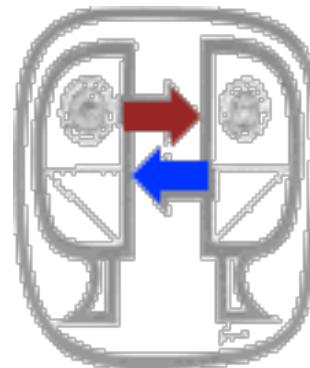
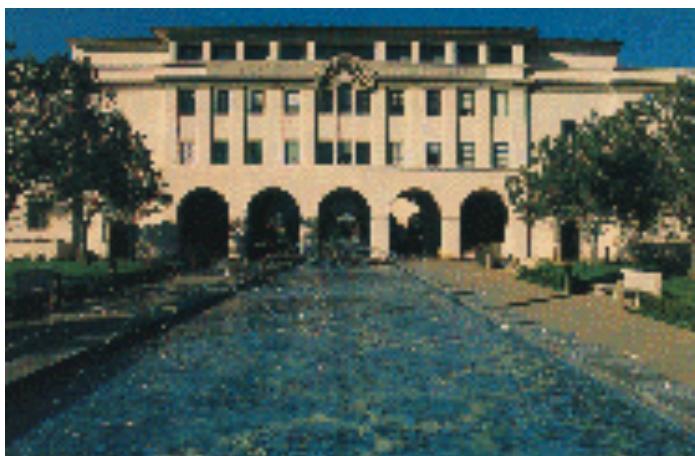


mothur tutorial STAMPS 2015

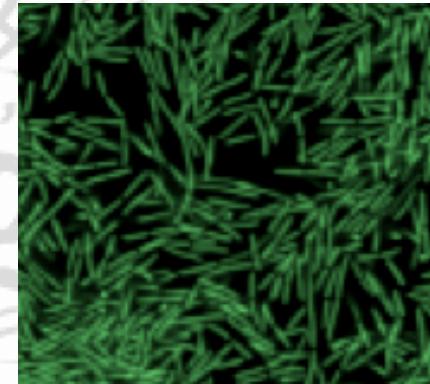
Tracy K. Teal, PhD
Data Carpentry, Executive Director
Adjunct Professor, Kellogg Biological Station



Cybernetics



Mathematical ecology Artificial life



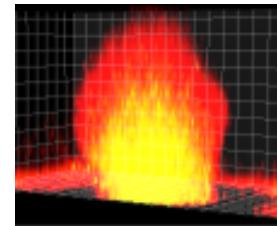
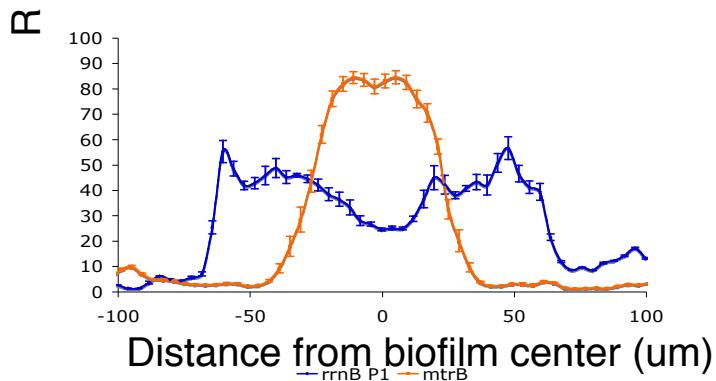
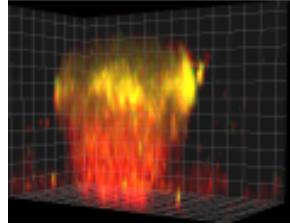
Shewanella oneidensis MR-1

OligoSelect

MotifSearch

Textpresso

An information extracting and processing package for biological literature



Schmidt Lab
Power, efficiency and microbial communities

Home | Research | Data | Software | Protocols

454 Replicate Filter

This tool clusters and filters out artificially replicated sequences in 454 data. It returns a fasta file of unique sequences and a list of the sequences in each cluster. This tool is described in Gomez-Alvarez V, Teal TK, Schmidt TM, **Systematic artifacts in**

Note:
Sequences that cluster together by CD-HIT and start with the same beginning base pairs are identified as replicates and



DATA CARPENTRY

MAKING DATA SCIENCE MORE EFFICIENT

datacarpentry.org

Workshops designed to teach basic concepts, skills and tools for working more effectively with data.

mothur

Pat Schloss

Microbiology & Immunology
University of Michigan

Sarah Westcott

<http://www.mothur.org>



Outline

- Case study – microbial communities in soil
- mothur workflow
- mothur MiSeq SOP

http://www.mothur.org/wiki/MiSeq_SOP

How to get your data in, get your data out
(an OTU table), and get help

- Some preliminary statistics

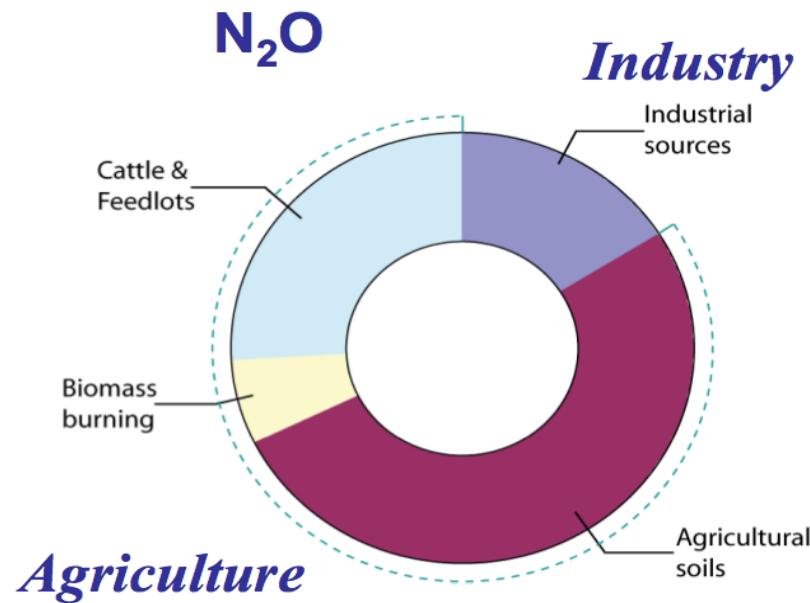
The functional potential of soil microbial communities shifts with agricultural management, increasing their capacity to produce greenhouse gases

