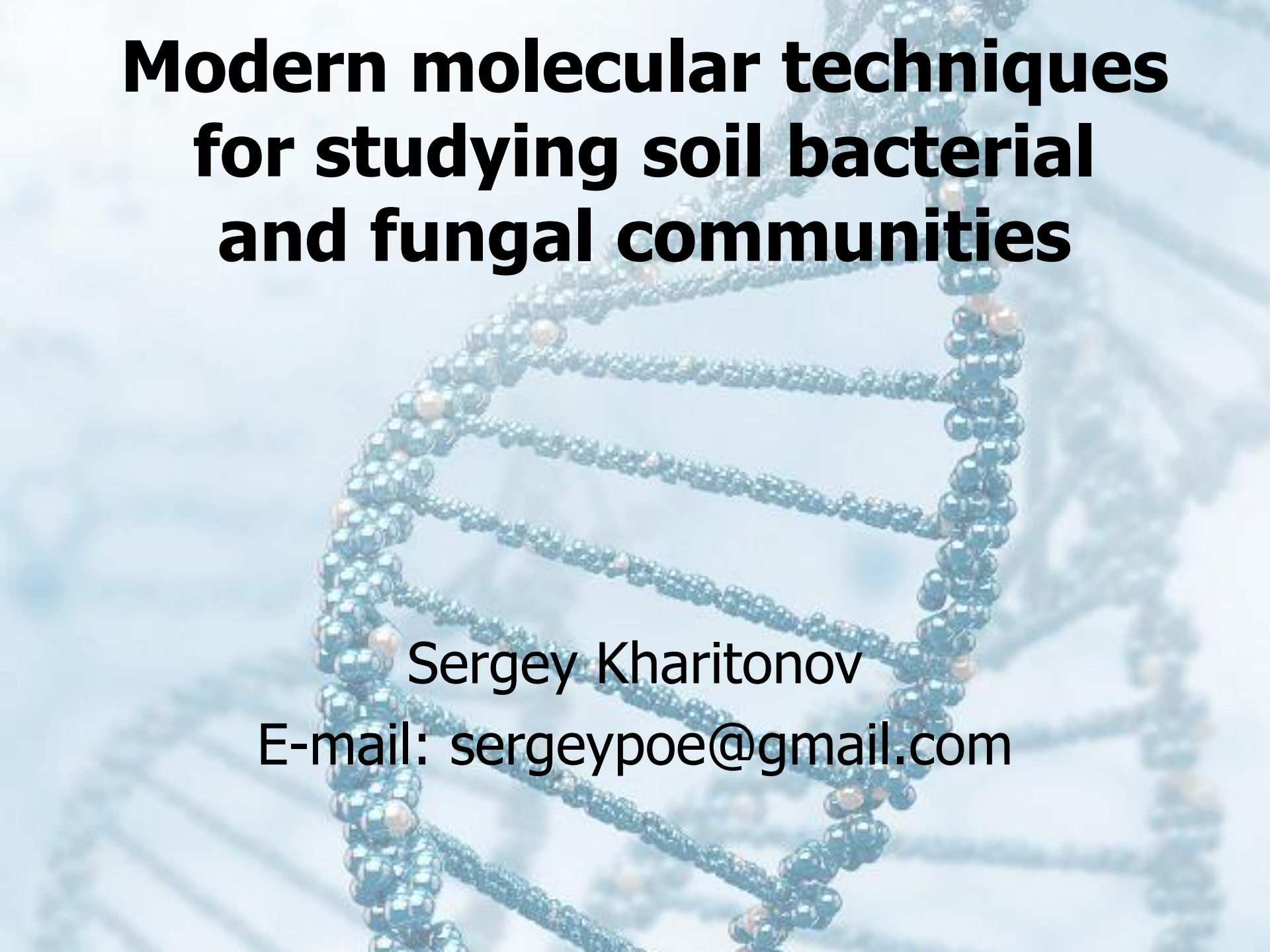
Modern molecular techniques for studying soil bacterial and fungal communities



