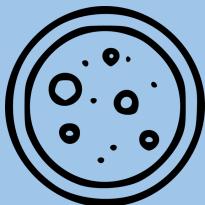


Je suis un microbe dans le lac, me voyez-vous ?

Écologie microbienne aquatique



Présenté par
Naíla Barbosa da Costa, Paula Reis et Patricia Tran

Les Ateliers Automnaux GRIL-EcoLac 2017



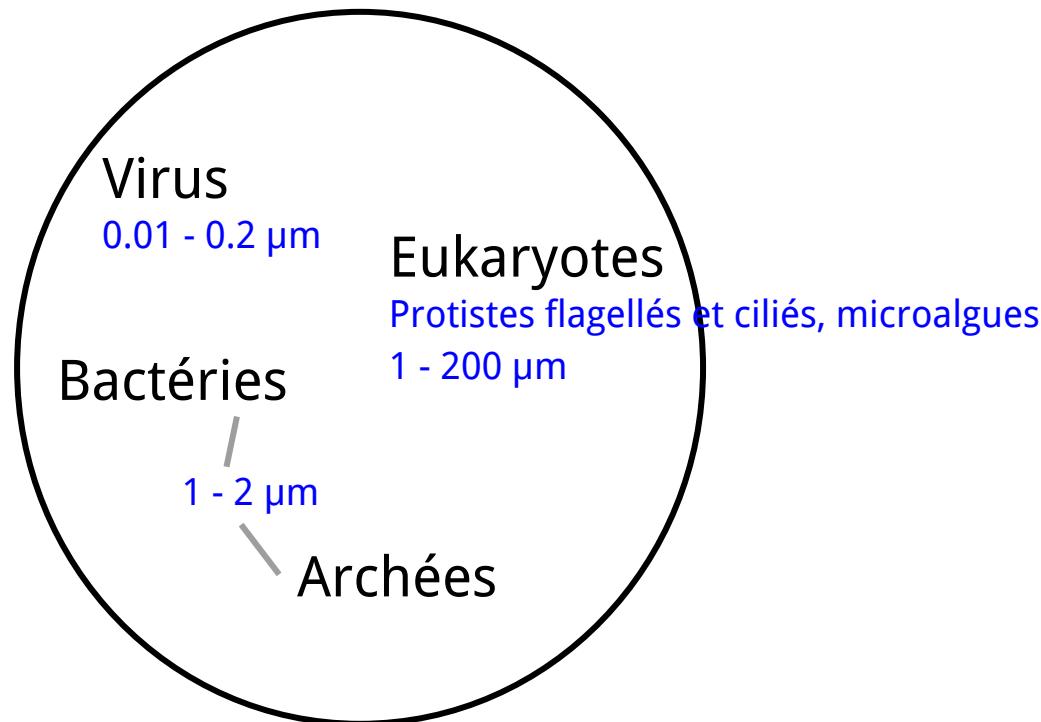
Groupe de recherche
interuniversitaire en limnologie
et en environnement aquatique



Programme de formation FONCER du
CRSNG en écologie lacustre et fluviale

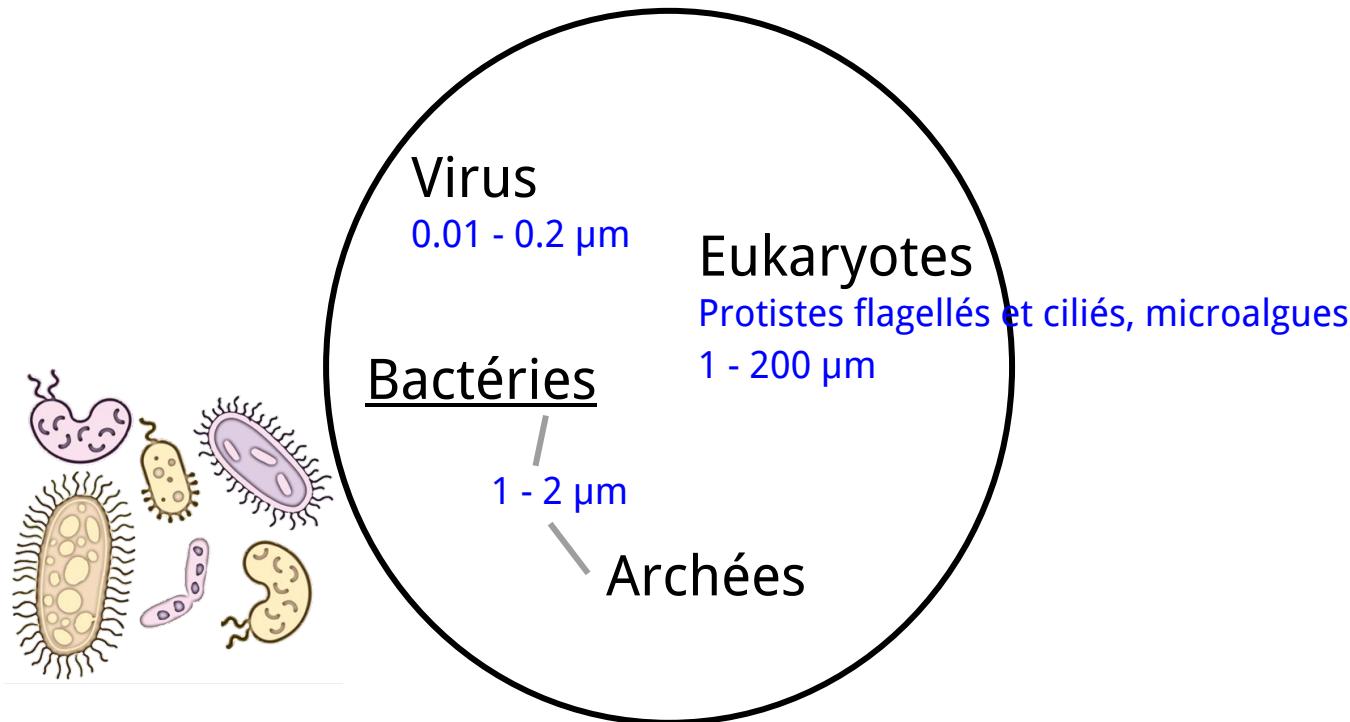
Qu'est-ce qu'un microbe ?

Microbe: organisme microscopique



Qu'est-ce qu'un microbe ?

Microbe: organisme microscopique



Outline

Intro à l'écologie microbienne

Focus sur les bactéries dans les lacs:

Que font-elles ?

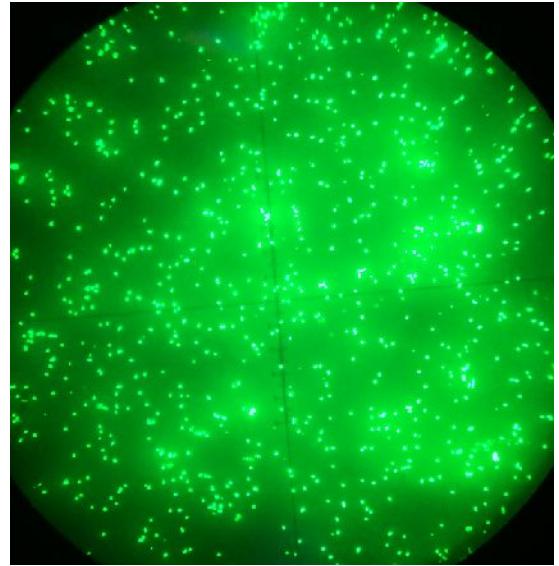
- Diversité de métabolismes

Sont-elles actives ?

- Déetecter leur activité

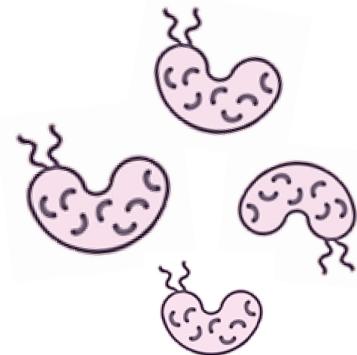
Les identifier (qui sont-elles ?)

- Méthodes traditionnelles (DNA sequencing)
- Méthodes NGS (“next-generation sequencing”)

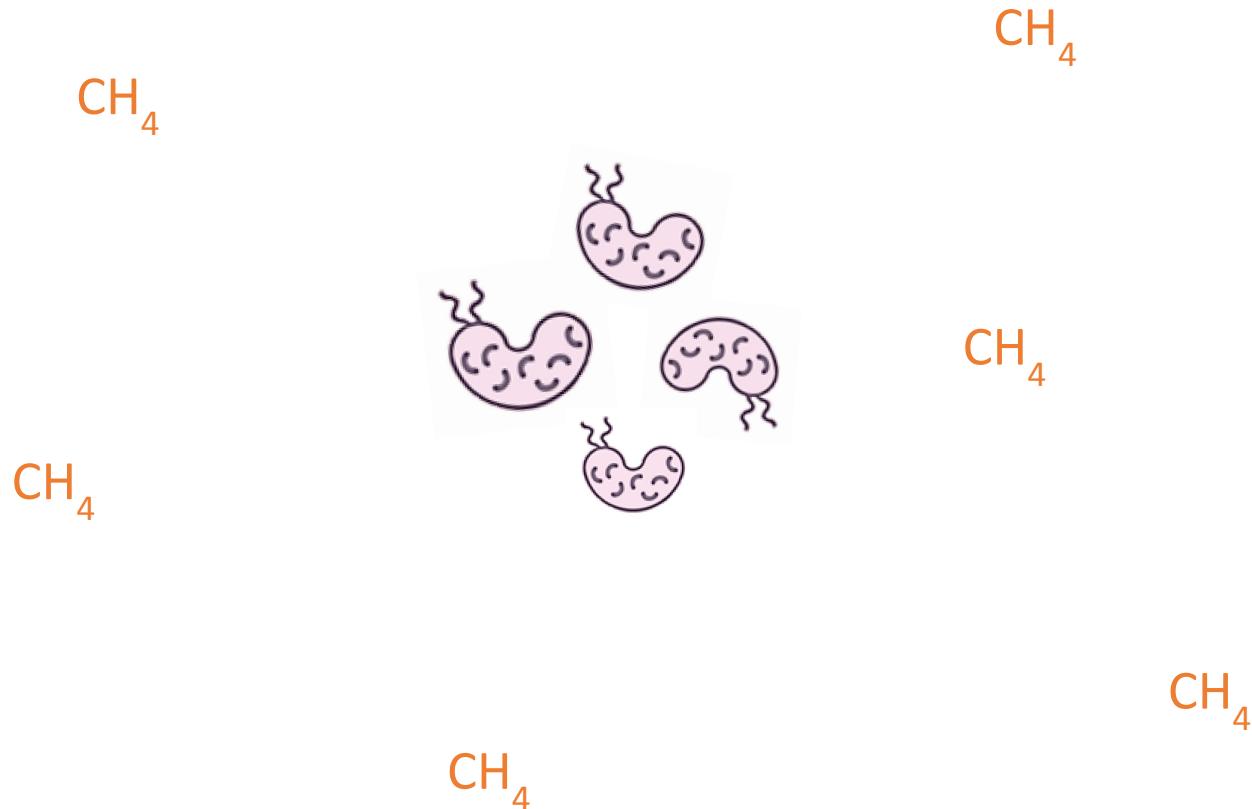


Belles bactéries méthanotrophes

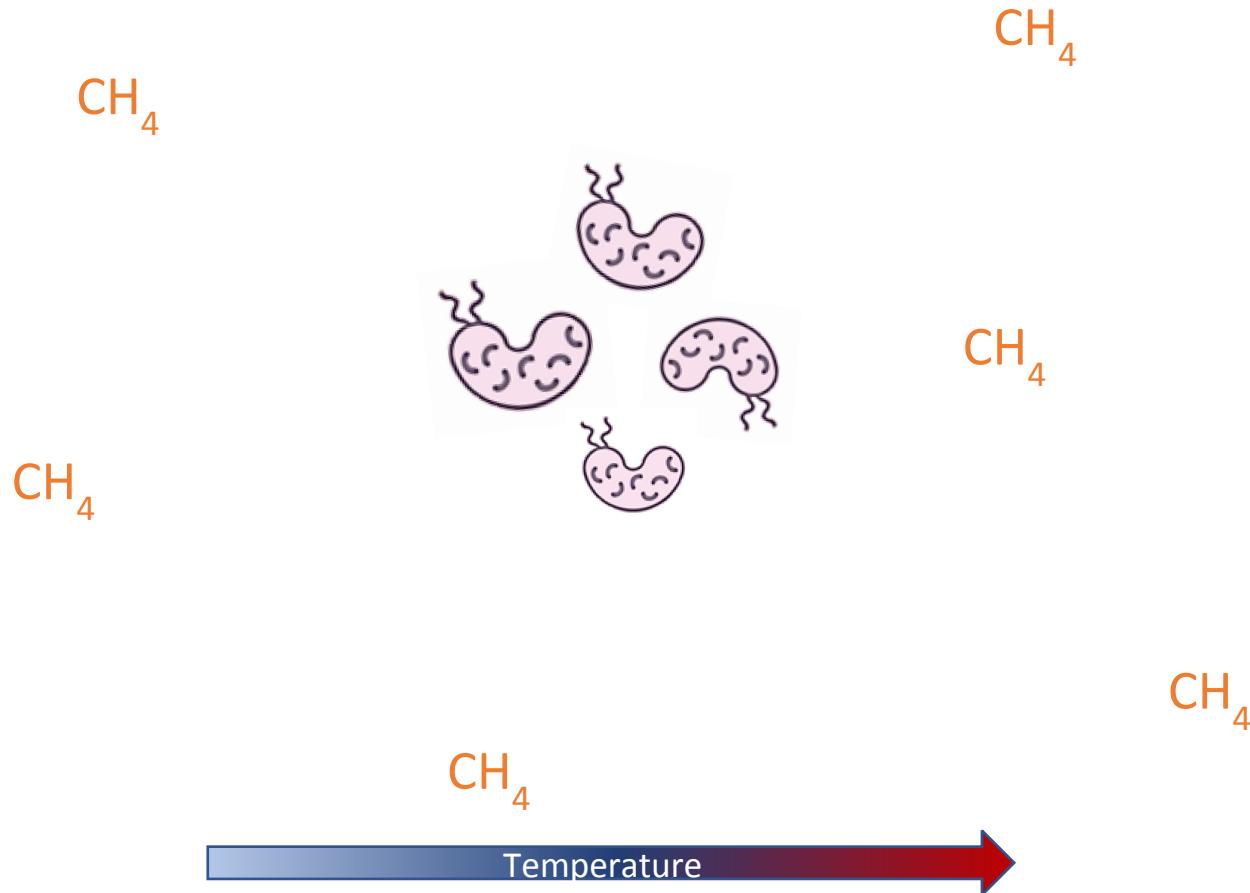
Différents régulateurs d'abondances bactériennes