Tracy Teal, Michigan State University

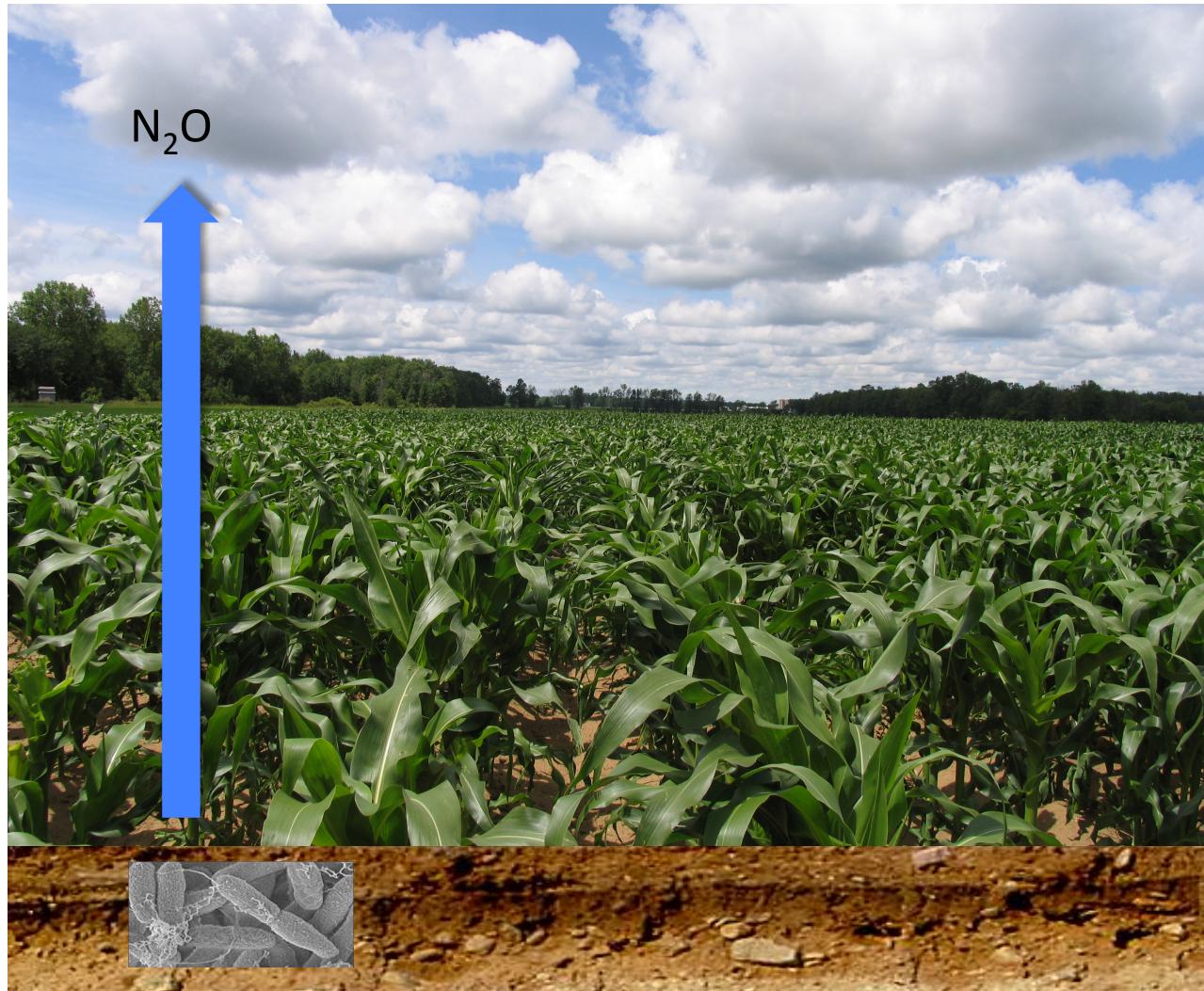
Vicente Gomez-Alvarez, Environmental Protection Agency

Tom Schmidt, University of Michigan

Land use change and intensive agriculture increase greenhouse gas fluxes



Microbes are the primary mediators of nitrous oxide



Land management, sustainability and microbial communities

- How do microbial communities change with land management?
- What is the relationship between denitrifying bacteria and N₂O in agricultural and native soils?

Experimental sites

Kellogg Biological Station LTER



How do microbial communities change with land management?

Kellogg Biological Station LTER

✖ Vicente Gomez-Alvarez

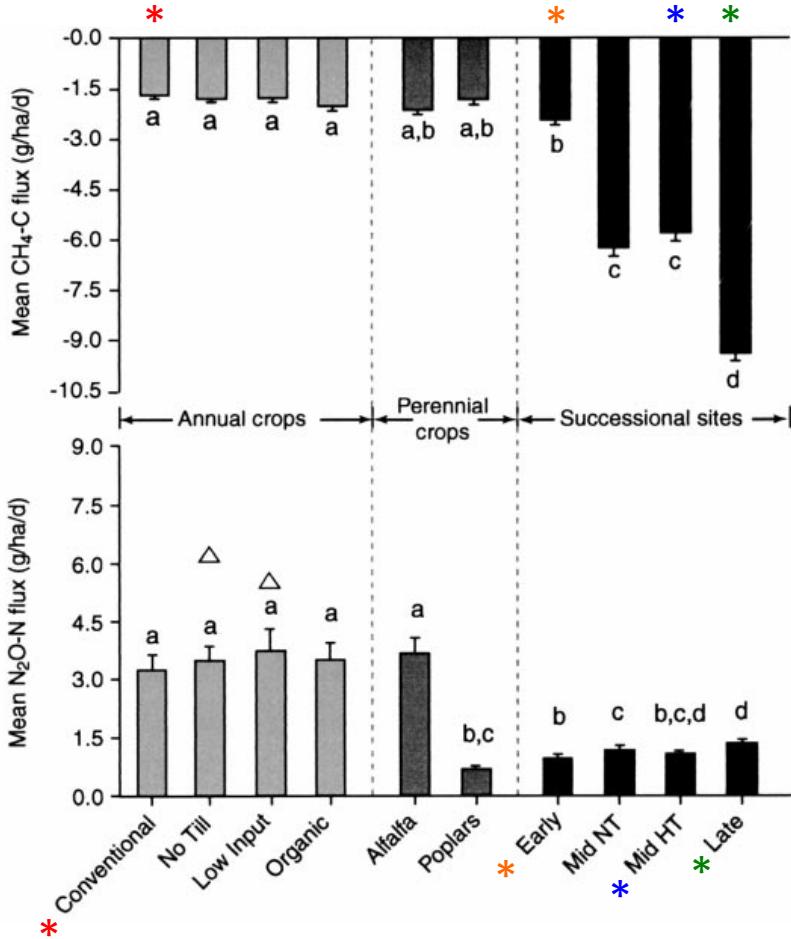


- AG □ Conventional Agriculture (3 crop rotation)
- ES □ Early Successional (20 years abandoned)
- SF □ Successional Forest (40 years abandoned)
- DF □ Deciduous Forest (native forest, never tilled)

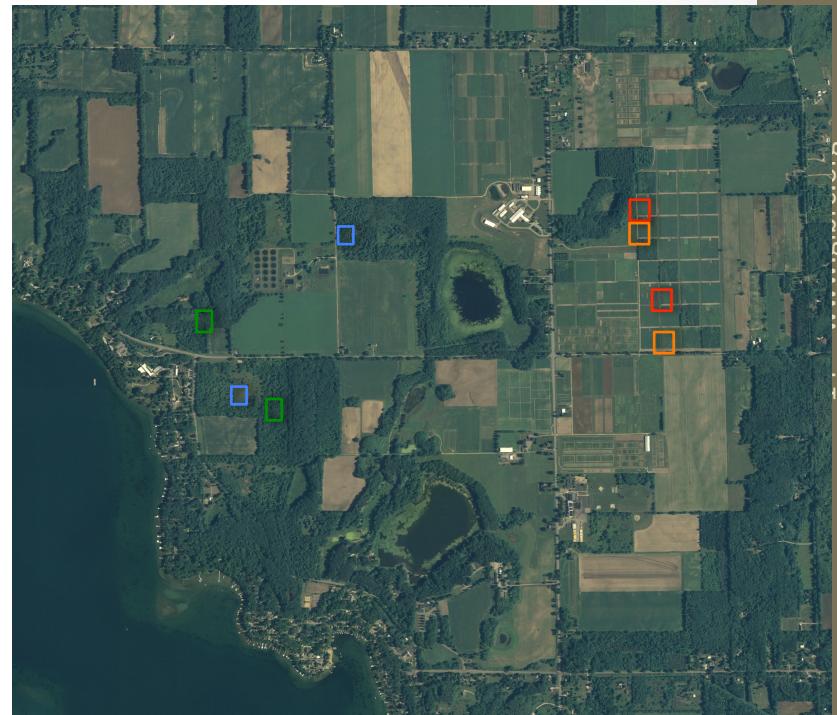
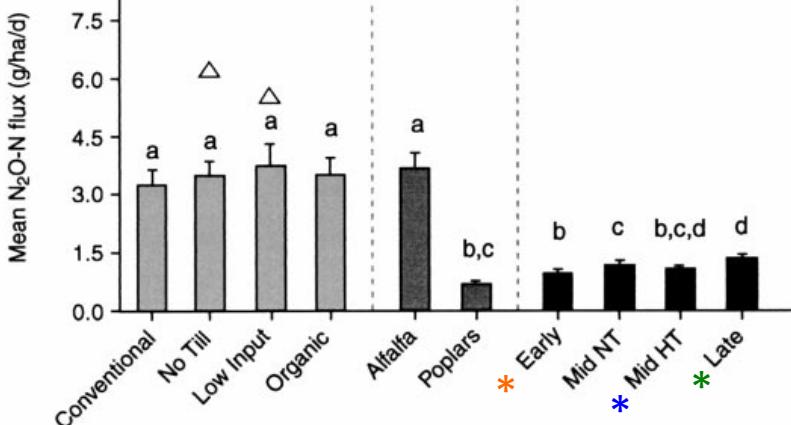
How do microbial communities change with land management?

