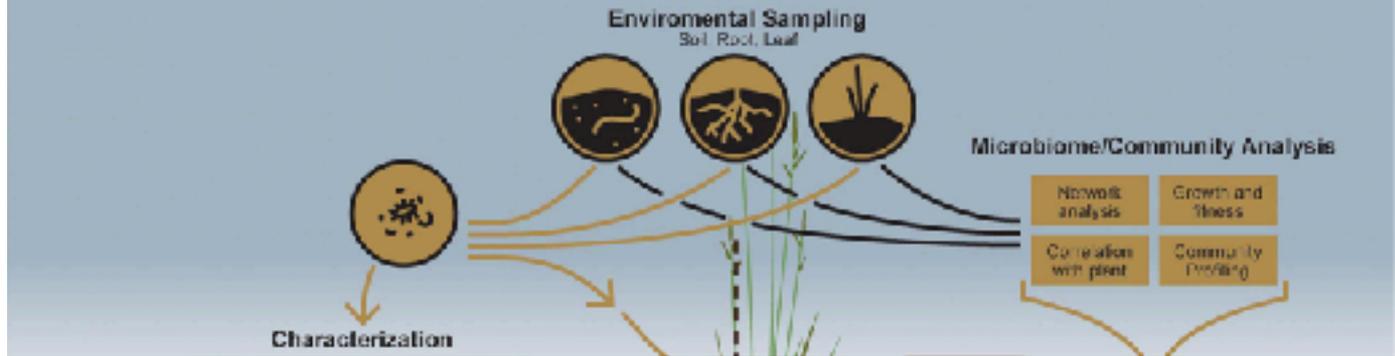
T. Jung, M. Blaschke

First published: 28 April 2004 | <https://doi.org/10.1111/j.0032-0862.2004.00957.x> | Citations: 75