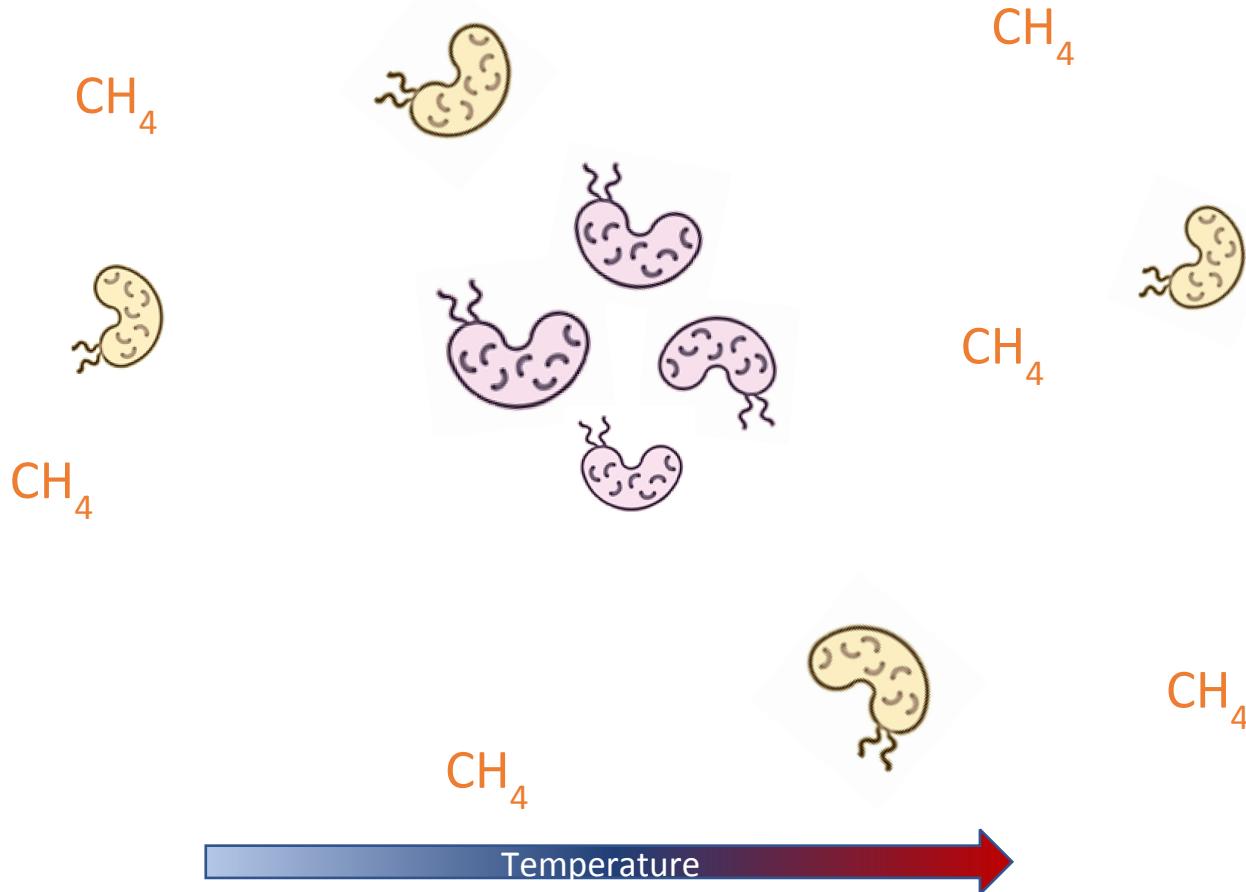
Différents régulateurs d'abondances bactériennes



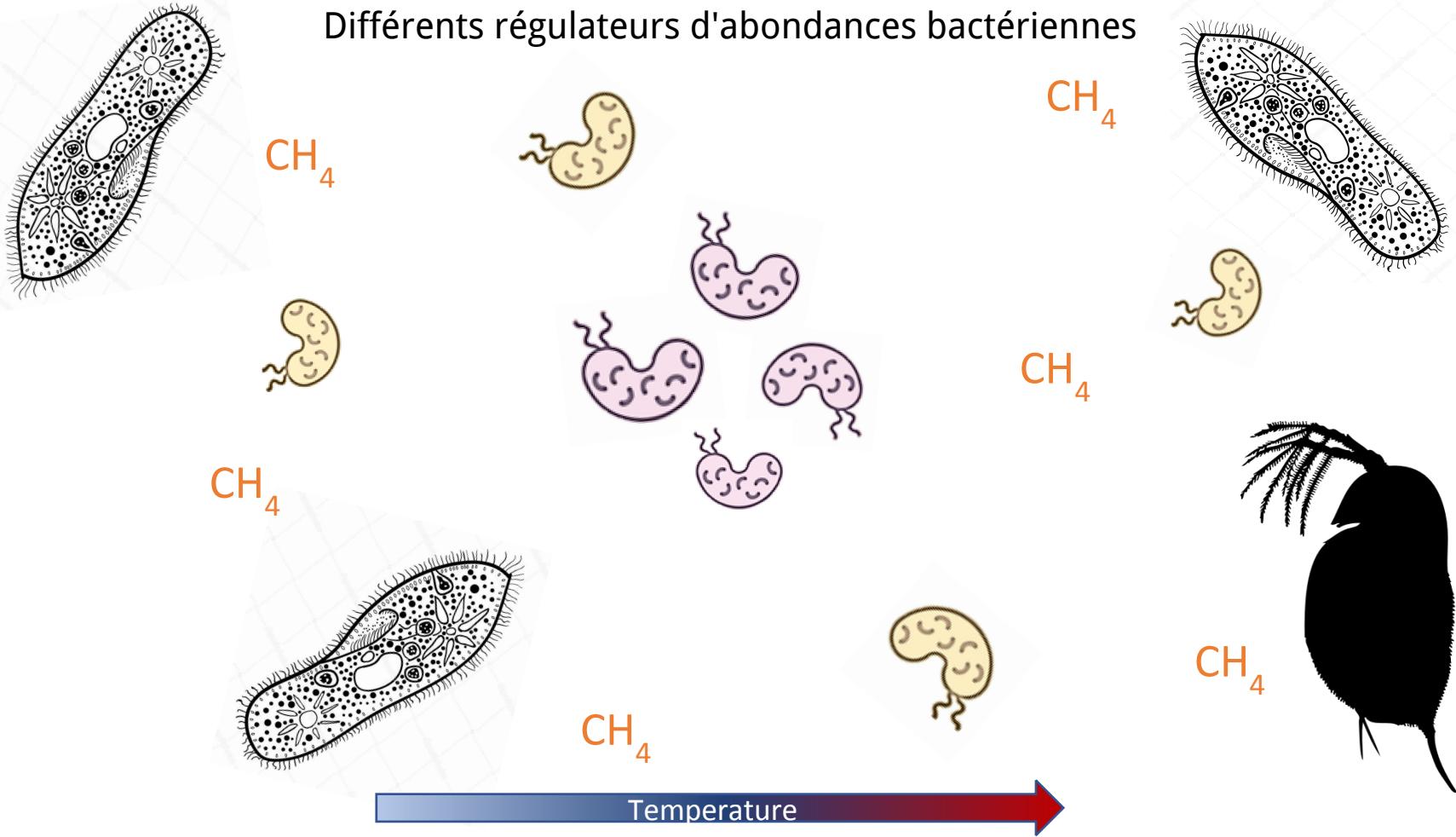
Différents régulateurs d'abondances bactériennes



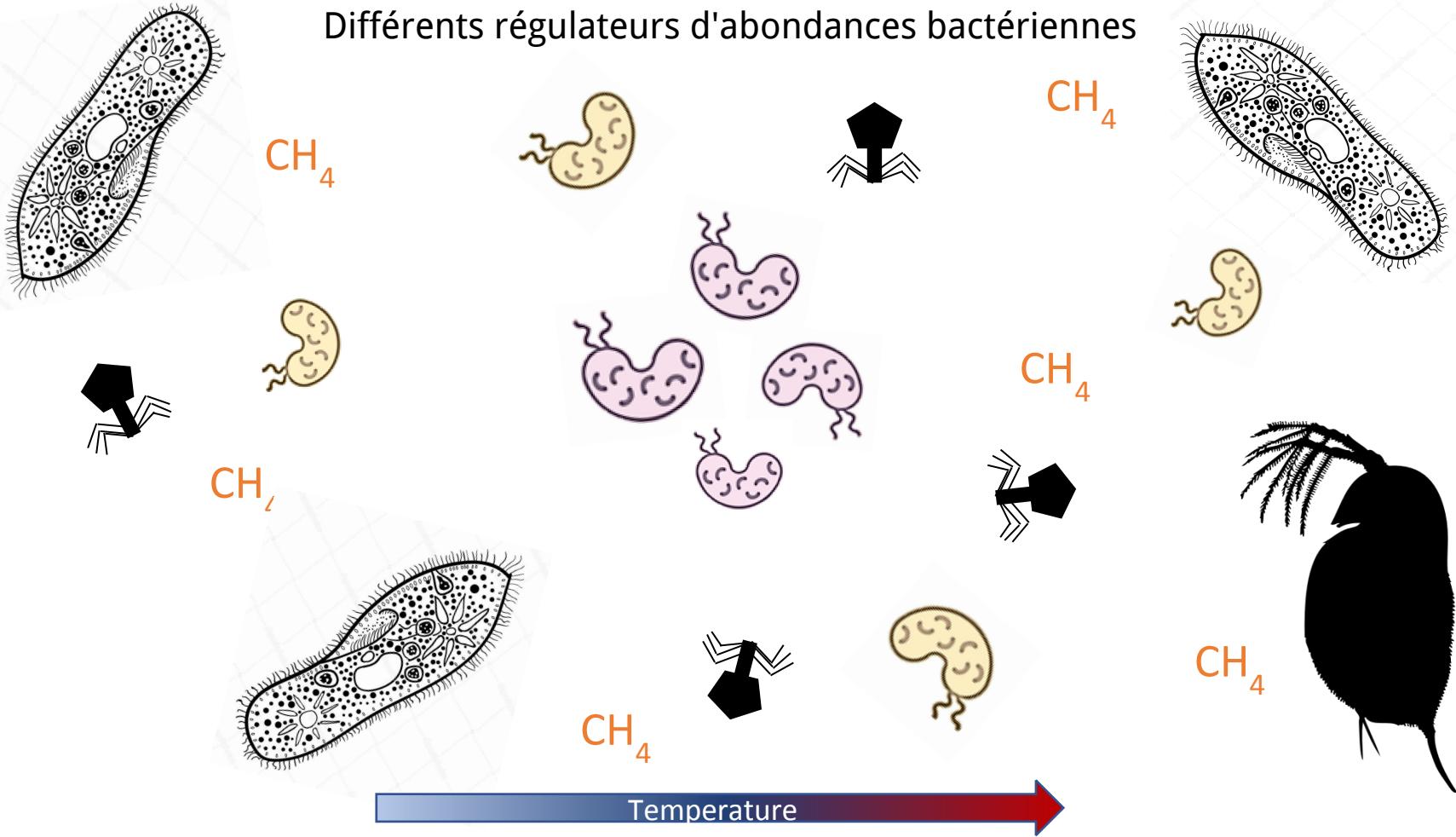
Différents régulateurs d'abondances bactériennes



Différents régulateurs d'abondances bactériennes



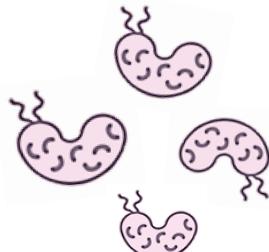
Différents régulateurs d'abondances bactériennes