Kellogg Biological Station LTER

Methane



Nitrous oxide



- AG Conventional Agriculture
- ES Early Successional
- SF Successional Forest
- DF Deciduous Forest

Metagenomics approach



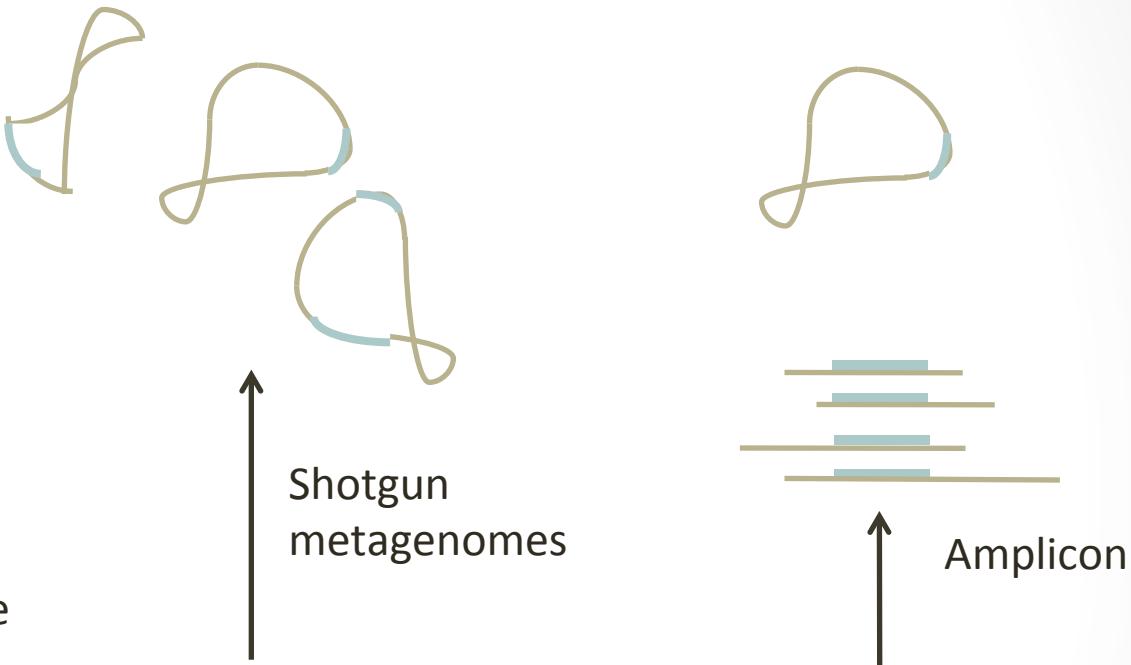
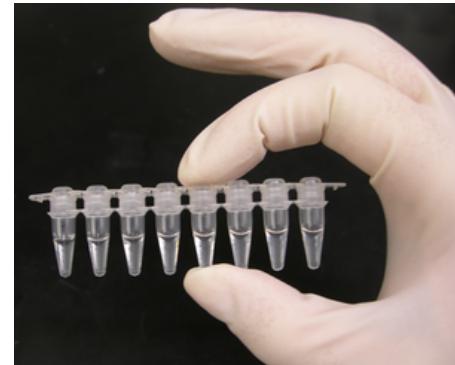
↓ Collect, composite
and sieve