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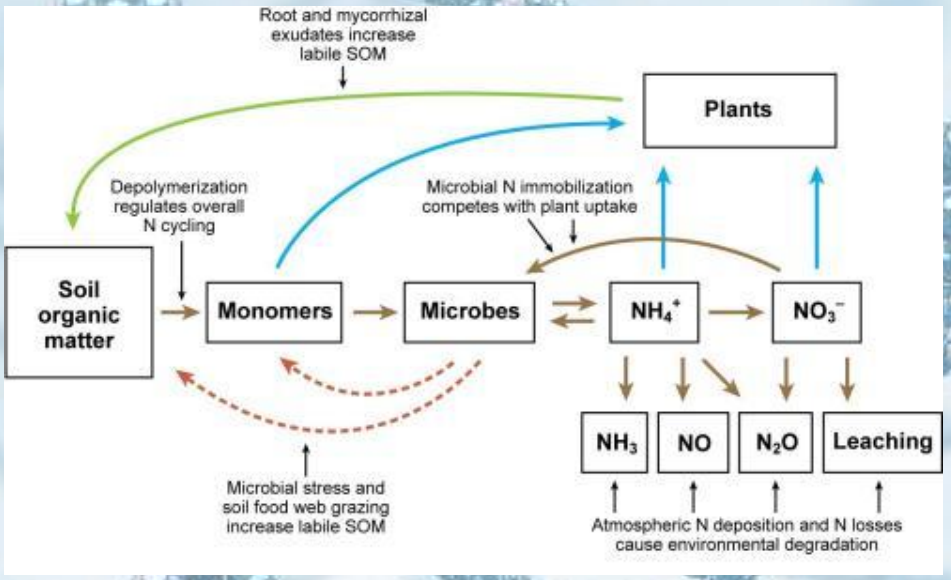
Microorganisms plays the central role in soil functioning

- Biogenic elements cycles (C, N, P, S)
- Trophic chains
- Soil structure and composition
- Destruction of pesticides and xenobiotics