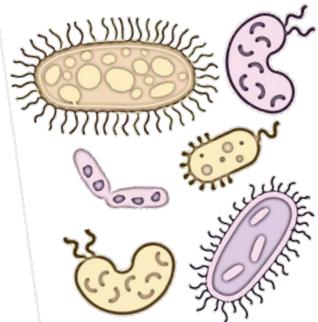


Les échelles

Génomique



Population



Communauté



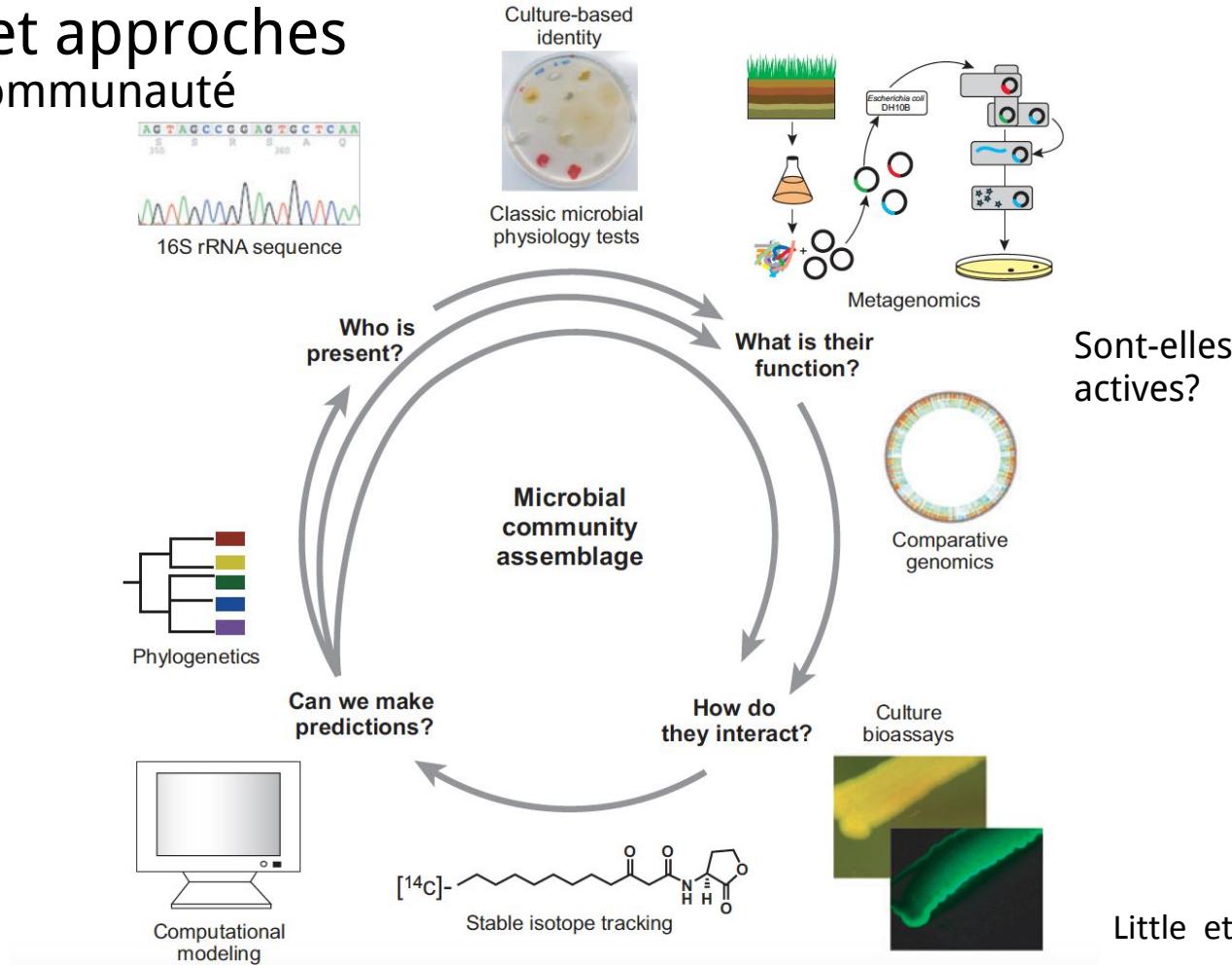
Écosystème



Paysage

Questions et approches

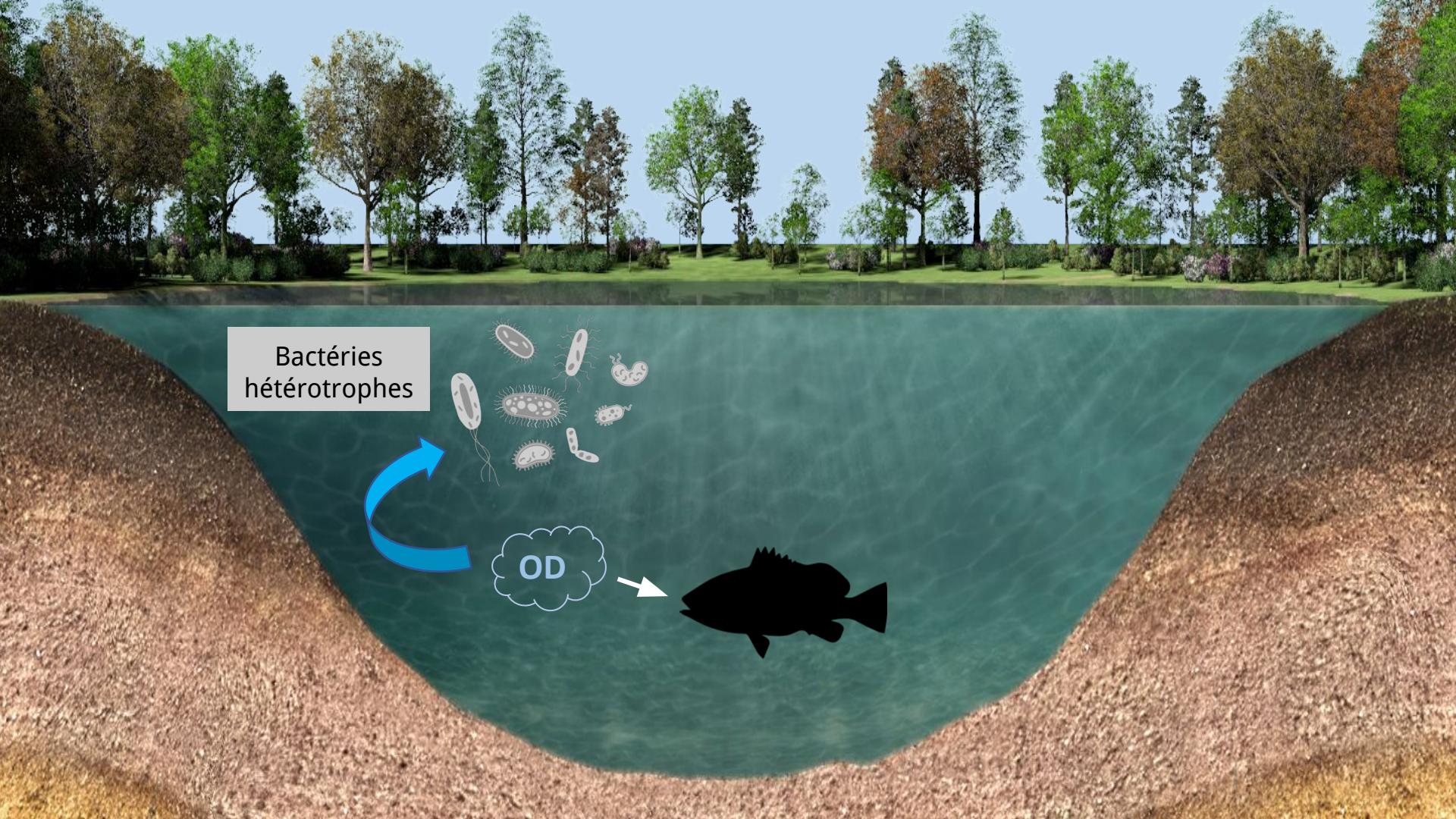
Niveau de la communauté



Little et al. 2008

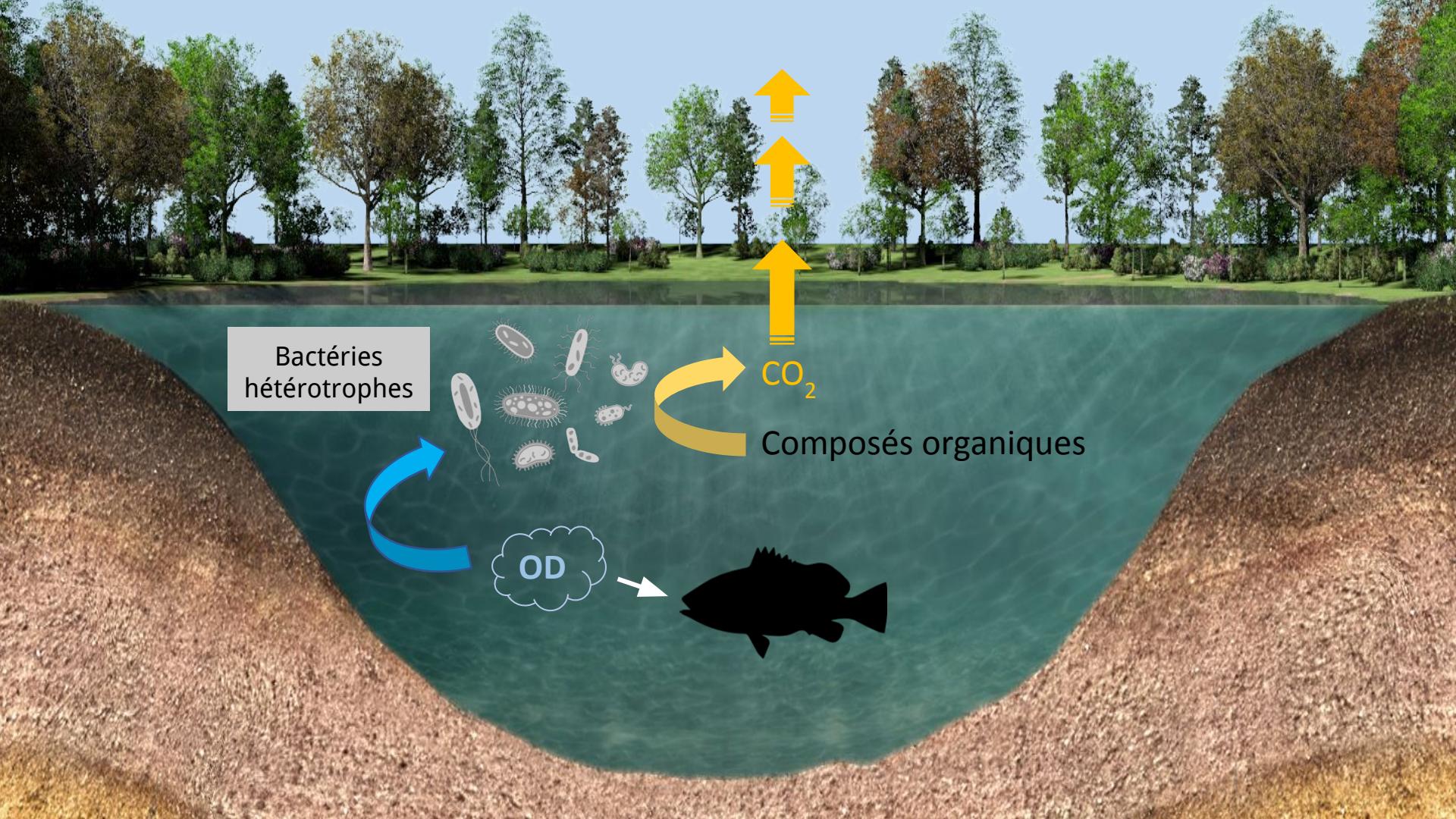


Pourquoi sont-elles importantes ?



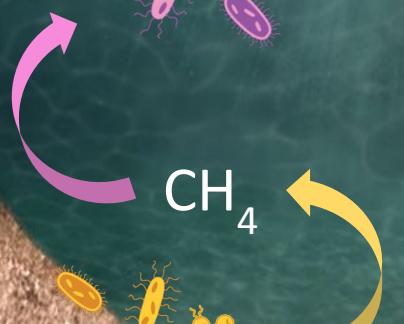
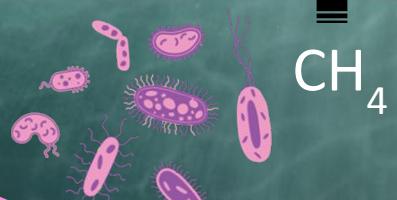
Bactéries
hétérotrophes