Extract
DNA
→

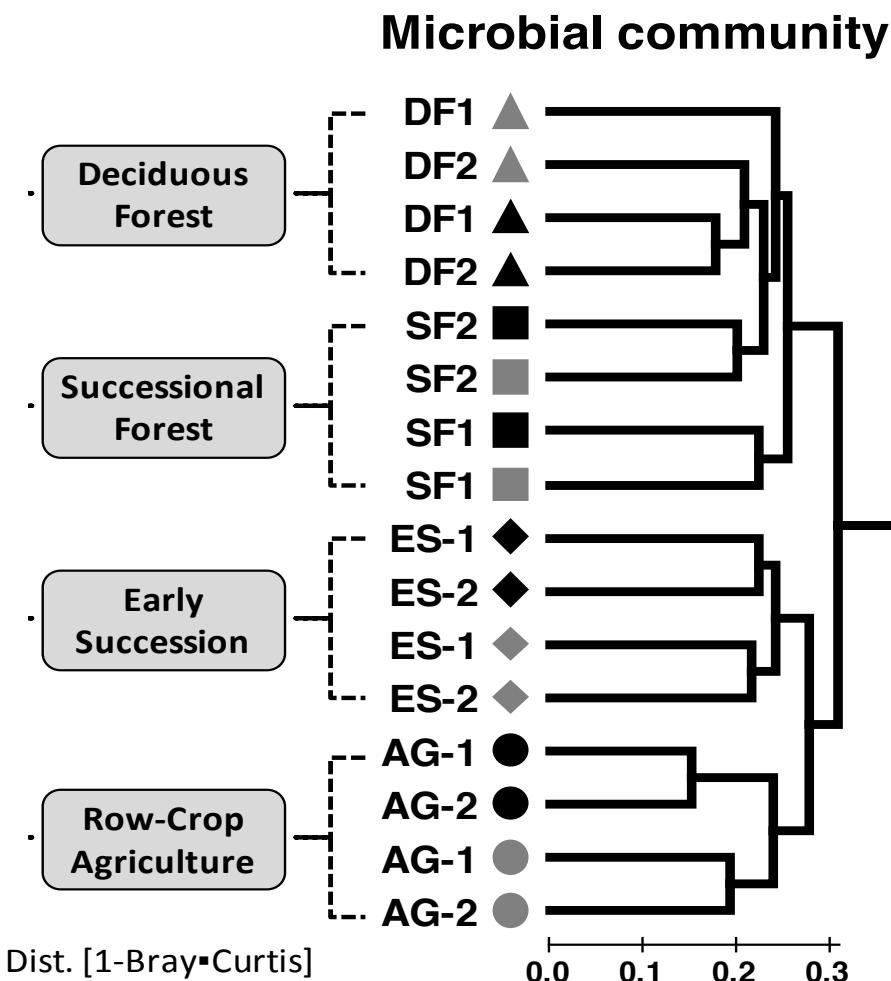


PCR
→

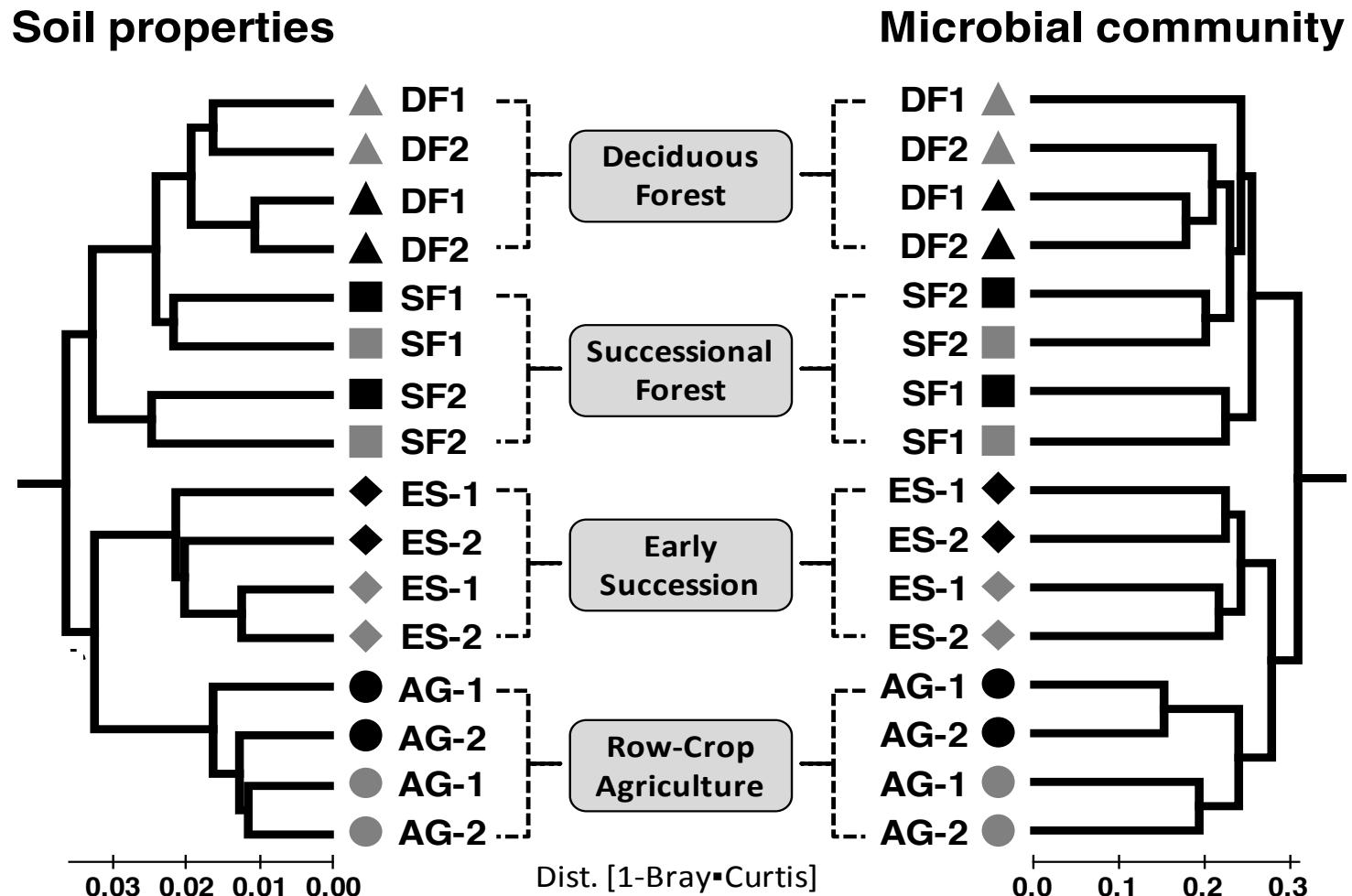


2 replicates of two treatments in each of two years

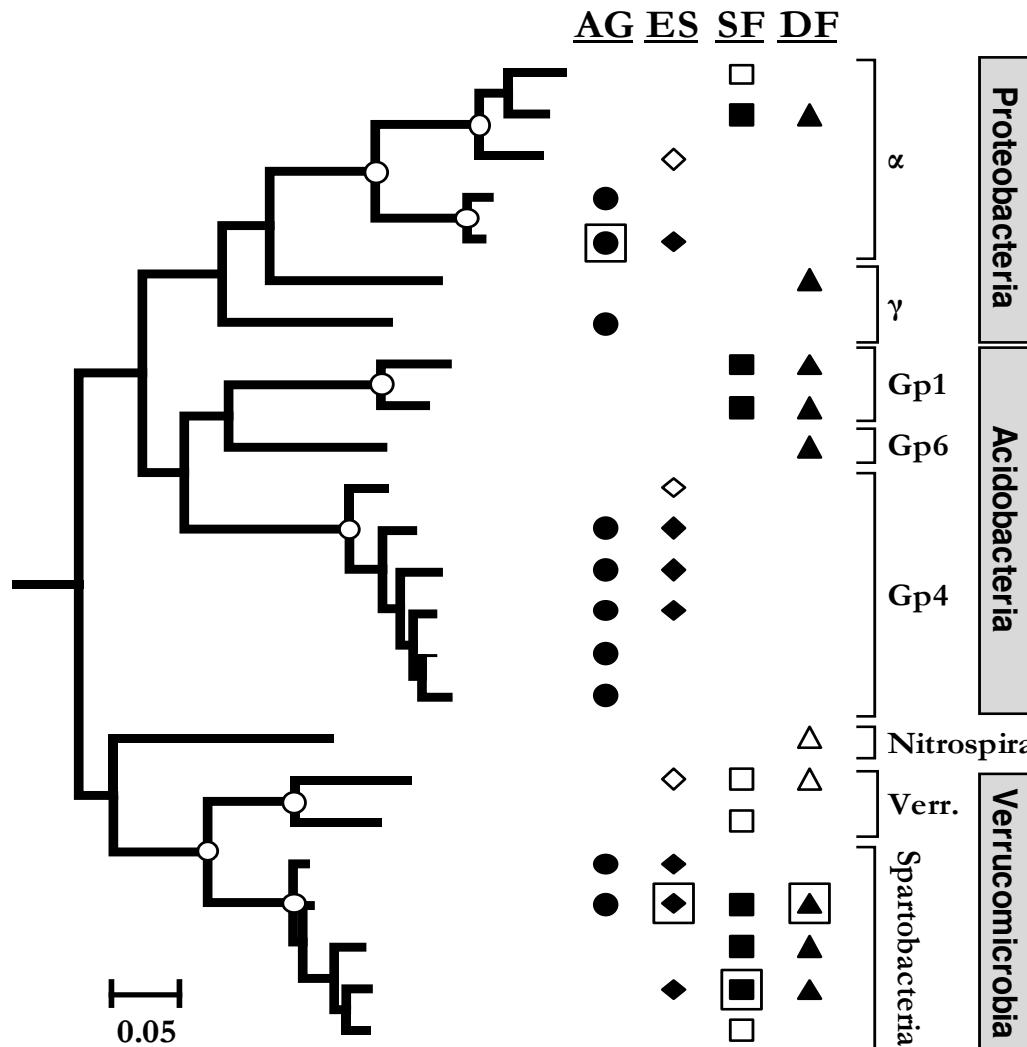
Taxonomic composition of bacterial communities changes with treatment



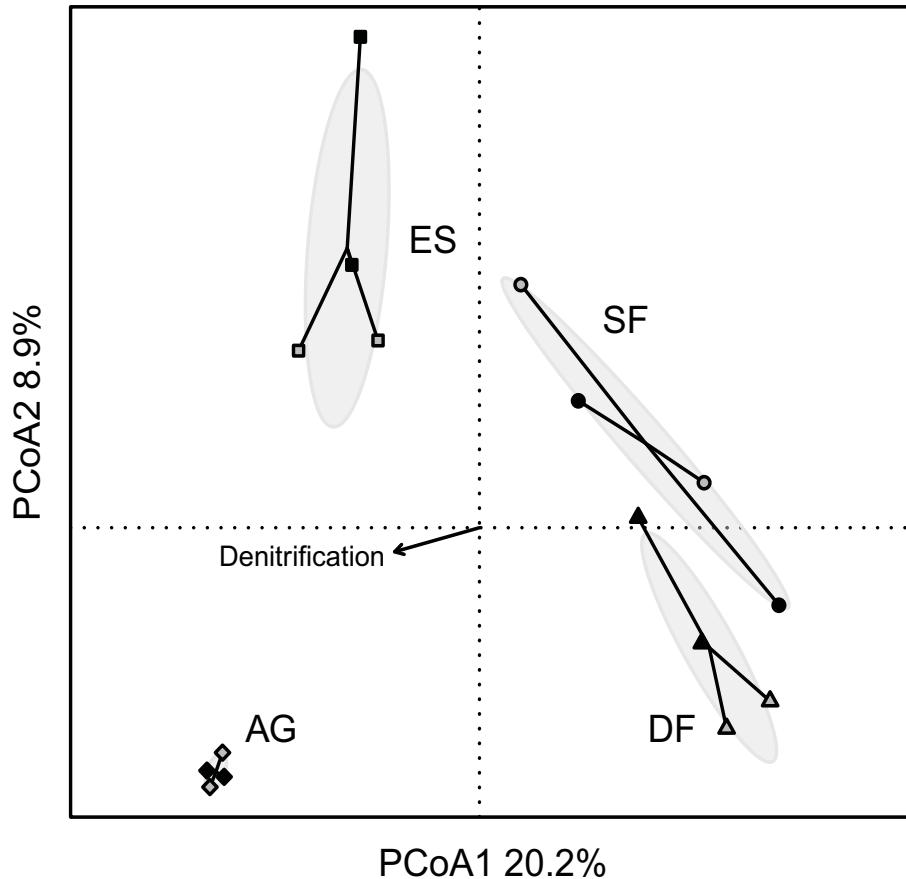
Biogeochemistry and bacterial community change concomitantly