Reductionist Approach

Holistic Approach



FUNGAL COMMUNITIES ILLUMINA-ITS



Co-culture

tests

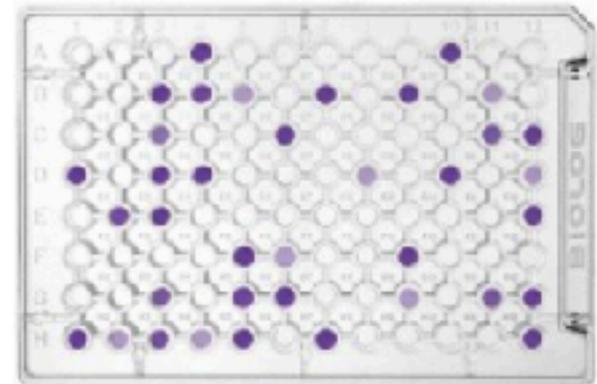


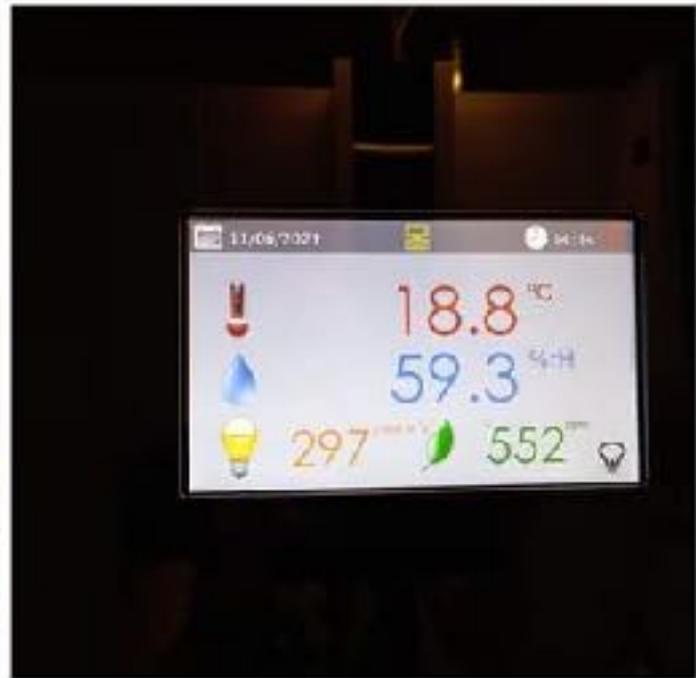
Antagonisms observed



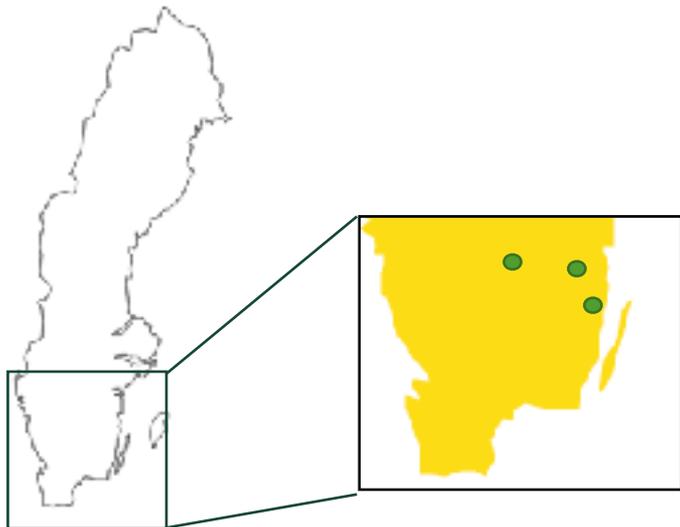
Eco-Plates

- Measure the metabolism of 31 carbon sources per assay
- Each assay panel tests in triplicate
- Simple colorimetric readout
- Readable with any microplate reader





Example Metabarcoding



Sampling sites: 3 locations in Southern Sweden

41 seedlings were collected in Nov 2019

2 yr old seedlings of Scots pine (*Pinus sylvestris*)