OD

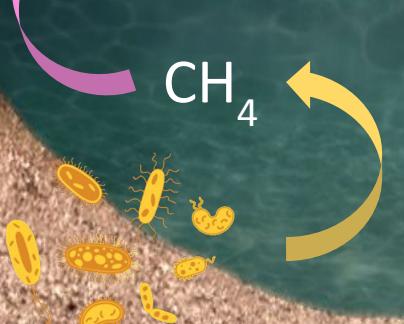




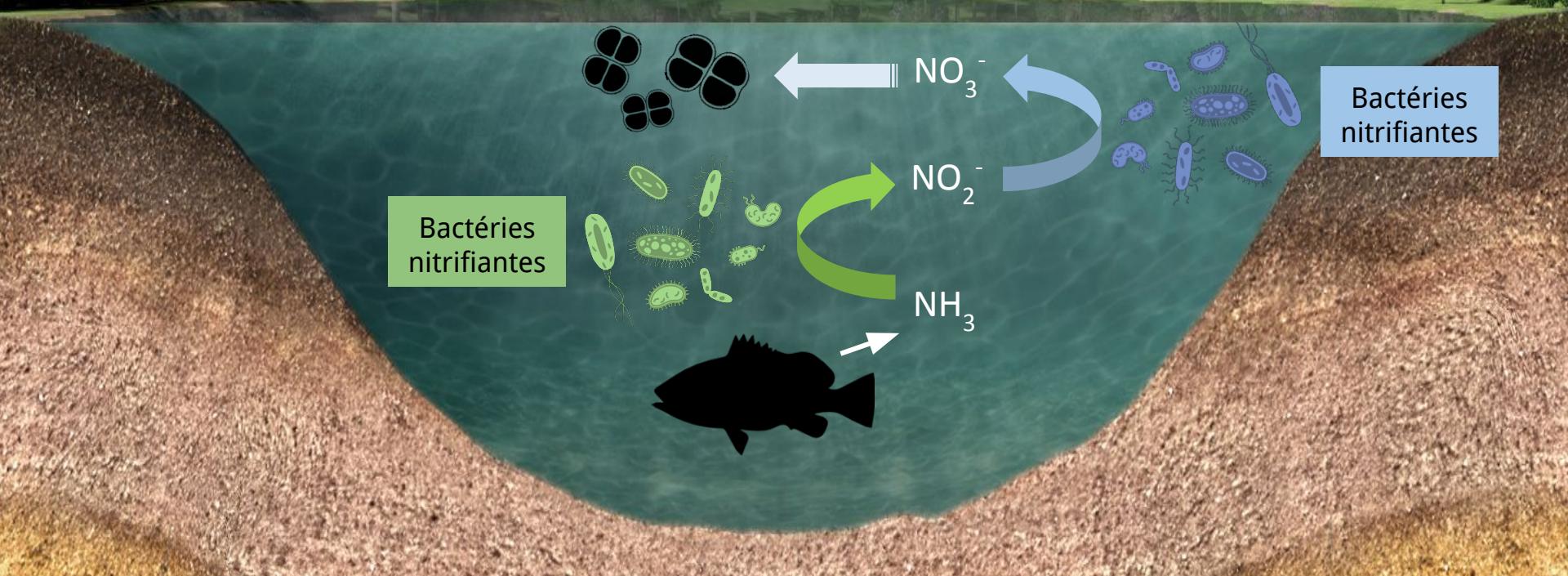
Bactéries
méthanotrophes

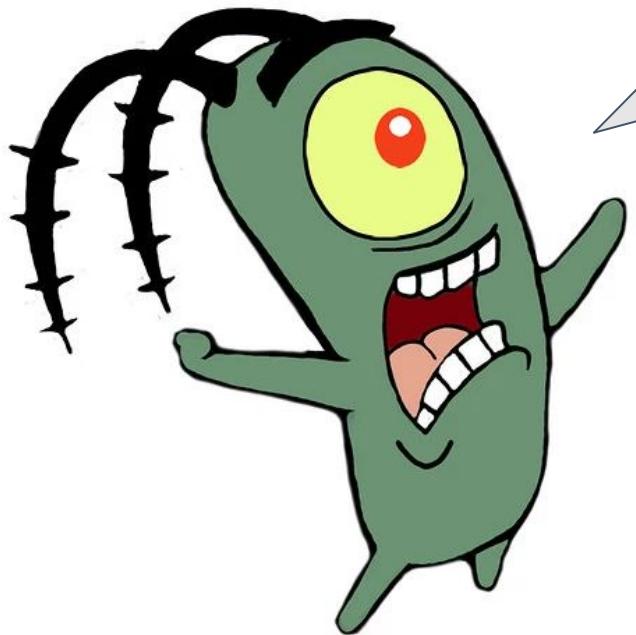


MO



Archées
méthanogènes

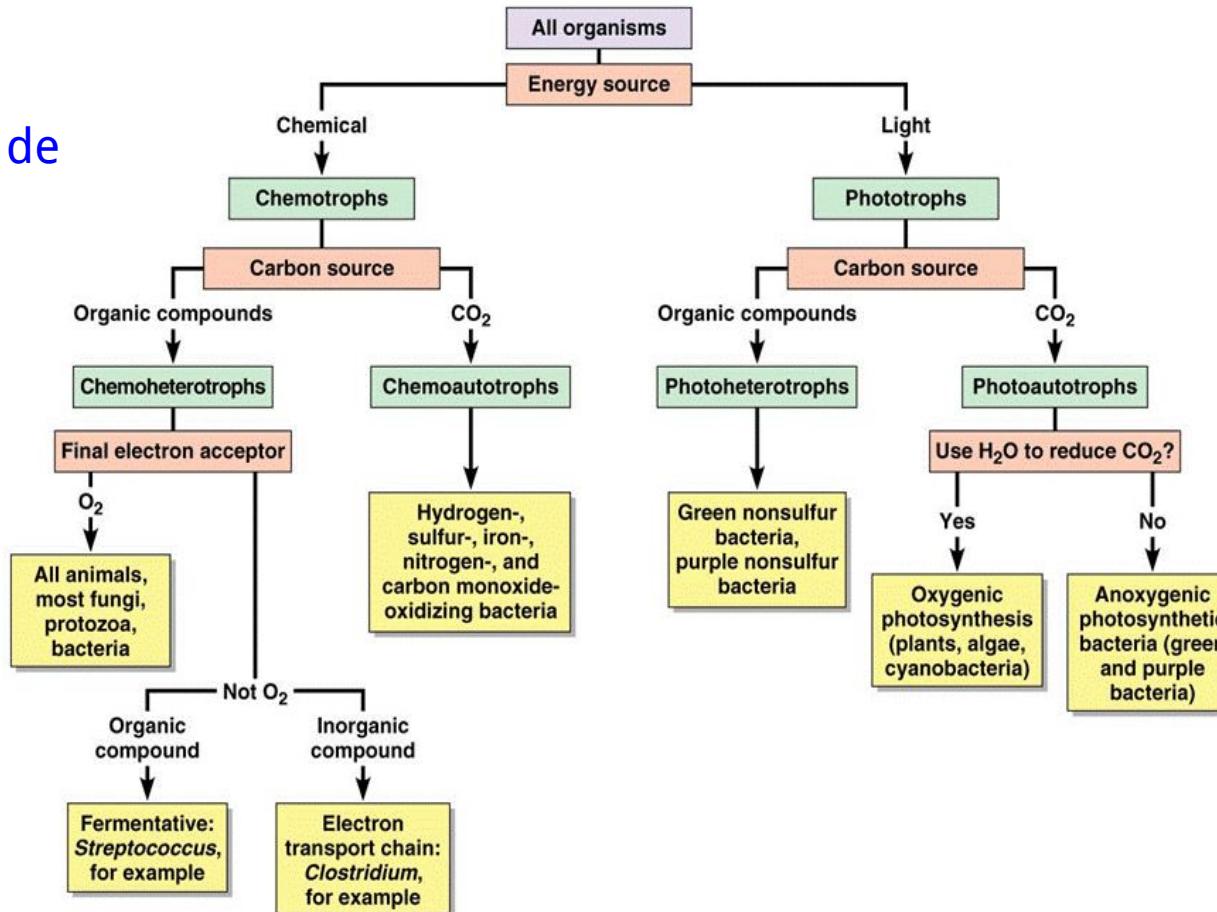




I ☐ bacteria!

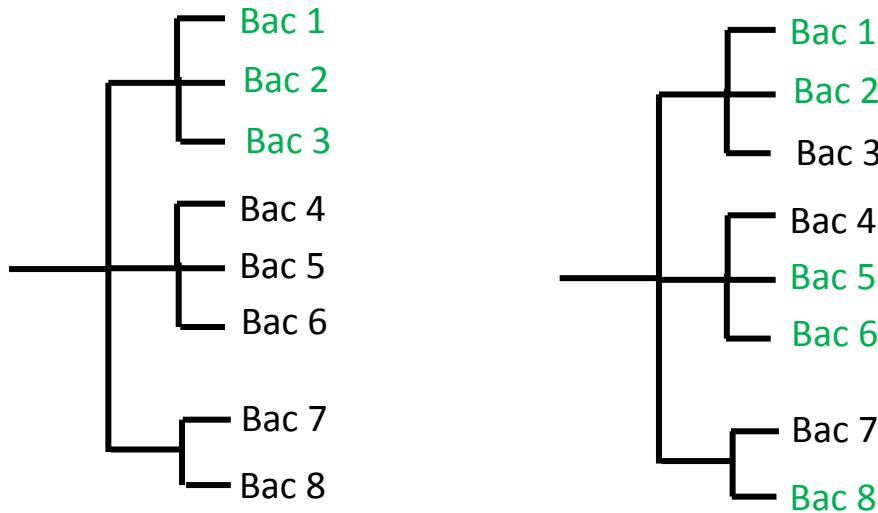
Chez les bactéries, le métabolisme est très varié!

Sources d'énergie et de carbone



Chez les bactéries, le métabolisme est très varié!

Le métabolisme peut être associé ou non avec l'affiliation phylogénétique.



L'activité microbienne au centre des processus de l'écosystème

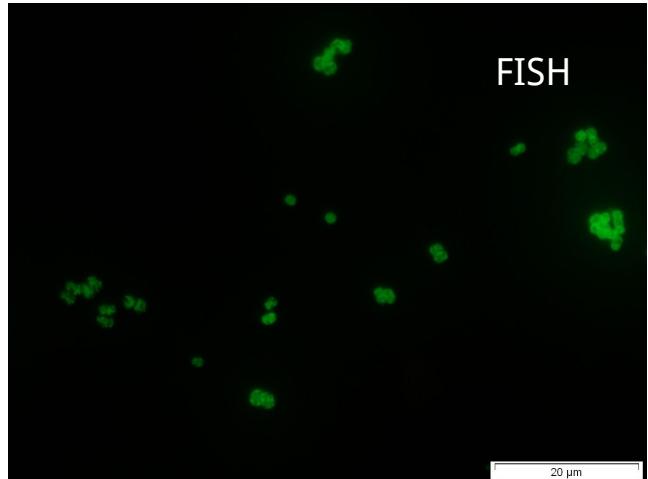
- Qu'est ce qui régule leur présence, abondance, et activité ?
- Comment est-ce que l'affiliation taxonomique est reliée à les processus/fonctions ?

Comprendre ces mécanismes est important pour prédire les changements dans l'écosystème dû à plusieurs facteurs d'impacts : l'eutrophisation, le réchauffement climatique, la construction de barrages, l'invasion d'espèces exotiques, etc...

Mesurer le microbiome

Quantifier les bactéries:

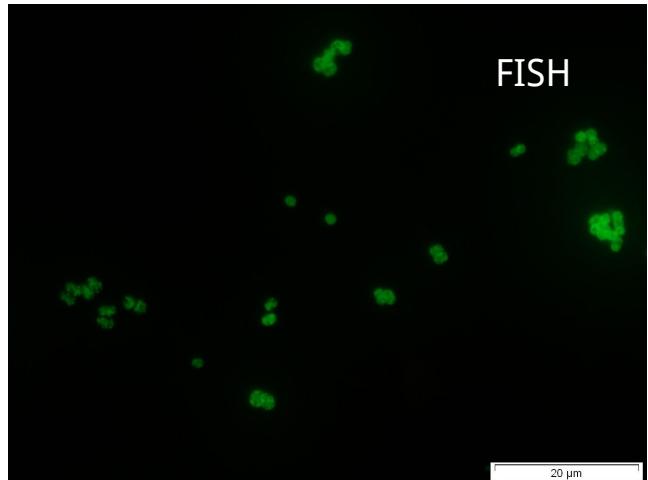
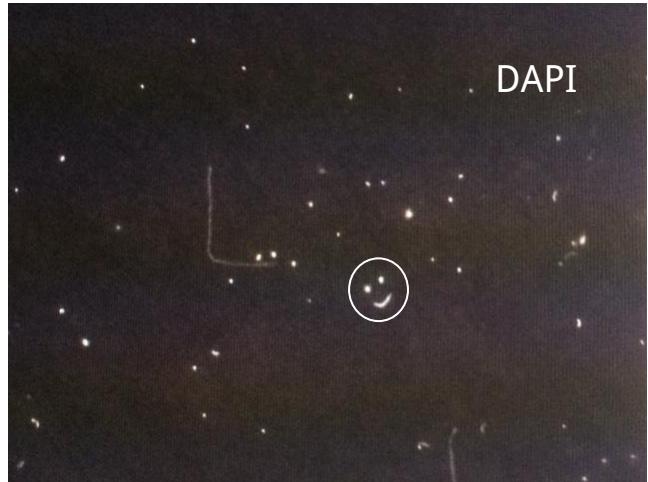
- Microscopie
 - DAPI - toutes les cellules
 - FISH - groupes spécifiques



Mesurer le microbiome

Quantifier les bactéries:

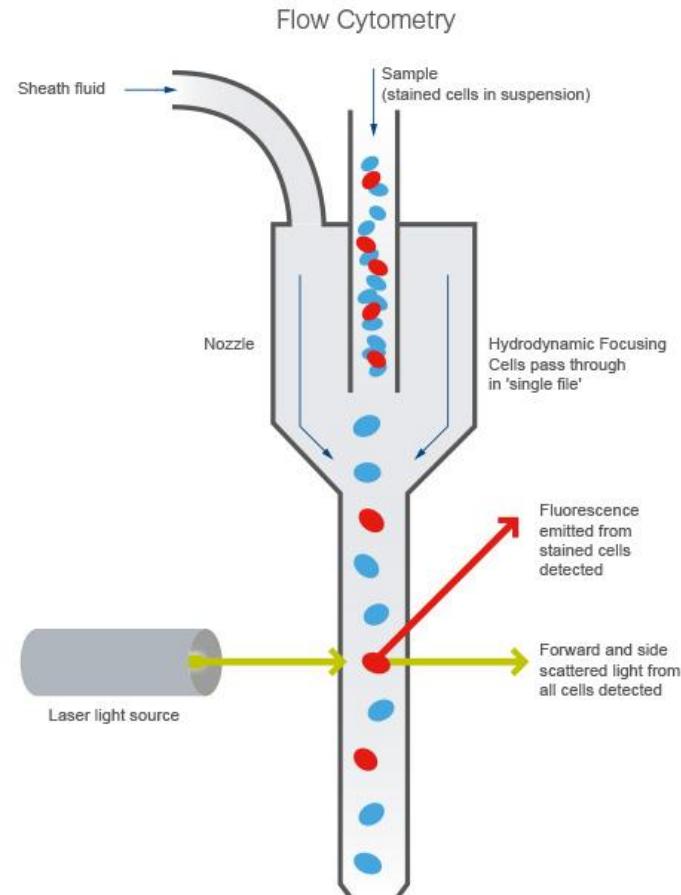
- Microscopie
 - DAPI - toutes les cellules
 - FISH - groupes spécifiques



Mesurer le microbiome

Quantifier les bactéries:

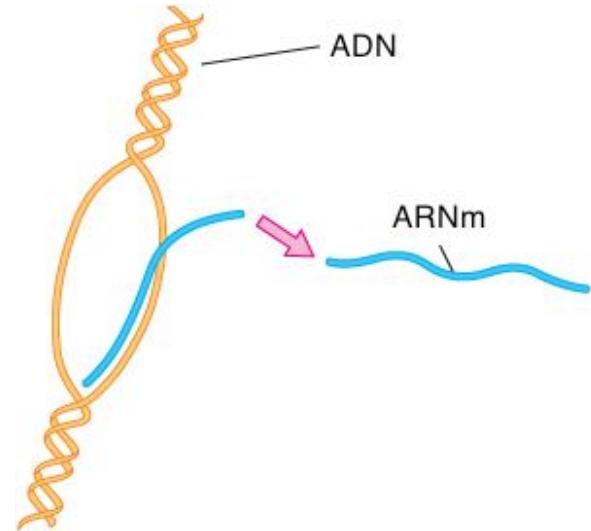
- Microscopie
 - DAPI
 - FISH - groupes spécifiques
- Cytomètre de flux



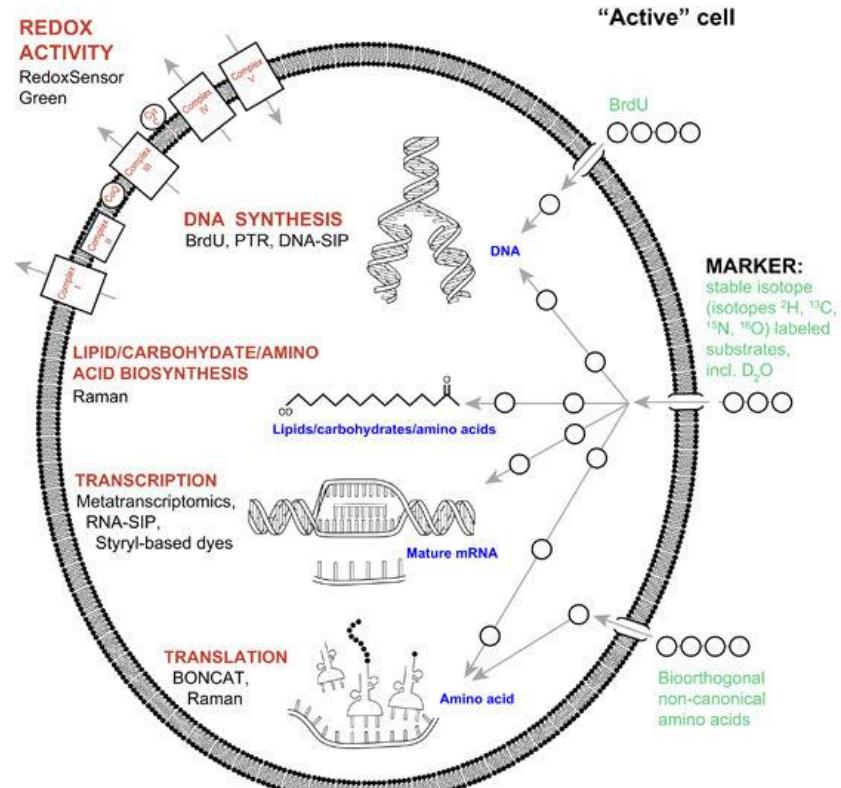
Mesurer le microbiome

Mesurer l'**activité** microbienne:

- Transcription des gènes fonctionnels
- qPCR – quantifier la transcription des gènes