Shifts within phyla differentiate communities



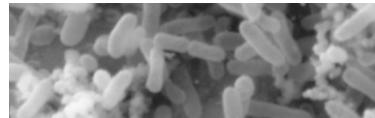
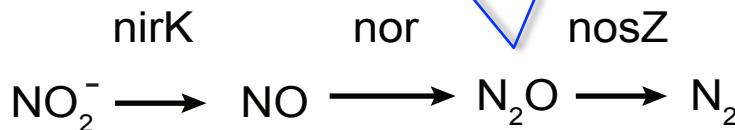
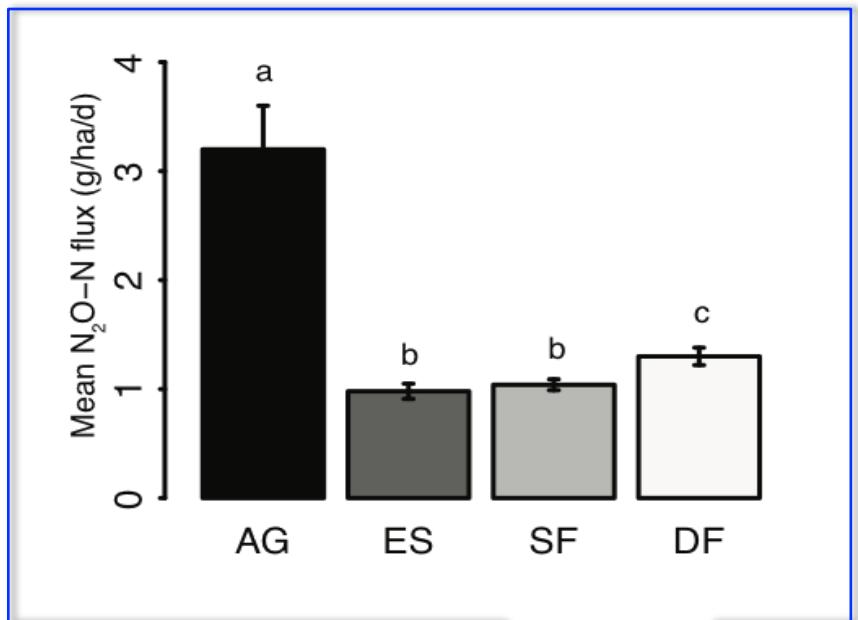
Functional potential changes with land management



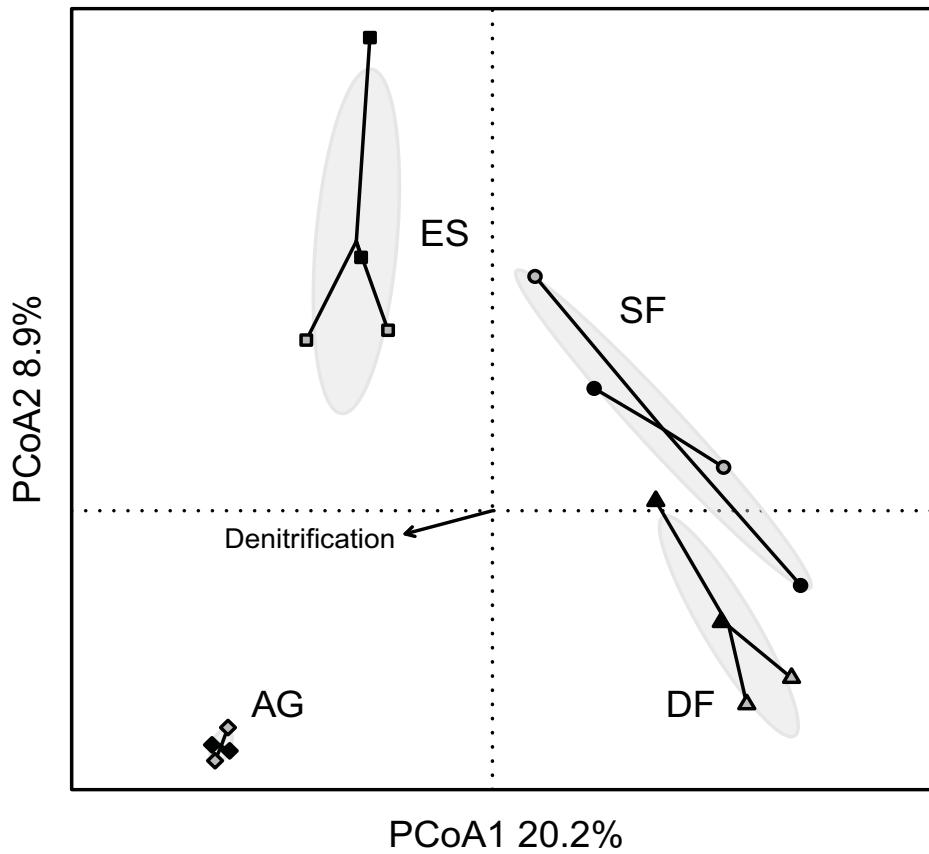
Analysis of 7058 genes annotated by MG-RAST against the SEED database

What is the relationship between
denitrifying bacteria and N₂O in
agricultural and native soils?