Great Plate Count Bias

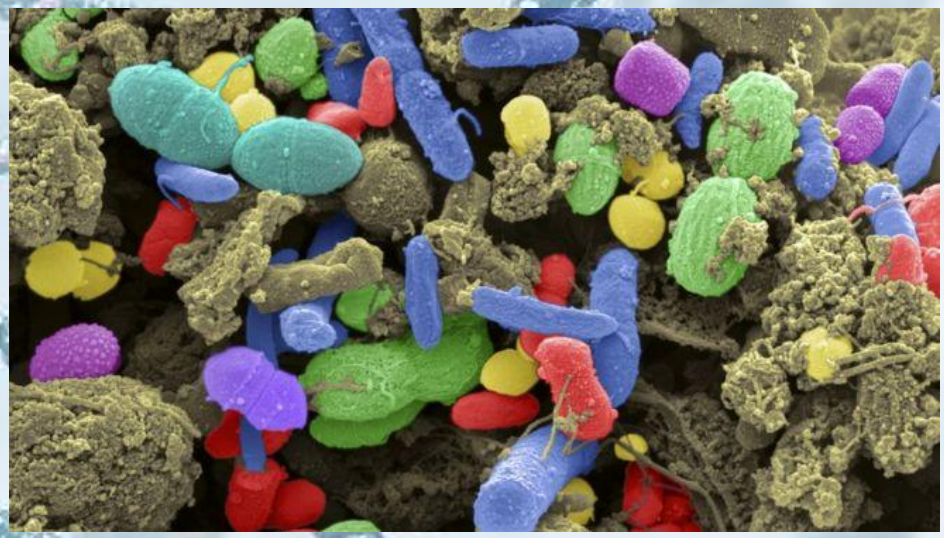
1g of soil

Petri Dish

Count: 10^5-10^7

Microscope

10^8-10^9



On the plate we can find only **1%** from the whole diversity

99% of soil microorganisms are **uncultivable**

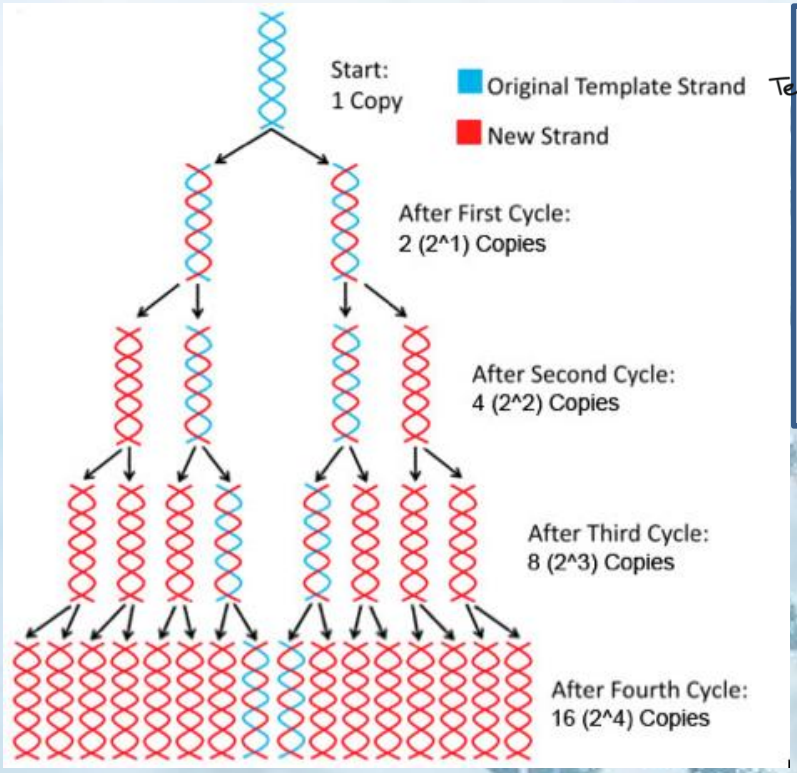
How can we study **any desirable species** or the **whole community**?

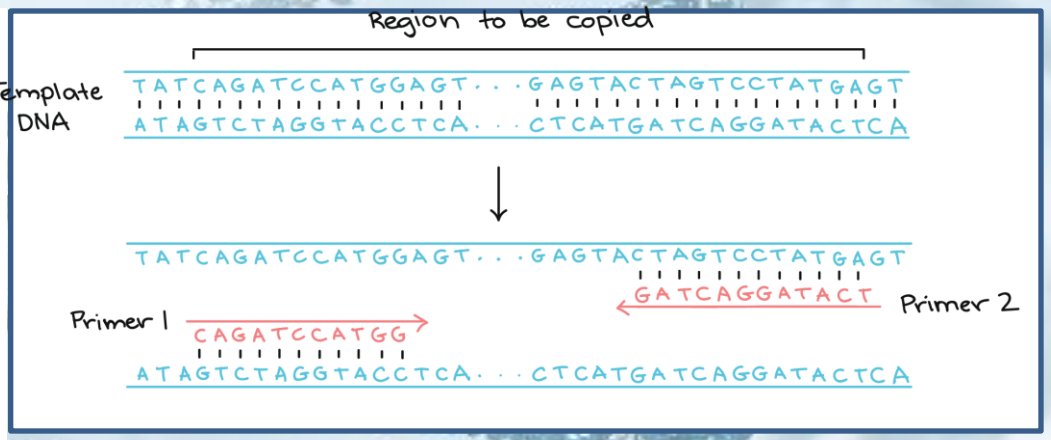
Molecular methods!

Molecular method are helping us to overcome limitations

- qPCR (detecting of 16s, 18s, functional and marker genes)
- Sequencing and NGS-based methods (shotgun-metagenomics)
- RNAseq

qPCR – targeted analysis in the black box.