Les techniques de marquage peuvent cibler différents processus cellulaires

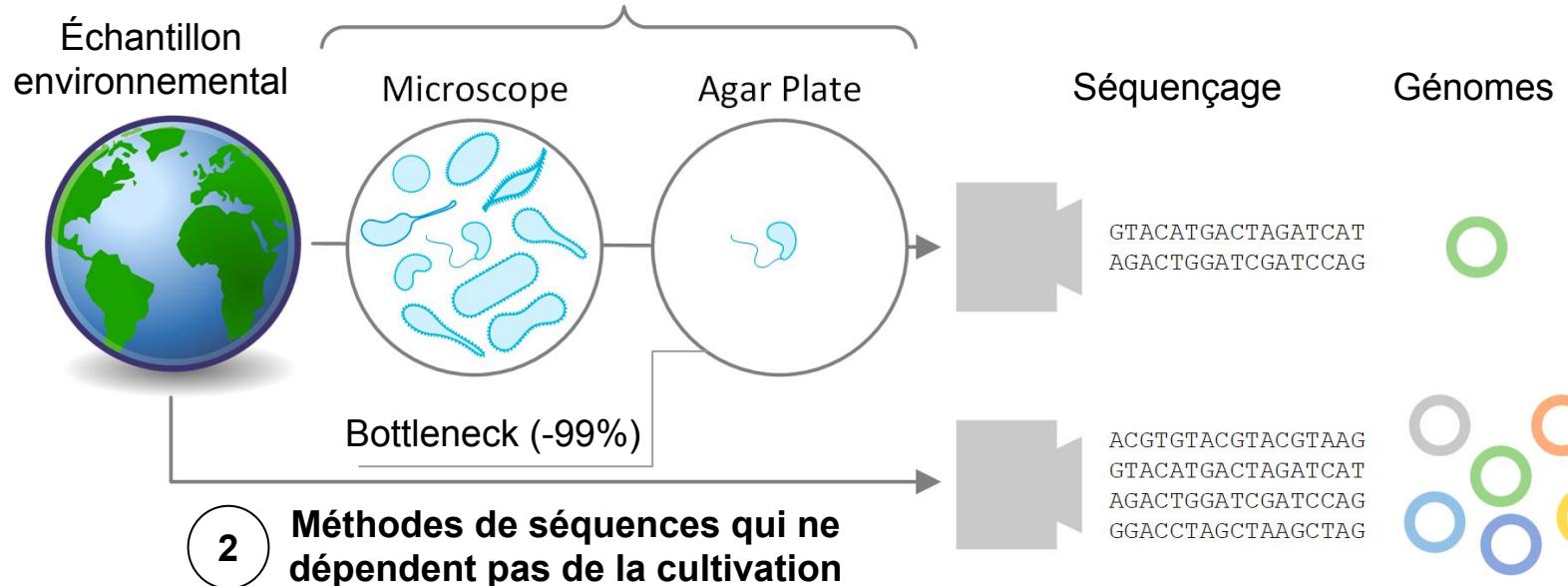


- Synthèse d'ADN
- Biosynthèse de lipide/ carbohydrate/ acides aminés
- Synthèse d'ARNm (transcription)
- Synthèse de protéines (traduction)

“The Great Plate Count Anomaly”

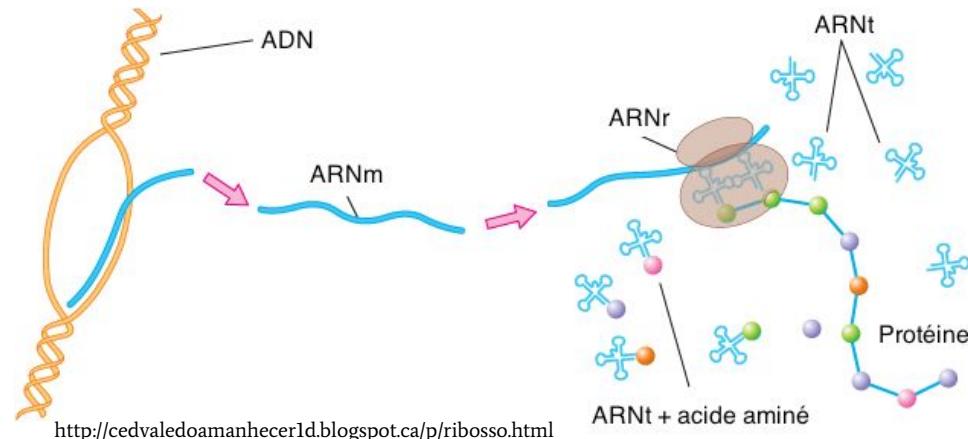
1 Méthodes qui dépendent de la culture

Seulement une fraction des bactéries sont cultivables



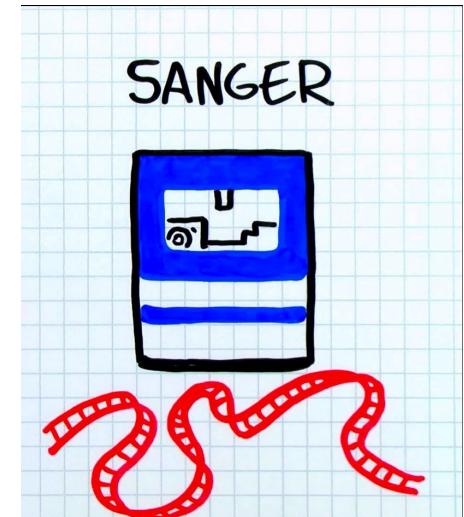
Méthodes indépendantes de la culture

- Basés sur la séquençage d'ADN
 - Dépendre d'extraction d'ADN ou ARN
 - ADN: composition de la communauté biologiques, relations évolutives et phylogénétiques
 - ARN: expression génétique



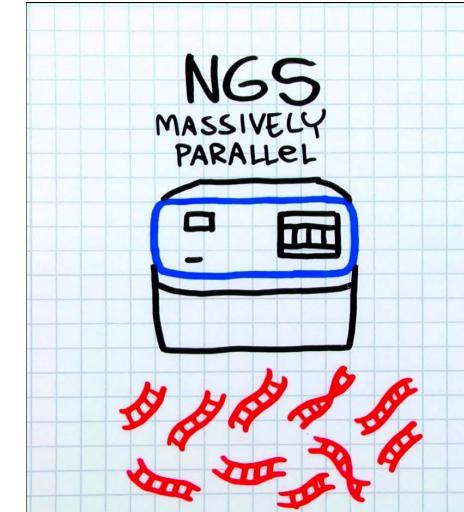
Méthodes basées sur le séquençage d'ADN

- Séquençage traditionnel (Sanger)
 - Une séquence à la fois
 - Isolation des séquences différentes avant le séquençage



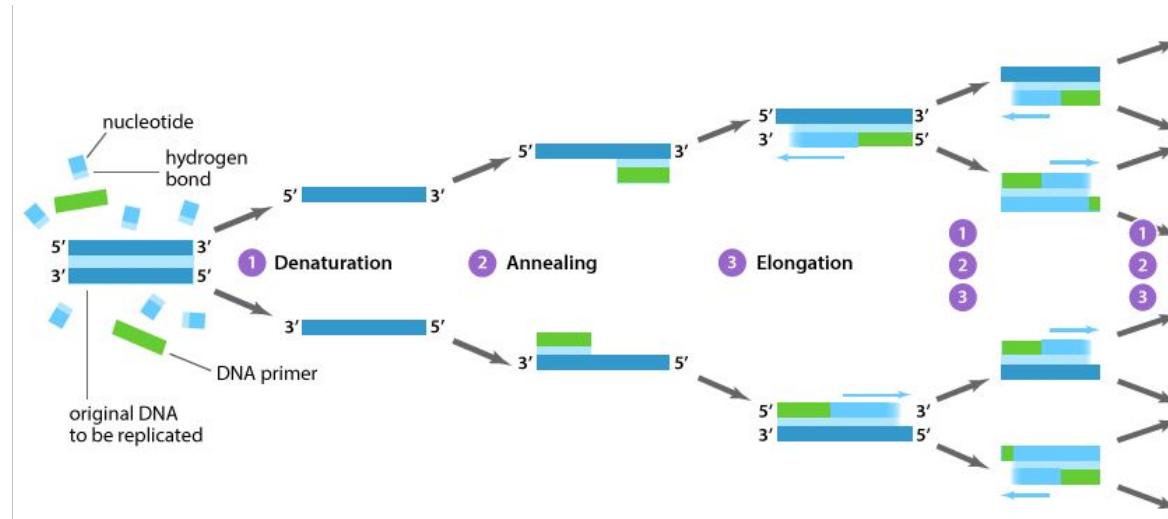
Méthodes basées sur le séquençage d'ADN

- Next Generation Sequencing (NGS)
 - Toutes les séquences en même temps (sequencing-by-synthesis)
 - L'isolation des séquences est faite après le séquençage (bioinformatique)



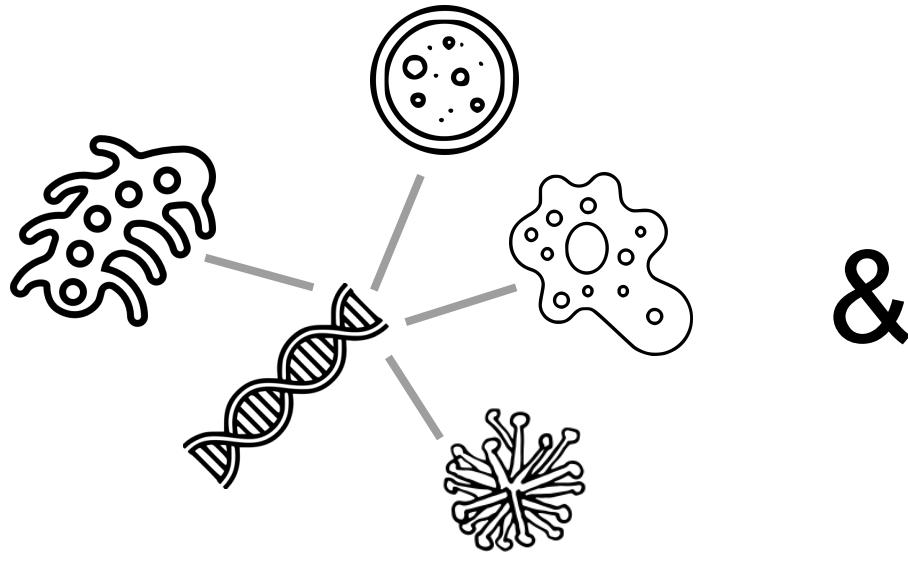
Méthodes basées sur le séquençage d'ADN

- Basés sur l'amplification par PCR en général
- PCR ⇒ de l'anglais “Polymerase Chain Reaction”
 - Amplification des séquences cibles d'ADN
- Amplification préférentielle des + abondantes ⇒ semi-quantitative

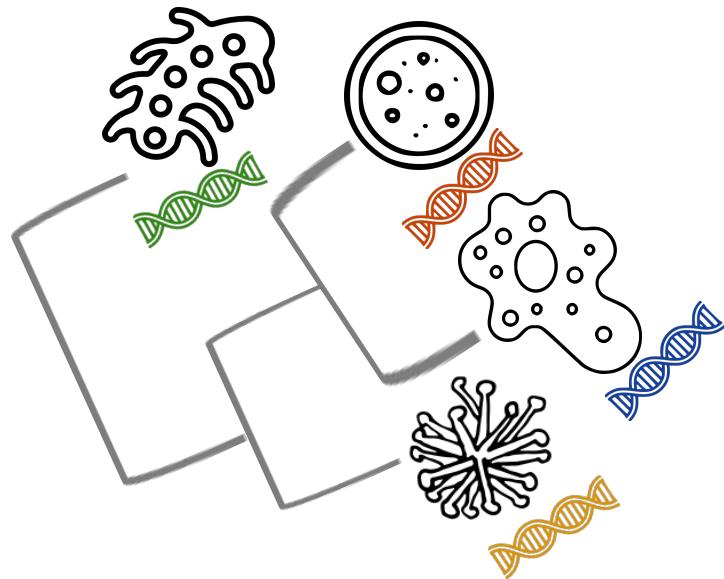


Quelle région amplifier?

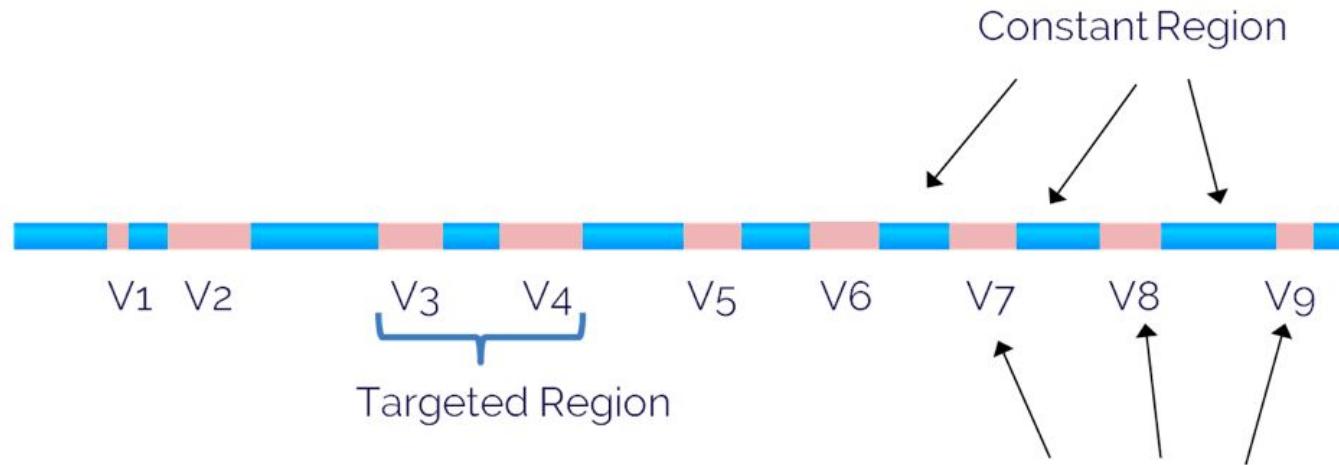
- Il faut choisir un gène:
 - Présent dans tous les organismes
 - Conservé dans l'espèce, mais avec des régions variables à travers les espèces



&

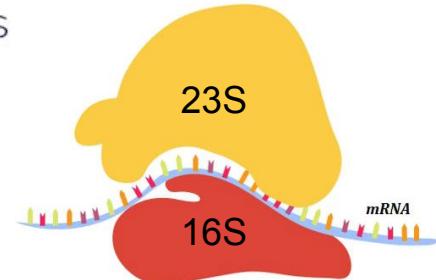


Exemple de régions cibles pour les études de diversité



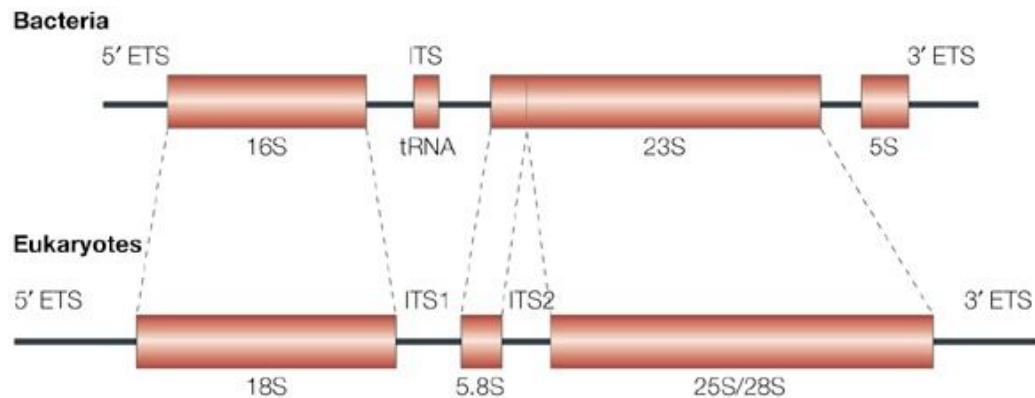
16S rRNA Gene

Variable Regions

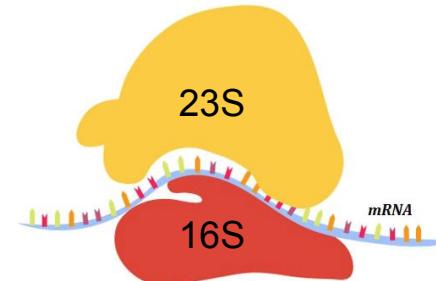
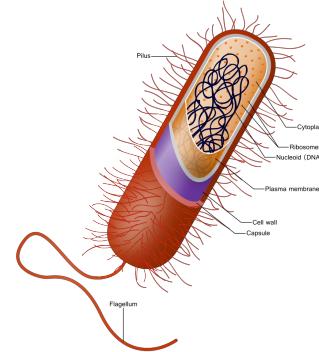