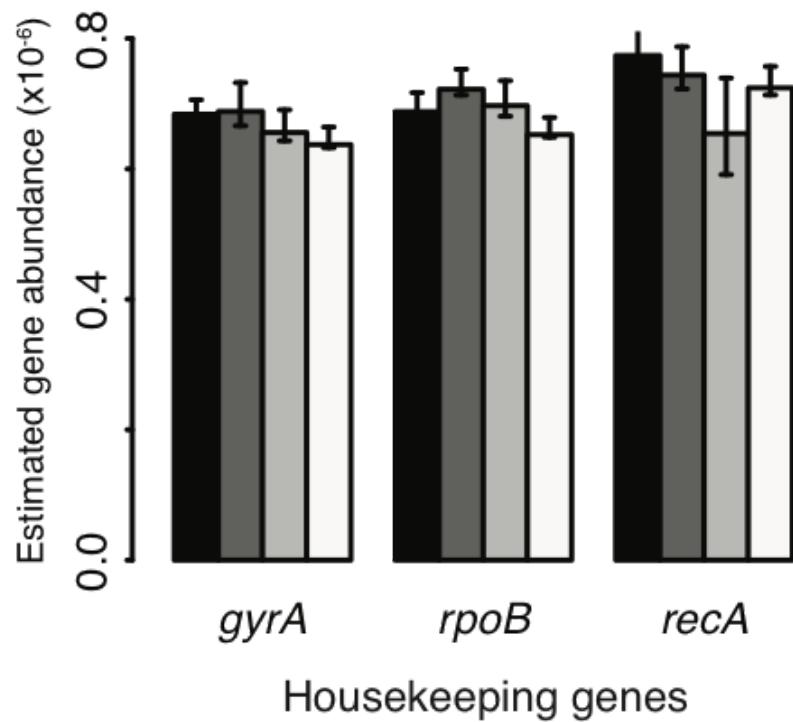
Denitrifying microbes



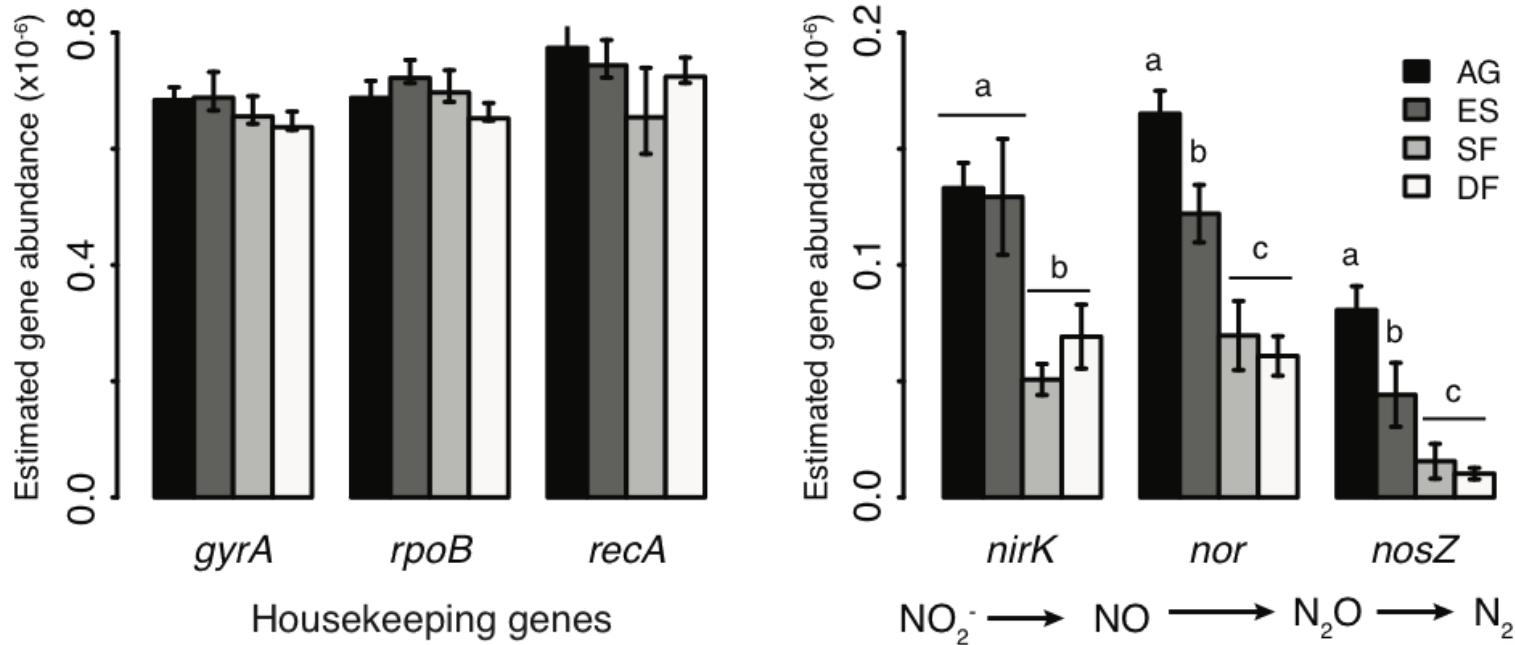
Nitrogen metabolism contributes to the differentiation of communities



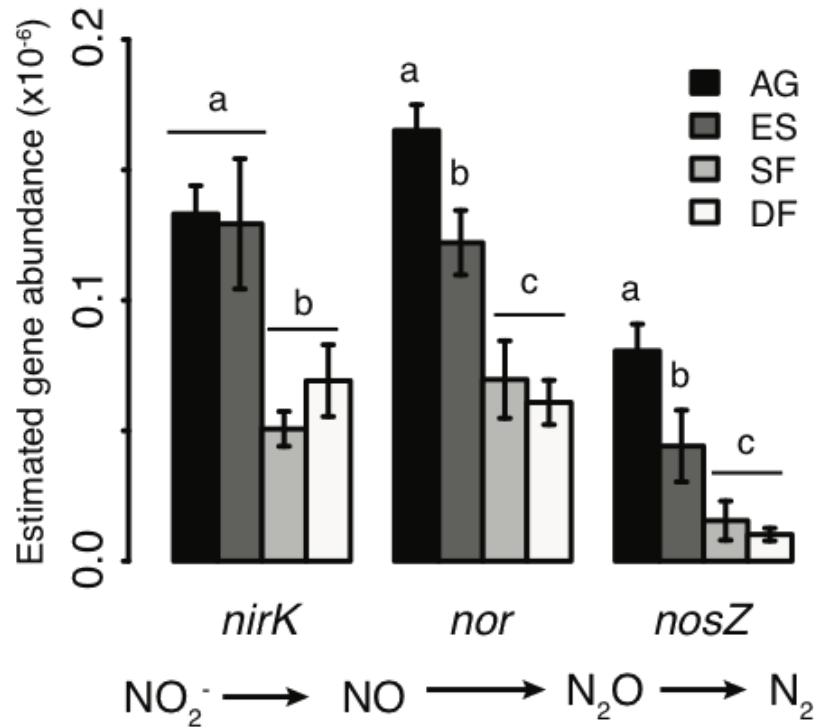
Gene abundances



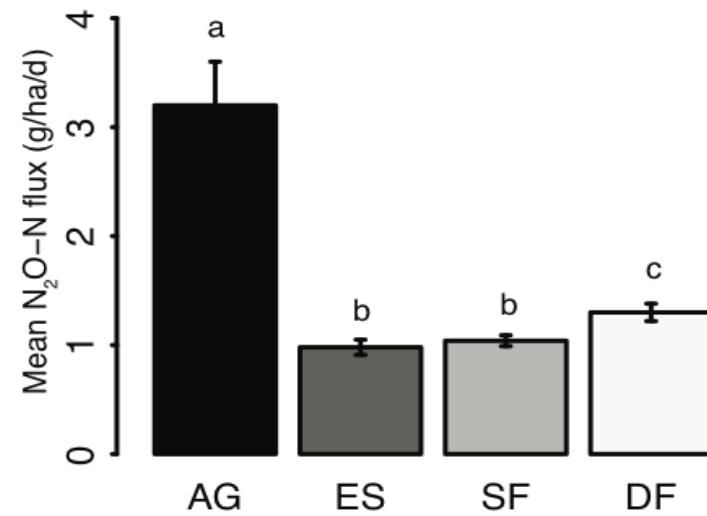
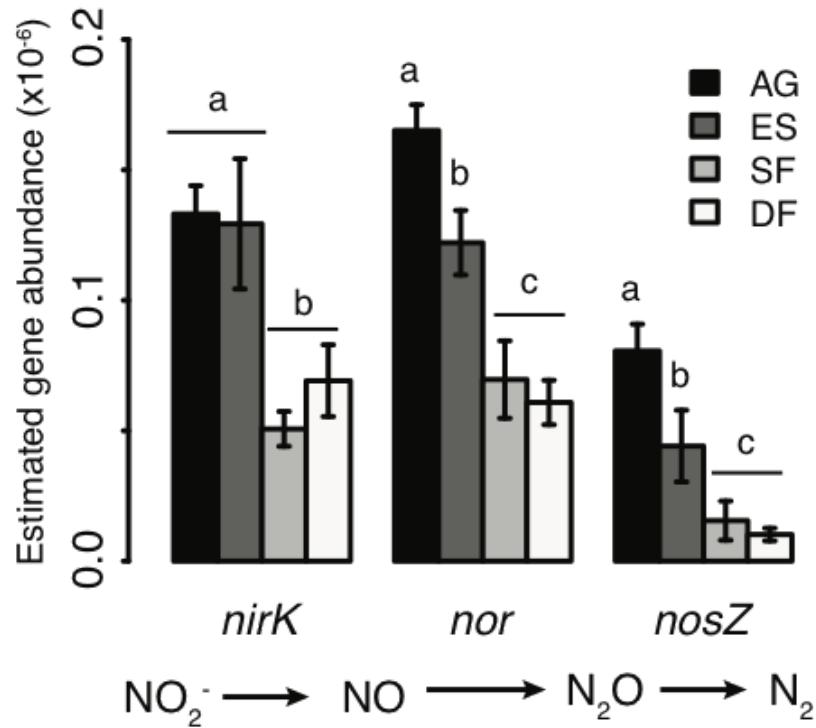
More denitrification potential in Ag soils