Different symptoms of growth disturbances



Source: Meshkova et al. 2018



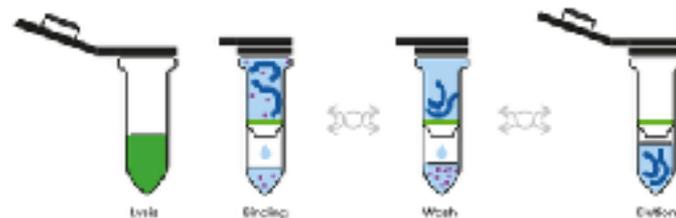
Source: etsy.com

Materials and methods

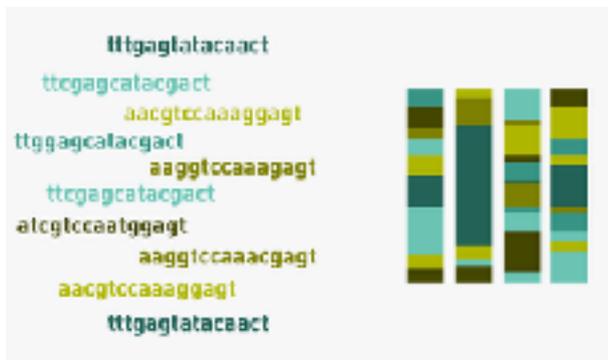


Liquid nitrogen

Source: wikipedia



Source: danagene.es



Source: allgenetics.eu

ITS region for fungi

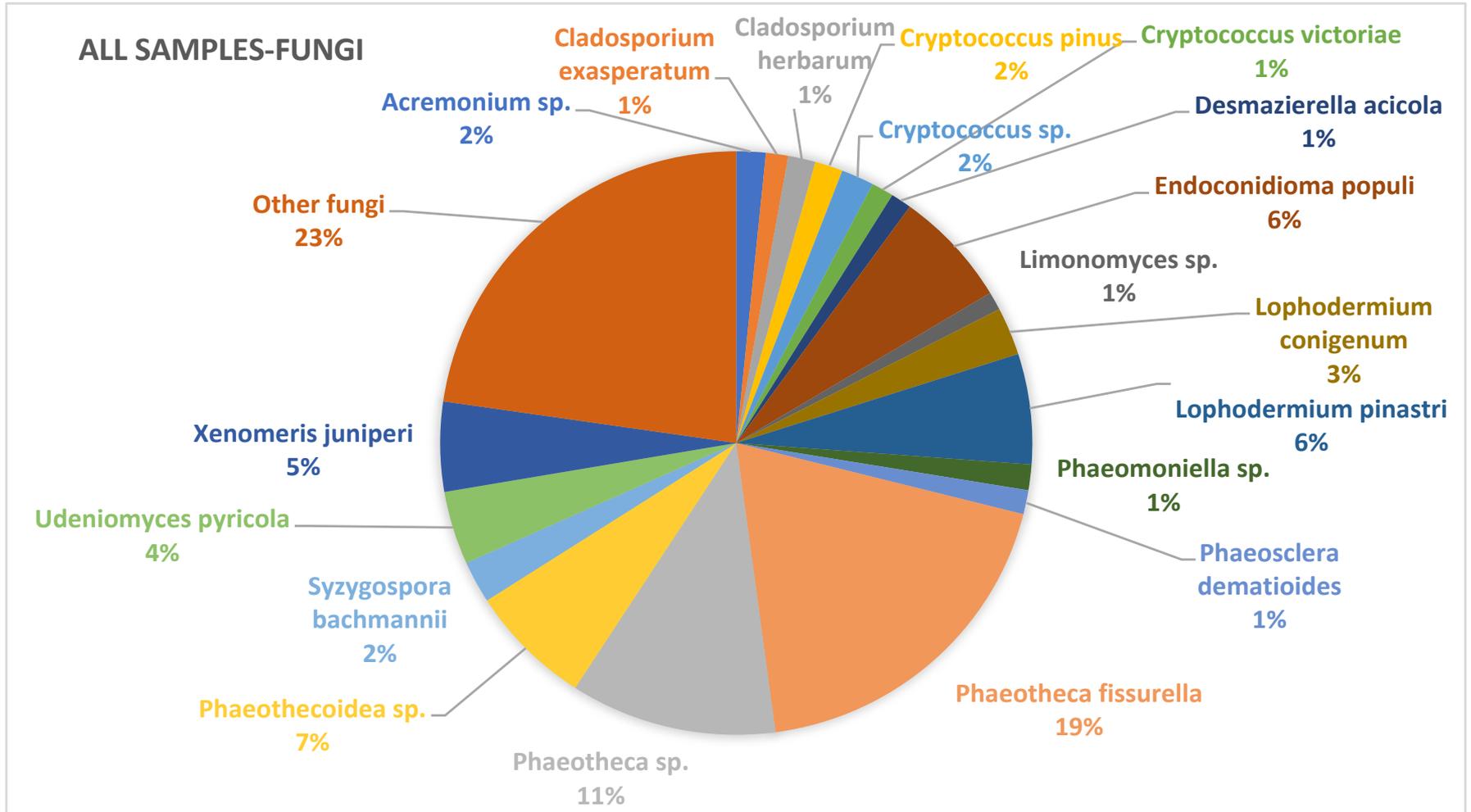
16S for bacteria



ILLUMINA

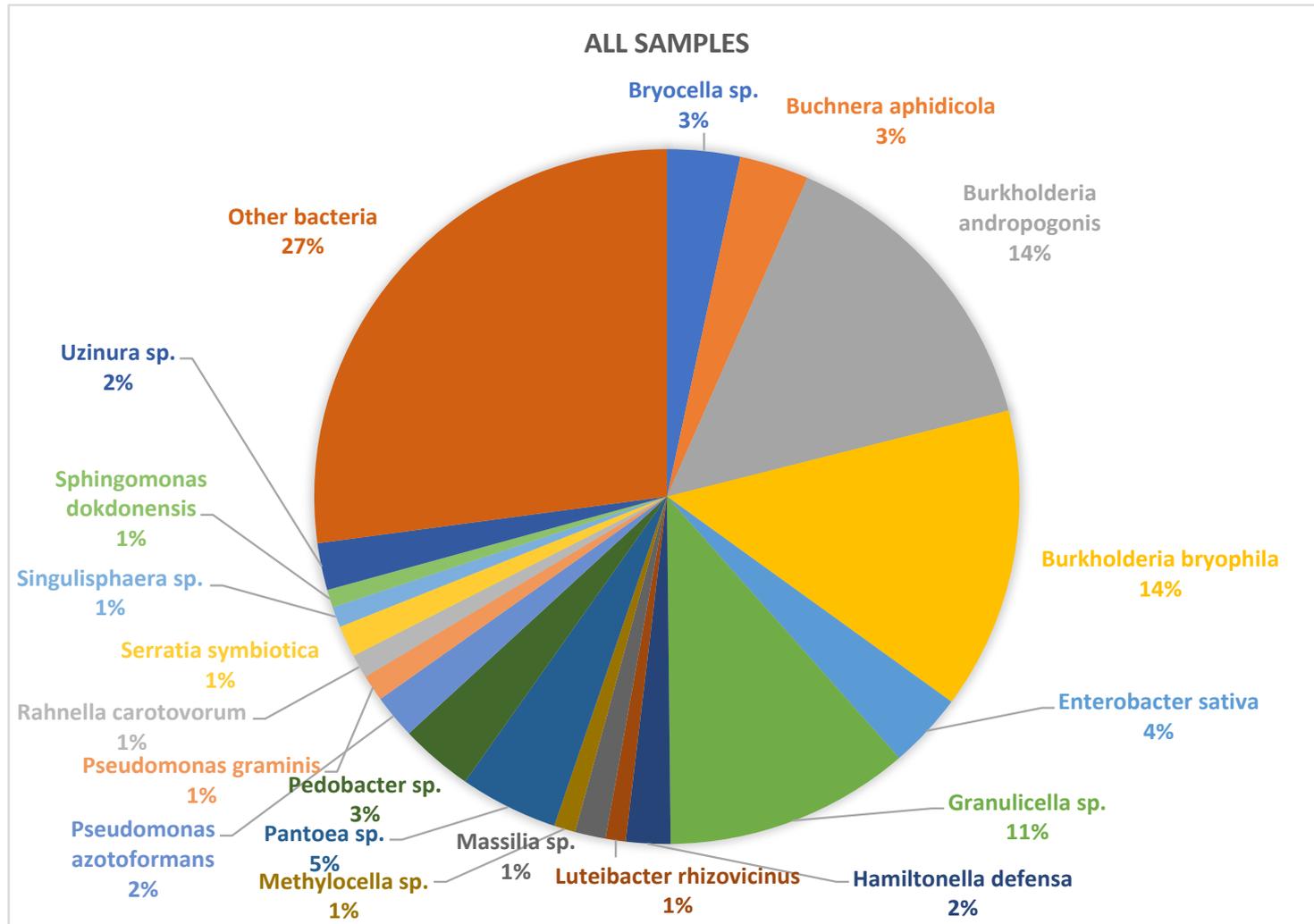
Results

878 Fungal OTUS



Results

573 Bacterial OTUS



Take home messages



- Plants rely on their microbiome for many important **life-support** functions (e.g. nutrient acquisition and protection against stress factors).
- Tree microbiome can **limit** pathogen damage.
- **NGS** is an important tool to characterize pathogens and microbial communities.
- **Phenomics** can be a useful tool for detecting **early stages of pathogen infection** and or resistant genotypes.

Videos

- Illumina: https://www.youtube.com/watch?time_continue=174&v=ZIPH9FzUOq8&feature=emb_logo
- DNA metabarcoding: <https://www.youtube.com/watch?v=kv09Mhegzuw>
- DNA Extraction from Plant Cells **Practical Experiment:** https://www.youtube.com/watch?v=_J_tB9PKNGI&t=8s
- What is DNA: <https://www.youtube.com/watch?v=GlzNIISbCxl&list=PLW0gavSzhMITeTj8290mOkZ0SOyNCkNz2>
- PCR: <https://www.youtube.com/watch?v=2KoLnlwoZKU>
 - https://www.youtube.com/watch?v=c07_5BfIDTw

Thanks for your attention!

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