Séquences les plus utilisées

- Pour les organismes prokaryotes
 - Le gène de la sous-unité 16S d'ARNr
 - Le 16S-23S ITS (Internal Transcribed Spacer)



Nature Reviews | Molecular Cell Biology



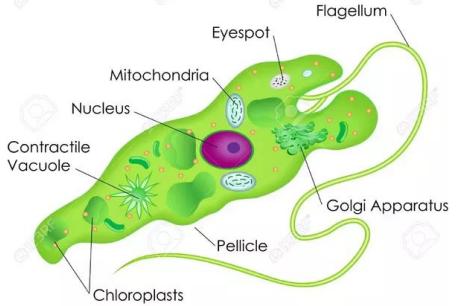
<http://biologianet.uol.com.br/biologia-cellular/ribosomos.htm>

Séquences les plus utilisées

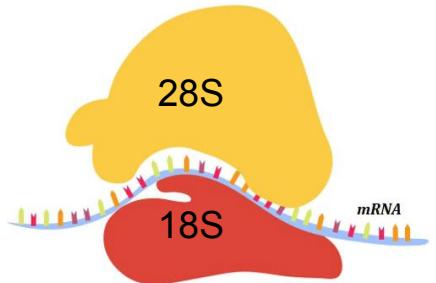
- Pour les microorganismes eukaryotes
 - Le gène de la sous-unité 18S d'ARNr
 - Le ITS (Internal Transcribed Spacer)
 - Le COI (cytochrome c oxidase I) de l'ADN mitochondrial



<http://www.emforma.net/11966-mitocondria>



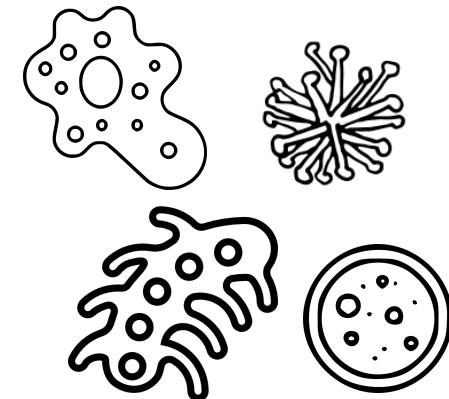
<https://www.quora.com/How-does-respiration-occur-in-the-Euglena>



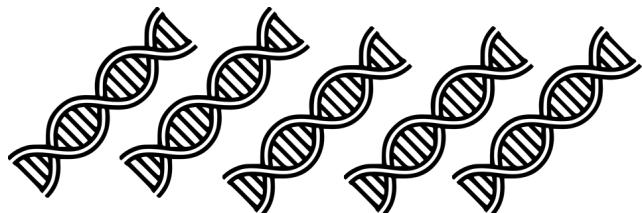
<http://biologianet.uol.com.br/biologia-celular/ribosomos.htm>



Méthodes d'isolation des séquences d'ADN

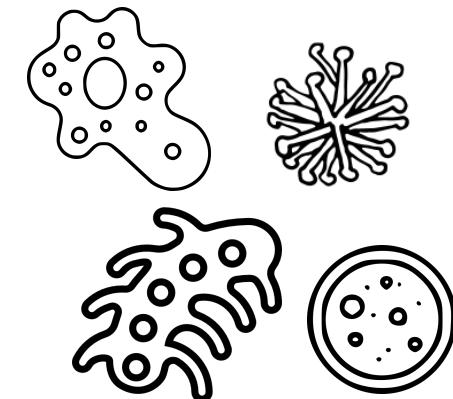


- Après l'amplification d'une région spécifique du génome trouvé dans tous les individus dans la communauté...

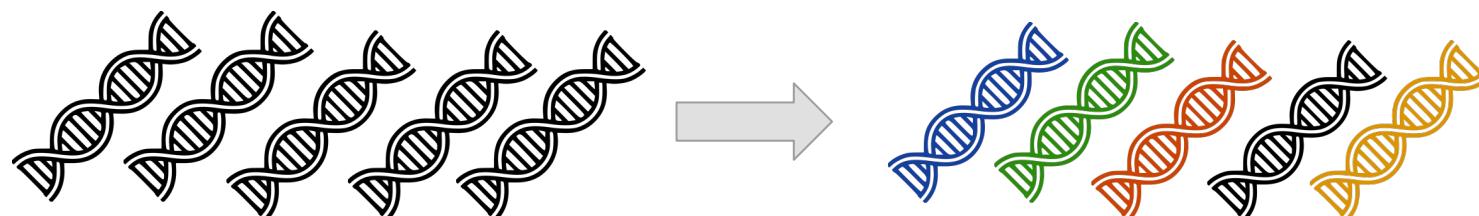




Méthodes d'isolation des séquences d'ADN



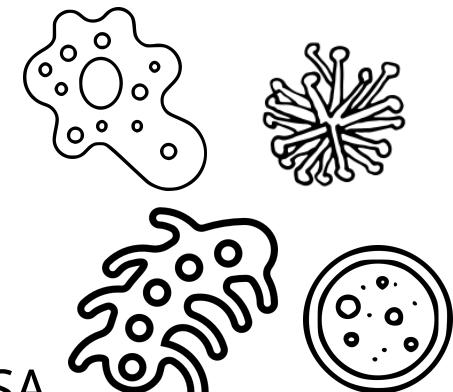
- Après l'amplification d'une région spécifique du génome trouvé dans tous les individus dans la communauté...
- Il faut séparer les espèces différentes



Community fingerprinting



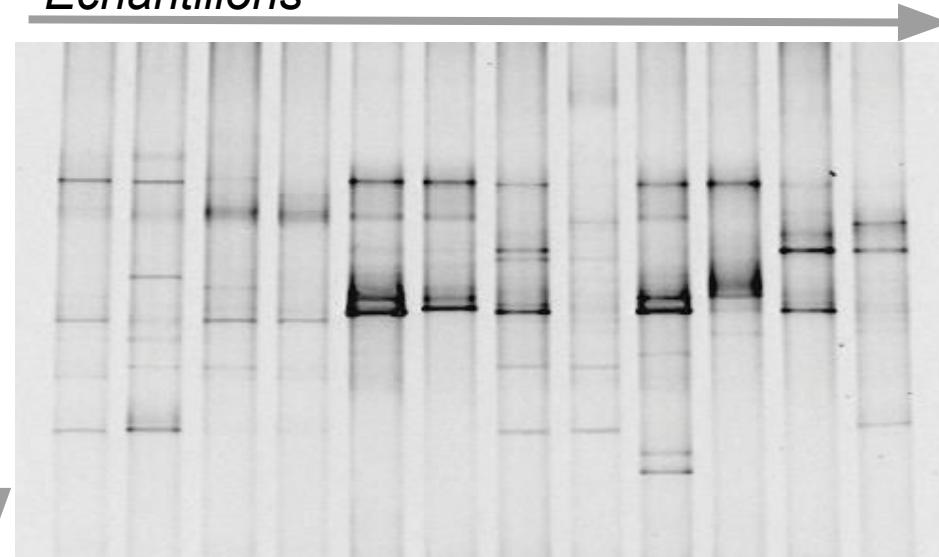
Community fingerprinting



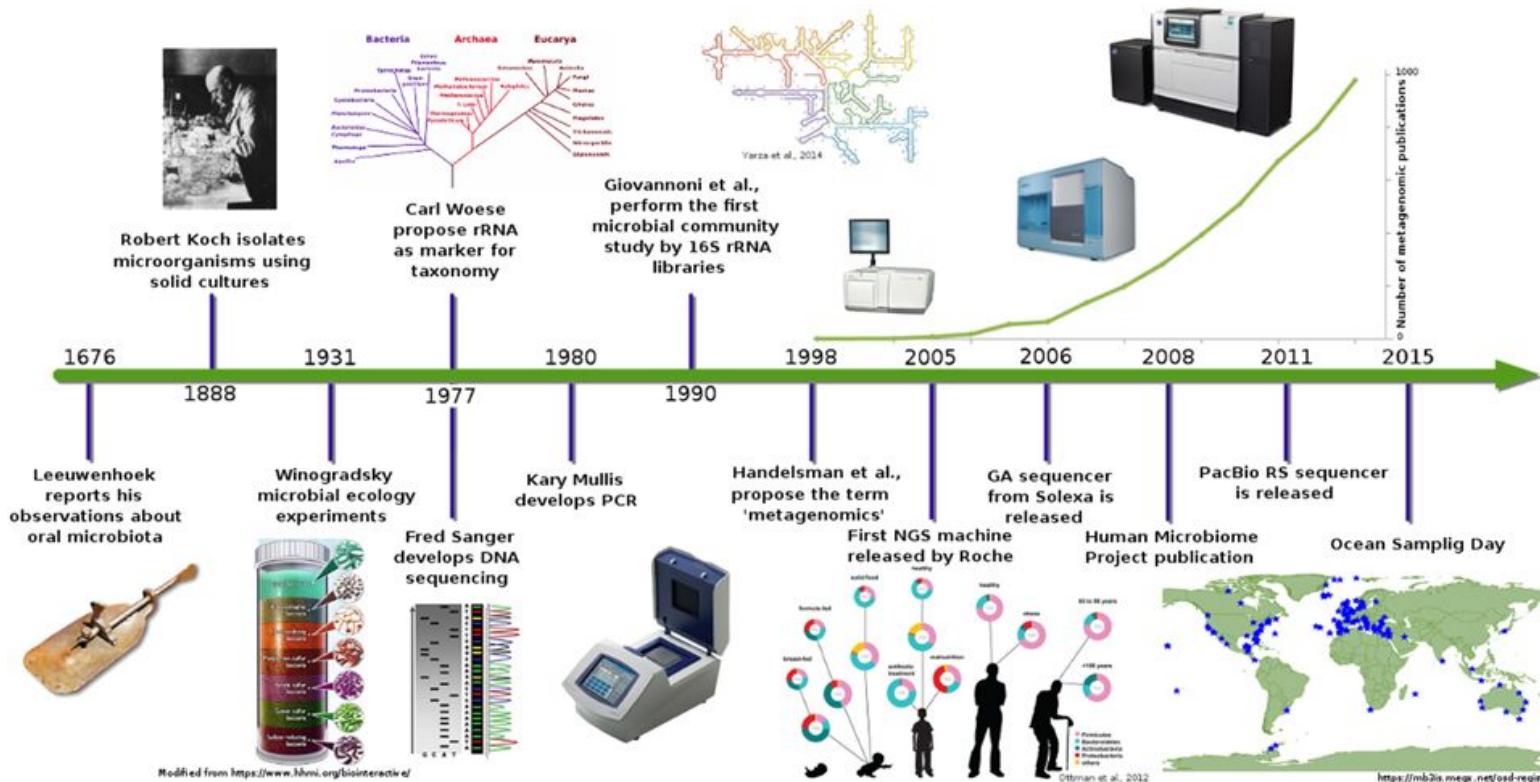
- Examples: DGGE/TGGE, ARDRA, RFLP, T-RFLP, ARISA...

Échantillons

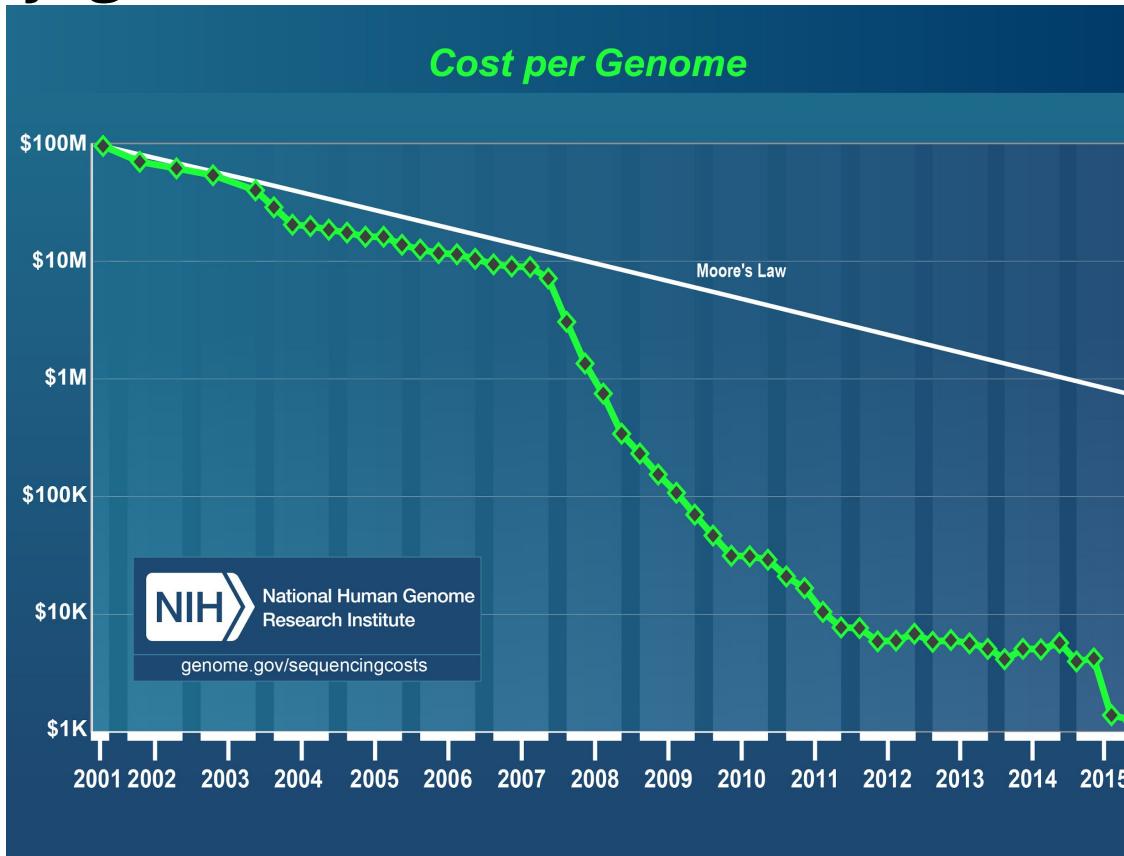
Espèces



Les méthodes à travers le temps



Le séquençage est de moins en moins cher!