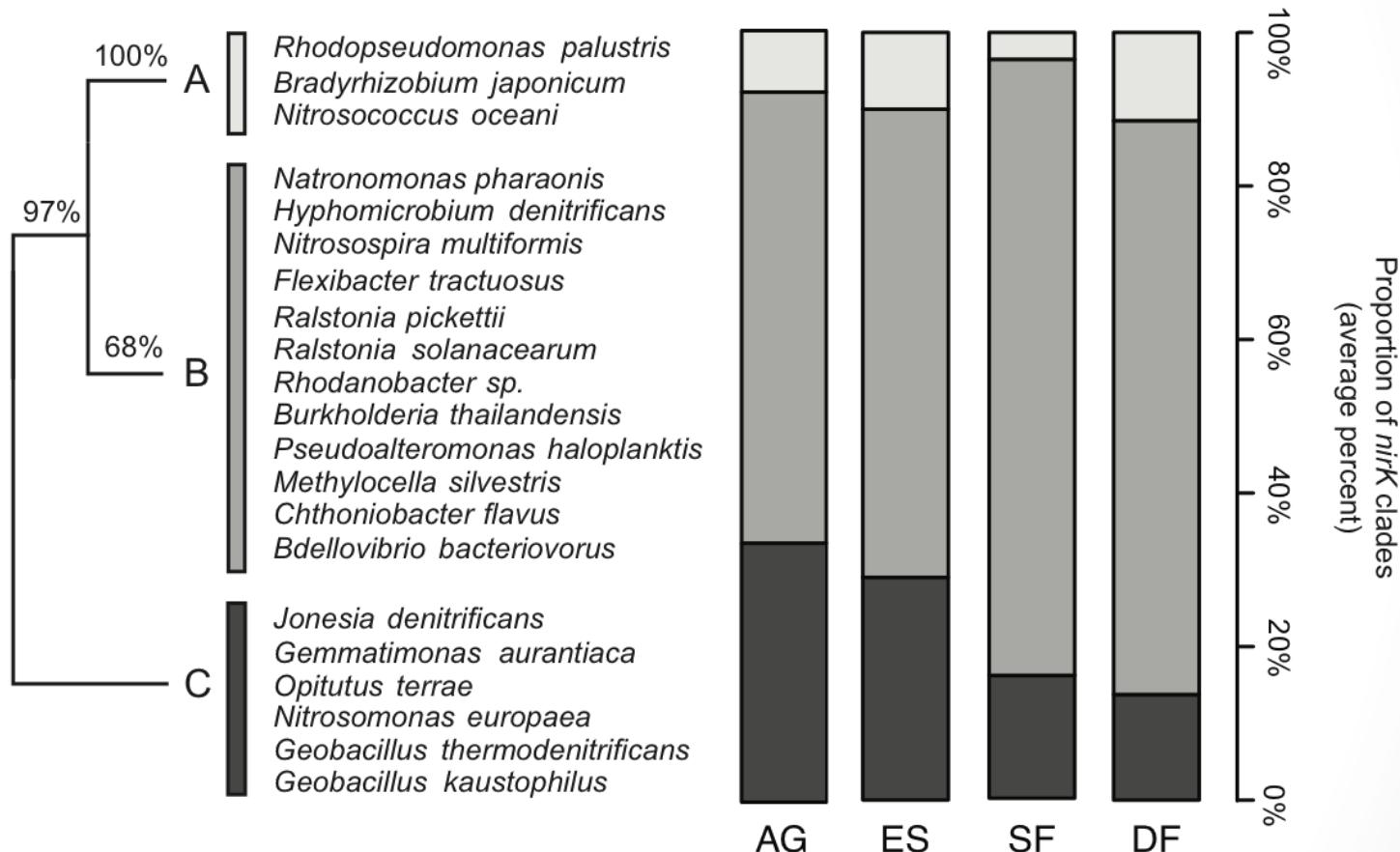
Not only abundance contributes to N₂O flux



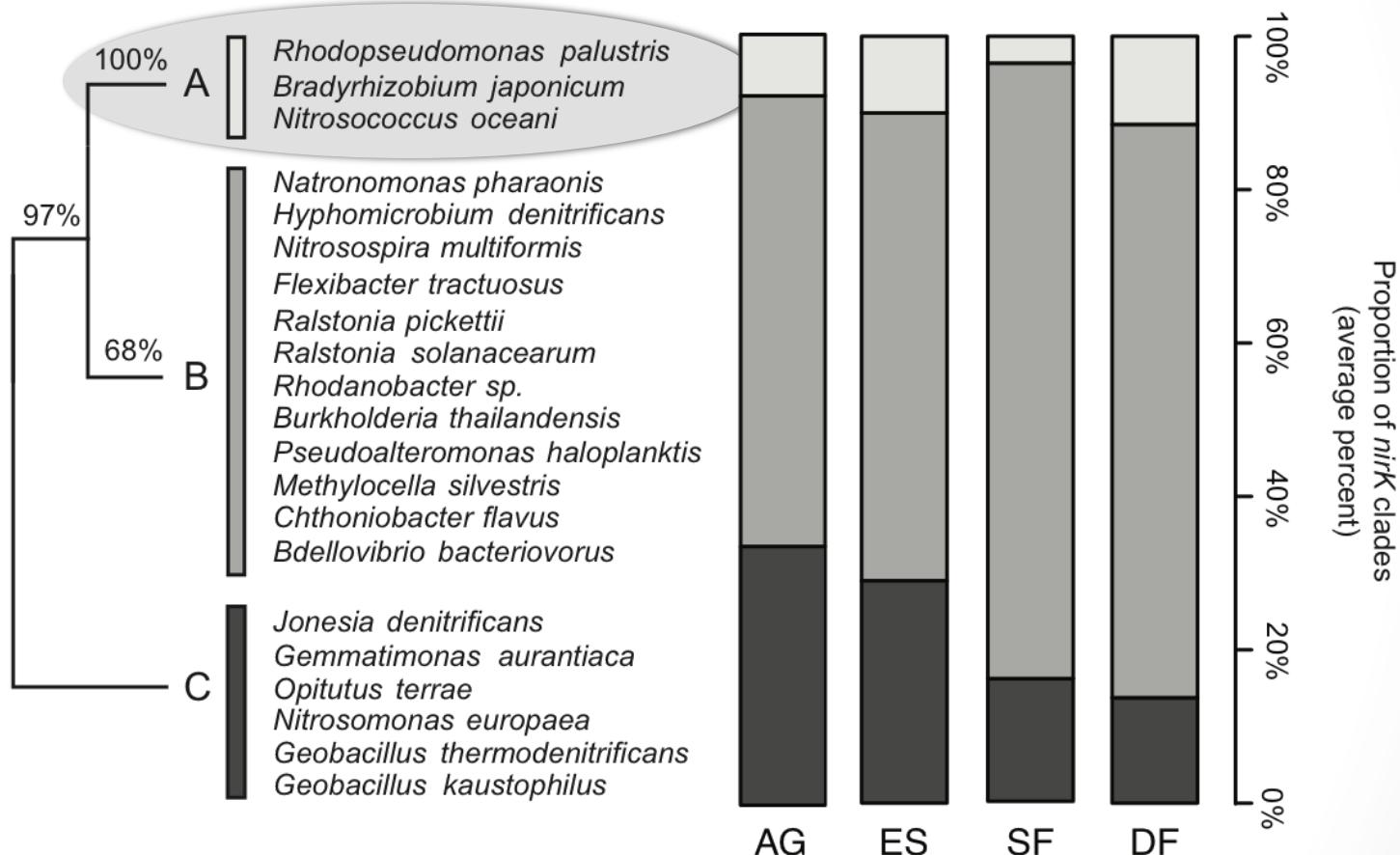
Not only abundance contributes to N₂O flux