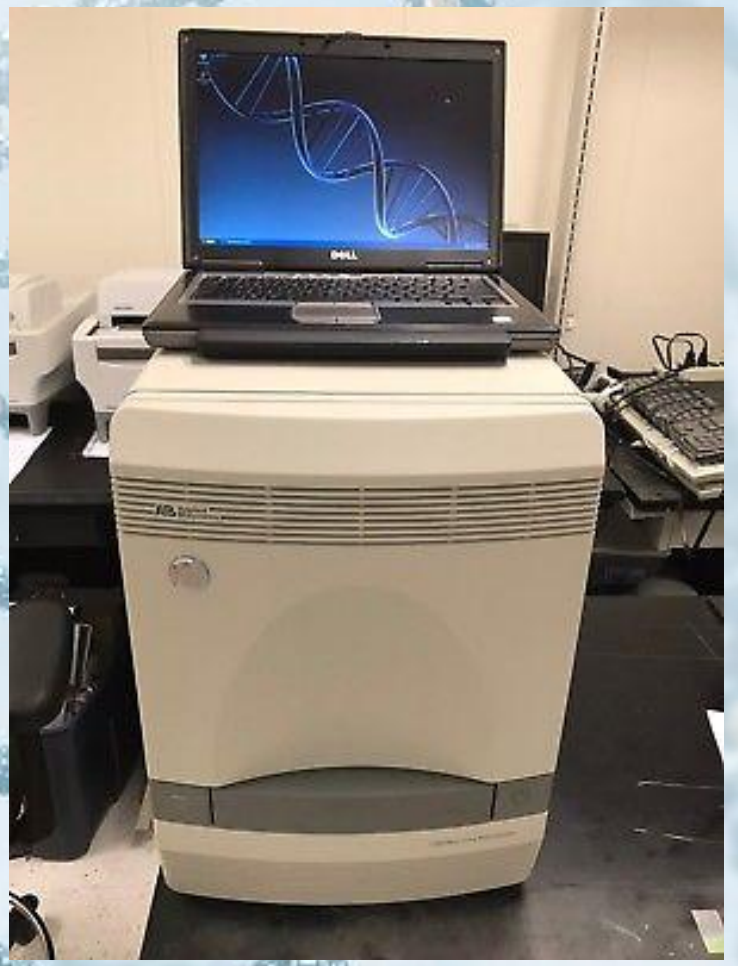


- Detect only desirable species from any community
- You can create primers for each genera or even species
- Widely used and effective
- Easy to apply

Platform

Amplificatory for real-time qPCR
AB 7500

Suitable for analysis **16s RNA and functional genes,** which are helping us **to count** desirable species



Sequencing

Allows us **read DNA** and, consequently:

- Describe new genes and new possible properties of known species
- Discover totally new species
- Describe diversity of whole microbial community without cultivation!

New Generation Sequencing – read all genomes in sample at the same time

Method demands a complicated sample preparation before start… But:

- Define **ALL** species in the sample in one experiment
- Allows us to compare the composition of **very complex** microbial communities and define core species in Wetlands, Soils, Rivers…

RNA-seq – reading of RNA

RNA is produced **only by active genes**

- Identify **biological processes** in community
- Understand which **xenobiotics** and other dangerous substances could be destroyed in community
- Identify **functional, most important species** in complex community in soil, water or wetland.

Platform

Illumina

MiSeq | NextSeq 500 550 | HiSeq 2500 3000 4000 | HiSeq X Ten

Output	15 Gb	120 GB	1500 GB	1800 GB
Max Number of Reads/ Run	25 Million	400 Million	5 Billion	6 Billion
Max Read Length	2x300 bp	2x150 bp	2x125- 2x250 bp (RR mode)	2x150 bp
Cost	$99K	$250K	$740K	$10M (10 units)

Source: Illumina

Summary

- Define and describe new species in complex communities without cultivation
- Understand functions of certain members of communities
- Calculate a proportion of desirable species in community
- Describe microbial diversity and biological processes
- Predict useful properties of community in soil, treatment plant, rivers (destruction of xenobitics in wastewater, self-purification in rivers and so on)

Thanks for Your attention!

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