Séquençage de l'ADN à haut débit (HTS: High-throughput sequencing)

Aussi appelé “NGS” pour “Next Generation Sequencing”

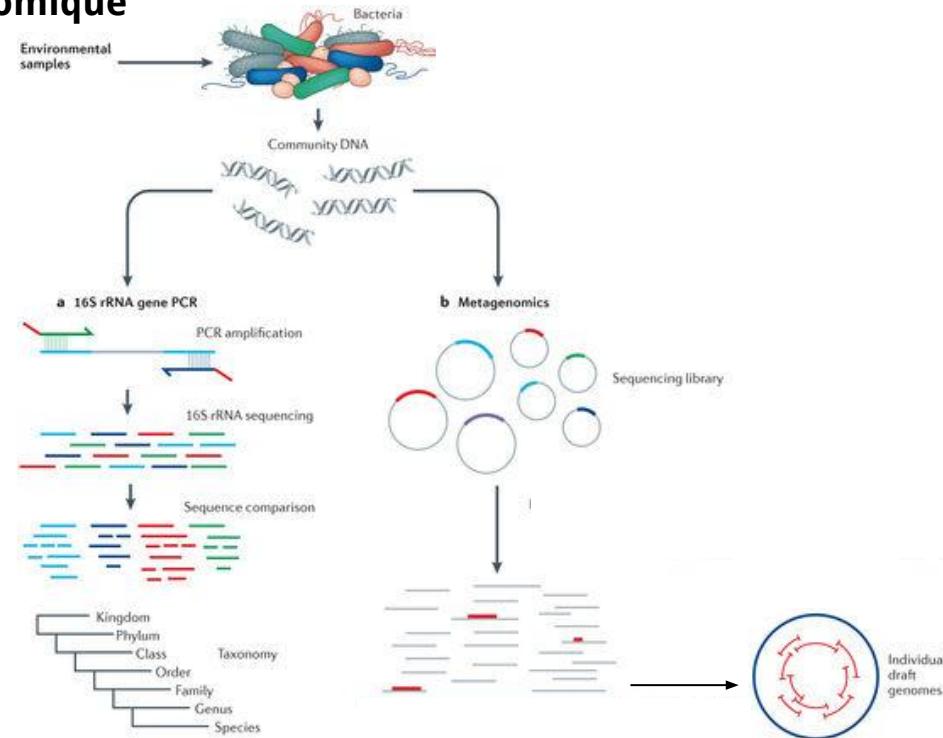
Les différentes méthodes varient par leur:

- Longueur de lecture (longueur de la séquence)
- Précision (base)
- Lecture par expérience (combien de séquences)
- Temps de lecture (la durée)
- Coût (\$\$!)

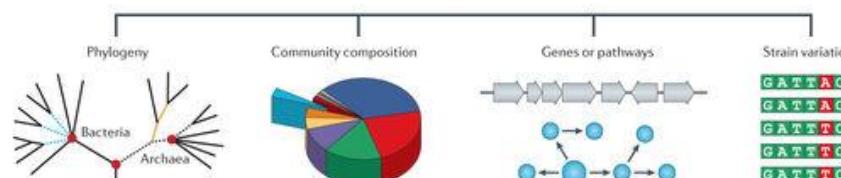
Chaque méthode a des avantages et inconvénients.

Deux méthodes populaires: 16S et la métagénomique

- 1) Échantillonnage:
environnement de votre choix
- 2) Séquençage NGS (high-throughput)
- 3) Amplification des séquences à l'aide
du PCR



- 4) Analyses



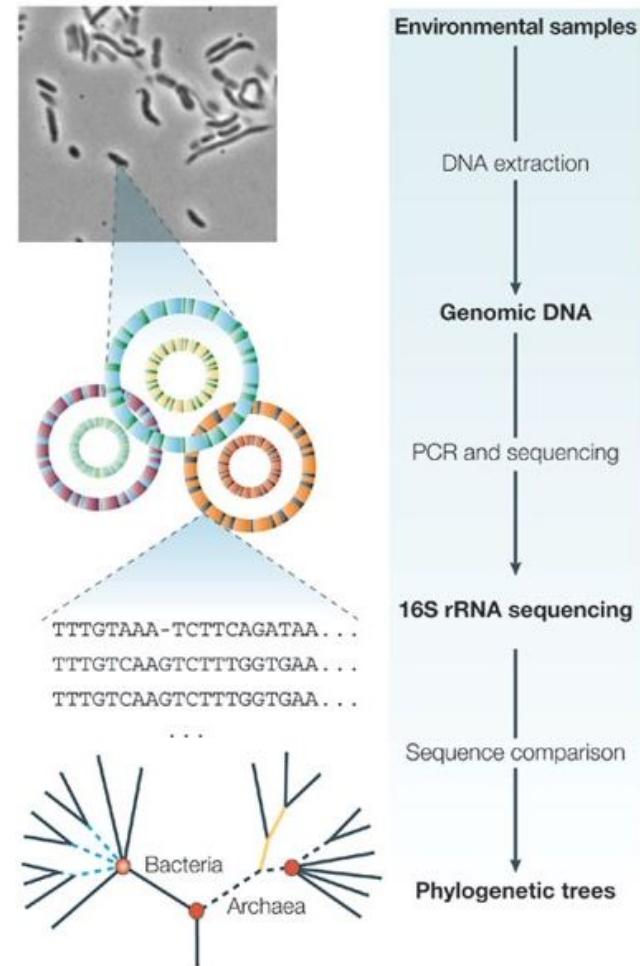
Séquençage de l'ARNr 16S (16S rRNA)

Qu'est-ce que c'est?

l'ARN ribosomique constituant la petite sous-unité des ribosomes des procaryotes. Les eukaryotes ont le 18S.

Pourquoi?

Un gène marqueur phylogénétique à cause de sa conservation à travers les domaines de la vie, mais juste assez variable entre les espèces

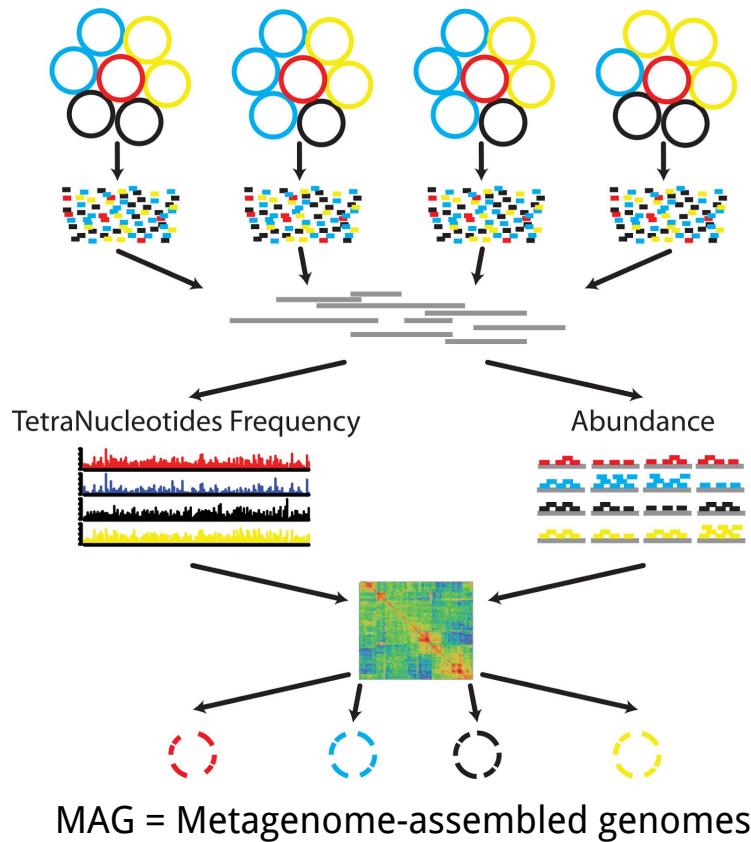


Metagénomique

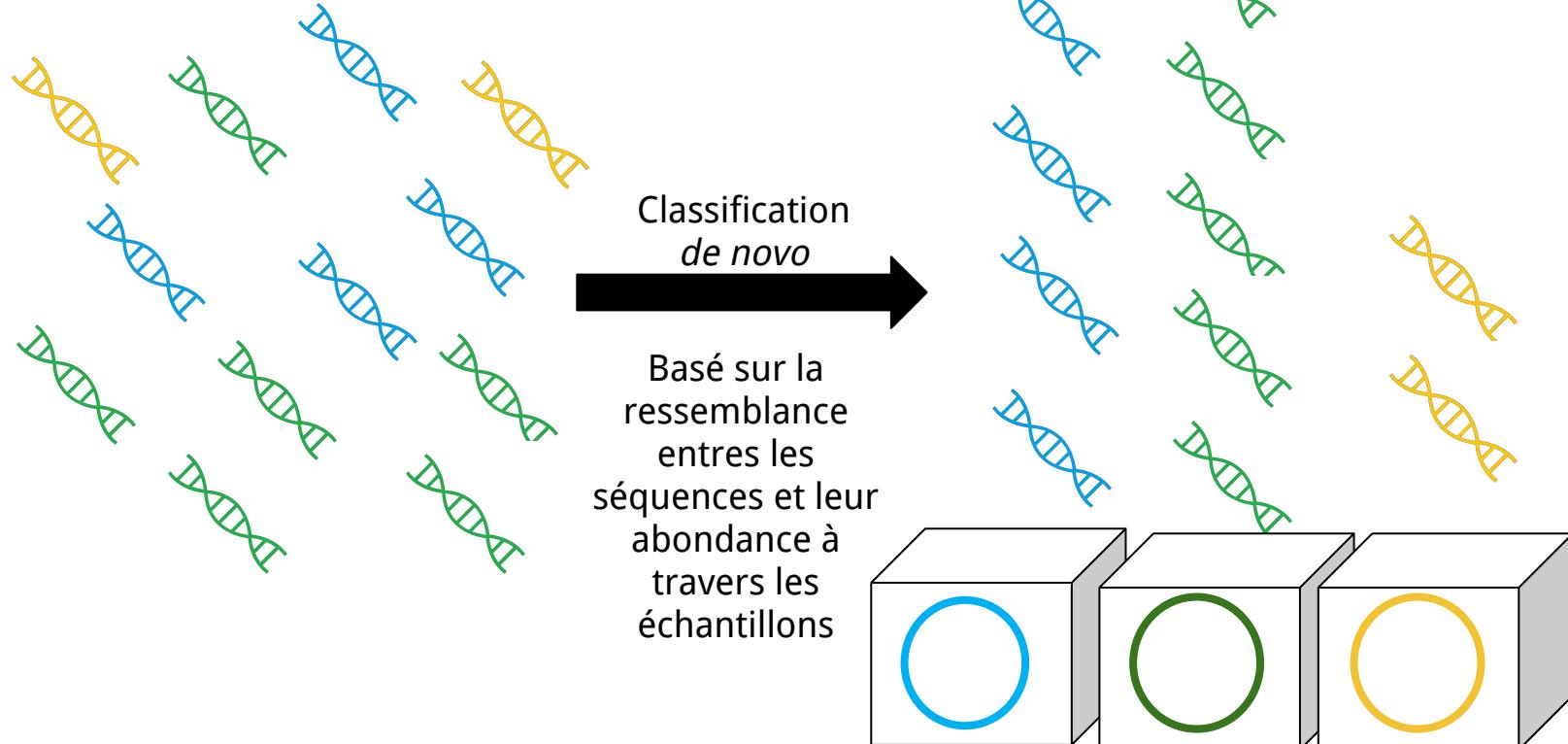
Définition: l'étude du contenu génétique d'échantillons issus d'environnement complexes.

Contrairement à la méthode du aRNA 16S, cette méthode ne cible pas seulement un gène, mais tout l'ADN contenu. Cette méthode permet d'étudier **tout le potentiel génomique et métabolique** d'un échantillon environnemental.

Identification → Fonction



Metagenomic binning: la classification des séquences dans des paniers afin de reconstituer des génomes.



Metagenome-Assembled-Genomes (MAG) aussi appelé "bin"
(comme un panier)

Atelier pratique

Choisissez votre propre aventure!

3 scénarios, des vraies données 16S
rRNA provenant de:

- 1) Lac tempéré et lac tropical
- 2) Photosynthèse oxygénique et anoxigénique
- 3) Lac des statut trophique différent (eutrophique et oligotrophique)

The screenshot shows a GitHub repository page for 'MicrobialEcologyWorkshop'. At the top, there are navigation links for 'Code', 'Pull requests 0', 'Wiki', 'Insights', and 'Settings'. To the right are buttons for 'Unwatch 1', 'Star 0', 'Fork 1', and an 'Edit' button. Below the header, a description states: 'Files, instructions and presentation during a presentation and workshop we gave on microbial ecology during the Annual Fall Workshops in Limnology at the Interuniversity Research Group for Limnology Research (GRIL) - EcoLac.' There is also a link to 'Add topics'. Below this, a summary bar shows '31 commits', '1 branch', '0 releases', and '2 contributors'. A dropdown menu for the branch 'master' is open. At the bottom of the page, there is a list of files and their details:

File	Description	Last Commit
Scenario1	Delete Icon	5 days ago
Scenario2	Delete Icon	5 days ago
Scenario3	Delete Icon	5 days ago
Tutorial_instructions	linux tutorial	5 days ago
MicrobialEcology_AdditionReadings_Wor...	Add files via upload	13 hours ago
README.md	Update README.md	3 days ago
extra_info_how_to_retrieve_16S_sequenc...	How to retrieve 16S sequences from Silva Ref NR	a day ago
workshop_presentation.pdf	presentation	12 hours ago

Suivez le tutoriel ici!
github.com/patriciatran/MicrobialEcologyWorkshop

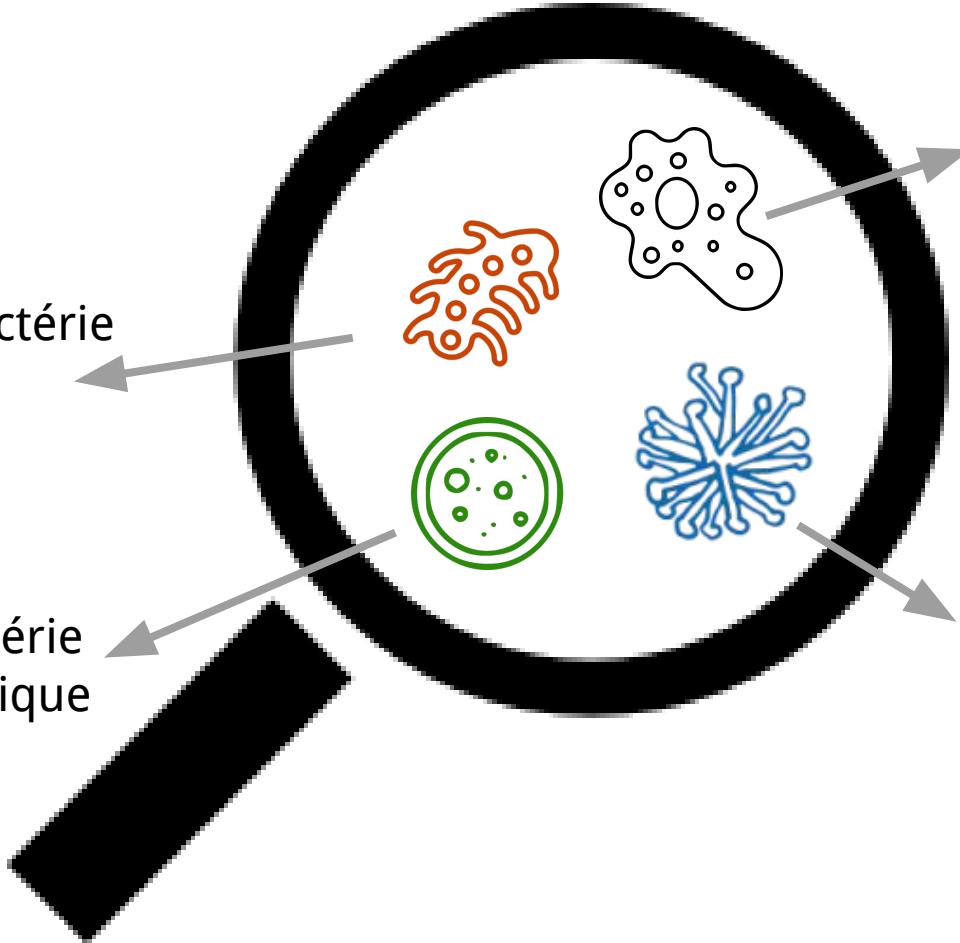
Merci!

Julie, la bactérie sulfureuse

Marie, la bactérie photosynthétique

Antoine, la bactérie méthanotrophique

Fred, la bactérie fixeuse d'azote



Introduction to the scenarios (Workshop Session)

Go to this page:

The screenshot shows a GitHub repository page for 'MicrobialEcologyWorkshop'. At the top, there's a header with the repository name, a 'Watch' button (1), a 'Star' button (0), and a 'Fork' button (1). Below the header, there are tabs for 'Code', 'Pull requests 0', 'Wiki', 'Insights', and 'Settings'. A main description states: 'Files, instructions and presentation during a presentation and workshop we gave on microbial ecology during the Annual Fall Workshops in Limnology at the Interuniversity Research Group for Limnology Research (GRIL) - EcoLac.' There's also an 'Edit' button. Below this, there's a section for 'Add topics'. A summary bar shows '31 commits', '1 branch', '0 releases', and '2 contributors'. A dropdown menu shows 'Branch: master' and a 'New pull request' button. To the right of these are buttons for 'Create new file', 'Upload files', 'Find file', and a prominent green 'Clone or download' button. The main content area lists various files and their details:

File	Description	Last Commit
nailabc presentation		Latest commit a22b3ac 12 hours ago
Scenario1	Delete Icon	5 days ago
Scenario2	Delete Icon	5 days ago
Scenario3	Delete Icon	5 days ago
Tutorial_Instructions	linux tutorial	5 days ago
MicrobialEcology_AdditionReadings_Wor...	Add files via upload	13 hours ago
README.md	Update README.md	3 days ago
extra_info_how_to_retrieve_16S_sequenc...	How to retrieve 16S sequences from Silva Ref NR	a day ago
workshop_presentation.pdf	presentation	12 hours ago
README.md		

Suivez le tutoriel ici!
github.com/patriciatran/MicrobialEcologyWorkshop

Scenario 1: Temperate versus tropical lakes

Quels sont les facteurs qui pourraient influencer la distribution des bactéries dans ces 2 écosystèmes?

- Saisonnalité
- Température
- Quantité de nutriments (phosphore, azote) (oligotrophique, mesotrophique, eutrophique)

Scenario 1: Temperate versus tropical lakes

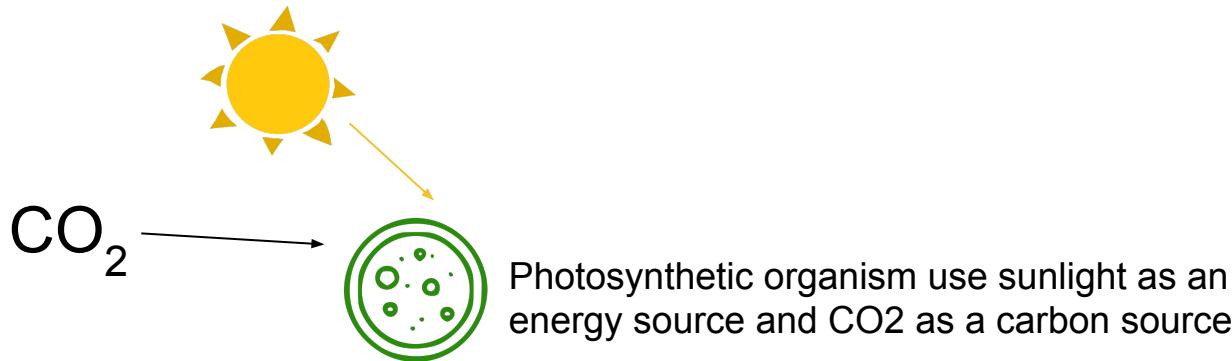


Lac Croche est un lac tempéré, avec une forte stratification durant l'été, et un hypolimnion anoxique à la fin de l'été. Ce lac est dimictique, une fois au printemps après la fonte des glaces et une fois en automne. Couvert de glace en hiver.

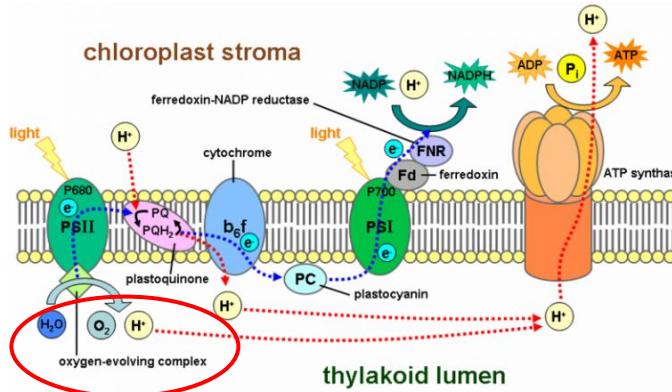


Lac Carioca is a mesotrophic lake. Mixes once a year in the dry-season ("winter"). It never freezes! 12m depth, like Croche. It is strongly stratified, and like Croche, it also gets anoxic almost year-round, except during mixing.

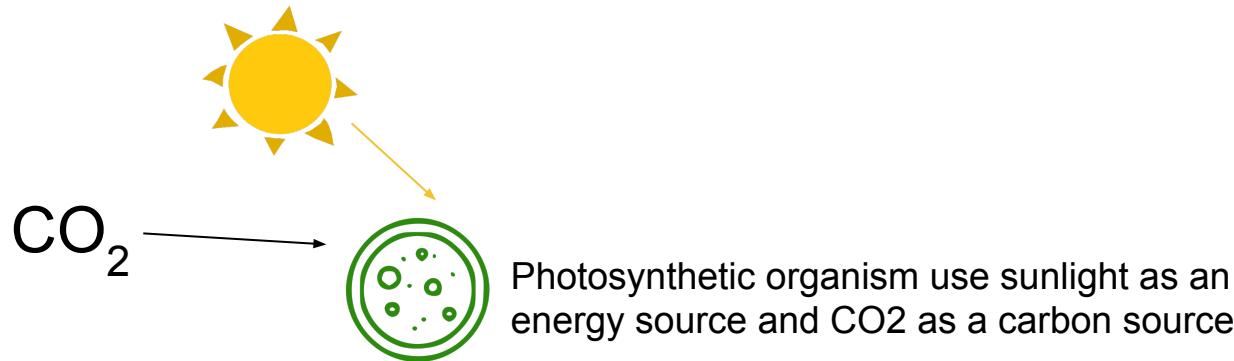
Scenario 2: Exploring anoxygenic and oxygenic photosynthesis



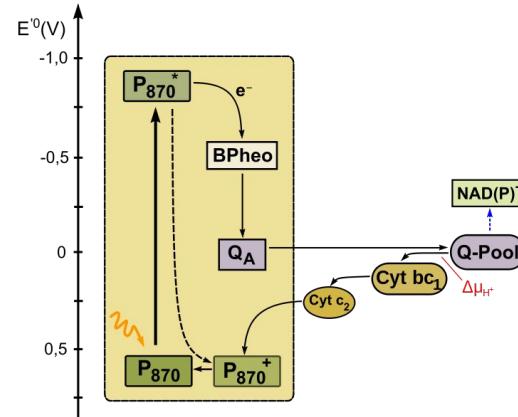
In oxygenic photosynthesis, water is hydrolysed, oxygen is released as a by product



Scenario 2: Exploring anoxygenic and oxygenic photosynthesis



But in anoxygenic photosynthesis, it is a cyclic process. Oxygen is not released, water is not used as an electron donor among other differences.



Scenario 2: Exploring anoxygenic and oxygenic photosynthesis

Oxygenic vs Anoxygenic Photosynthesis	
Oxygenic photosynthesis is the process which converts light energy to chemical energy by certain photoautotrophs by generating molecular oxygen.	Anoxygenic photosynthesis is the process which converts light energy to chemical energy by certain bacteria without generating molecular oxygen.
Generation of Oxygen	
Oxygen is released as a by-product.	Oxygen is not released or generated.
Organisms	
Oxygenic photosynthesis is shown by cyanobacteria, algae, and green plants.	Anoxygenic photosynthesis is mainly shown by purple bacteria, green sulfur and nonsulfur bacteria, heliobacteria, and acidobacteria.
Electron Transport Chain	
Electrons travel via several electron carriers.	It occurs via cyclic photosynthetic electron chain.
Water as an Electron Donor	
Water is used as the initial electron donor.	Water is not used as an electron donor.
Photosystem	
Photosystem I and II are involved in oxygenic photosynthesis	Photosystem II is not present in anoxygenic photosynthesis
Generation of NADPH (reducing power)	
NADPH is generated during the oxygenic photosynthesis.	NADPH is not generated because electrons cycle back to the system. Hence reducing power is obtained from other reactions.

Only 1 bacterial group: it should cluster in the tree

A lot more diversity

Scenario 2: Exploring anoxygenic and oxygenic photosynthesis

Purple sulfur bacteria: Chromatiales (Order)

Purple non sulfur bacteria: Rhodospirillaceae (Family)

Green sulfur bacteria: Chlorobi (family)

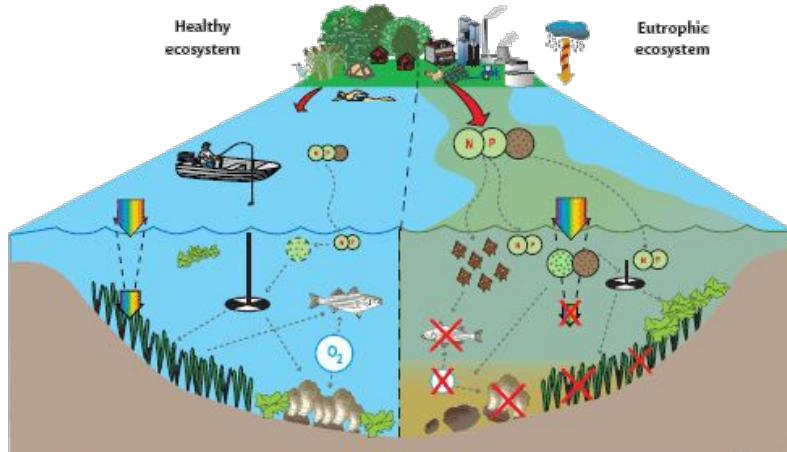
Green non sulfur bacteria: Chloroflexi (Class)

Heliobacteria: Heliobacteriaceae (Family)

Acidobacteria: Acidobacteria (Phylum)

Scenario 3: Healthy versus unhealthy lakes

Figure 1.1. Conceptual diagram comparing a healthy system with no or low eutrophic condition to an unhealthy system exhibiting eutrophic symptoms.



In healthy ecosystems, nutrient inputs, specifically nitrogen and phosphorus (●), occur at a rate that stimulates a level of macroalgal (●) and phytoplankton (chlorophyll a (●)) growth in balance with grazer biota. A low level of chlorophyll a in the water column helps keep water clarity high (●), allowing light to penetrate deep enough to reach submerged aquatic vegetation (●). Low levels of phytoplankton and macroalgae result in dissolved oxygen (O₂) levels most suitable for healthy fish (●) and shellfish (●) so that humans can enjoy the benefits (●) that a coastal environment provides.

In eutrophic ecosystems, increased sediment (●) and nutrient loads (●) from farming (●), urban development (●), water treatment plants (●), and industry (●), in combination with atmospheric nitrogen (●), help trigger both macroalgal (●) and phytoplankton (chlorophyll a (●)) blooms, exceeding the capacity of grazer control. These blooms can result in decreased water clarity (●), decreased light penetration (X), decreased dissolved oxygen (X), loss of submerged aquatic vegetation (X), nuisance/toxic algal blooms (●), and the contamination or die off of fish (X) and shellfish (X).



Lake Erie under cyano bloom

Scenarios

- References : sequences of known origin (cultures, other studies, etc.) to compare your “unknown” sequences to
- Outgroup : point of comparison for the ingroup, the set of organisms under study that specifically allows the phylogeny to be rooted
- Scenario 1 (sequences obtained from a sample 1)
- Scenario 2 (sequences obtained from a sample 2)

How to retrieve sequences: 16S rRNA database



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SILVA

Welcome to the SILVA rRNA database project

A comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data.

SILVA provides comprehensive, quality checked and regularly updated datasets of aligned small (16S/18S, SSU) and large subunit (23S/28S, LSU) ribosomal RNA (rRNA) sequences for all three domains of life (*Bacteria, Archaea and Eukarya*).

SILVA are the official databases of the software package ARB.

For more background information → [Click here](#)

SILVAngs



Check out our new service for Next Generation Amplicon data

SILVA Tree Viewer

The SILVA Tree Viewer is a web application to browse and query the SILVA guide trees.

A technical preview is available at www.arb-silva.de/treereviewer



News

28.10.2017

de.NBI Handbook ready for Download



The Handbook is the first comprehensive document that lists the work and effort of all de.NBI partners. Content: How de.NBI is structured, Presentations of all Partners, Index of Persons/Contact Details.

05.10.2017

SILVA TreeViewer published



SILVA TreeViewer: interactive web browsing of the SILVA phylogenetic guide trees now published in BMC Bioinformatics.

22.09.2017

Sneak-Preview: SILVA 132



The first statistics for SILVA release 132. The SILVA 132 release can be expected for November 2017.

12.09.2017

Registration for the Primer 2 Paper Workshop now open



The next SILVA Primer 2 Paper workshop takes place from November 6 to 10 in Bremen. If you are interested please register quickly since space is limited to 11 participants.

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User satisfaction survey

<https://www.arb-silva.de/>