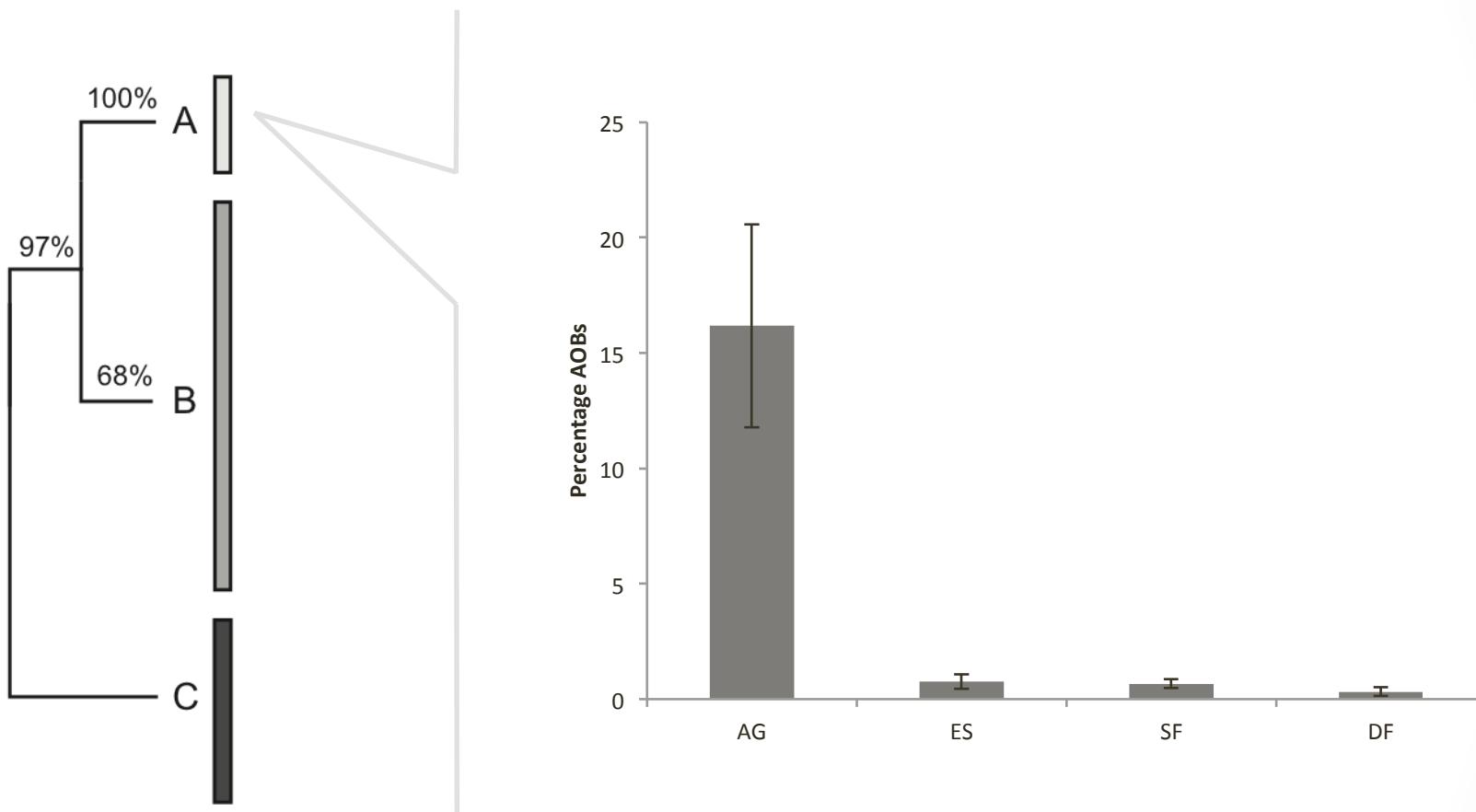
Denitrifier composition also changes



High denitrifier diversity

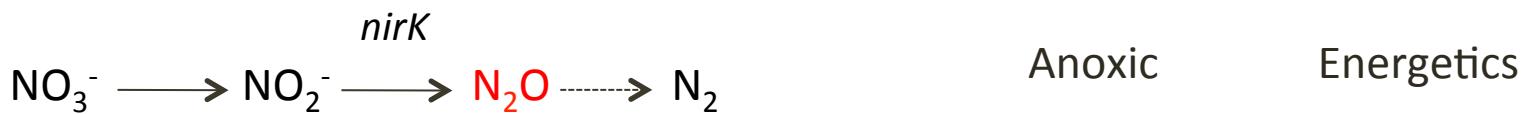


AOB proportion increased significantly in AG

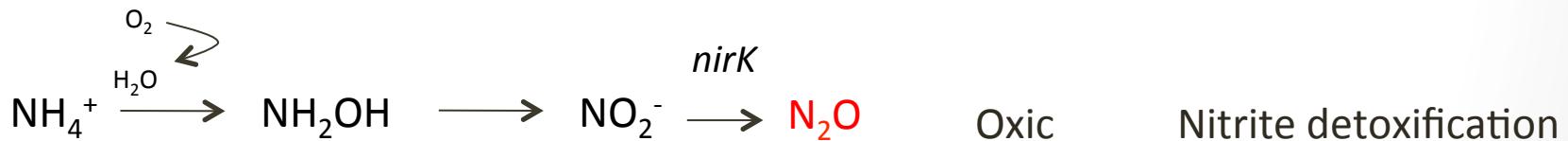


Types of denitrification

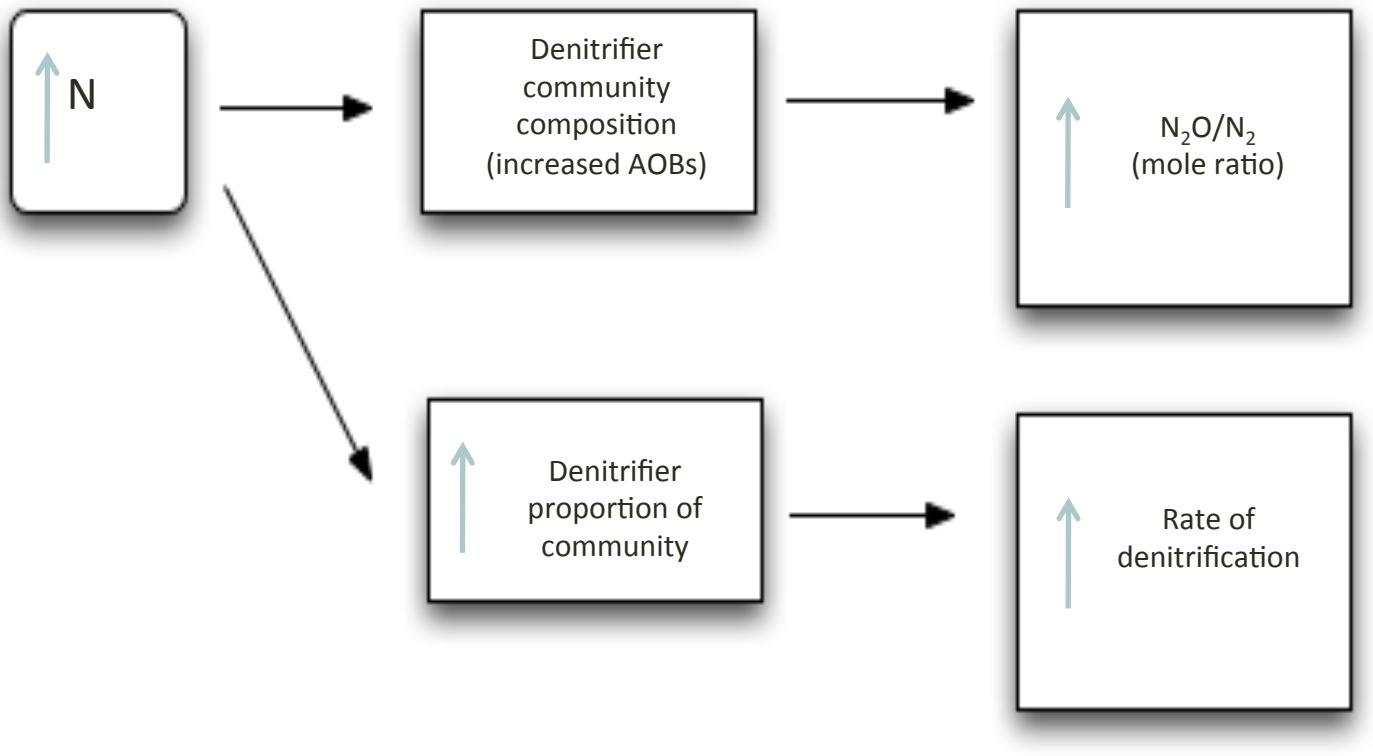
Heterotrophic denitrification



Autotrophic denitrification (AOBs)



Microbial communities in agriculture are poised for denitrification



What is the relationship between denitrifying bacteria and N₂O in agricultural and native soils?

- Denitrifier abundance and composition changes with agricultural management. Communities more diverse than previously thought and poised for denitrification.
- May be potential for microbial mediation



Tom Schmidt
University of Michigan



Other studies on:
Methanotrophs
Biofuel crops with the GLBRC
Longitudinal study of KBS LTER sites
Effects of fertilization
HGBF studies of soil health in sub-Saharan Africa



mothur

- Updated versions are released every few months
 - First release, v1.1.0, March 2009
 - Last release, v1.36.0, July 2015
- Approaches

Taxonomy

OTUs Phylogeny

- Sequencing systems

Sanger

454

Illumina

- Tutorials for OTU-based approach

- 454

http://www.mothur.org/wiki/454_SOP

- Illumina

http://www.mothur.org/wiki/MiSeq_SOP

mothur and QIIME

- Both are open source and on github
- Both aim to enable advances in microbial ecology and are actively maintained and developed
- Both require alignment
- mothur is not an acronym
- While QIIME connects multiple tools, mothur reimplements algorithms, so that it is all one program

Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities

Schloss et al, AEM, 2009

- mothur in C++, QIIME connections in Python
- QIIME has mothur and mothur has Unifrac, but the default behavior for mothur is to do clustering based on sequence distance
- mothur's clustering can be very memory intensive, Uparse as used in QIIME requires less memory

Outline

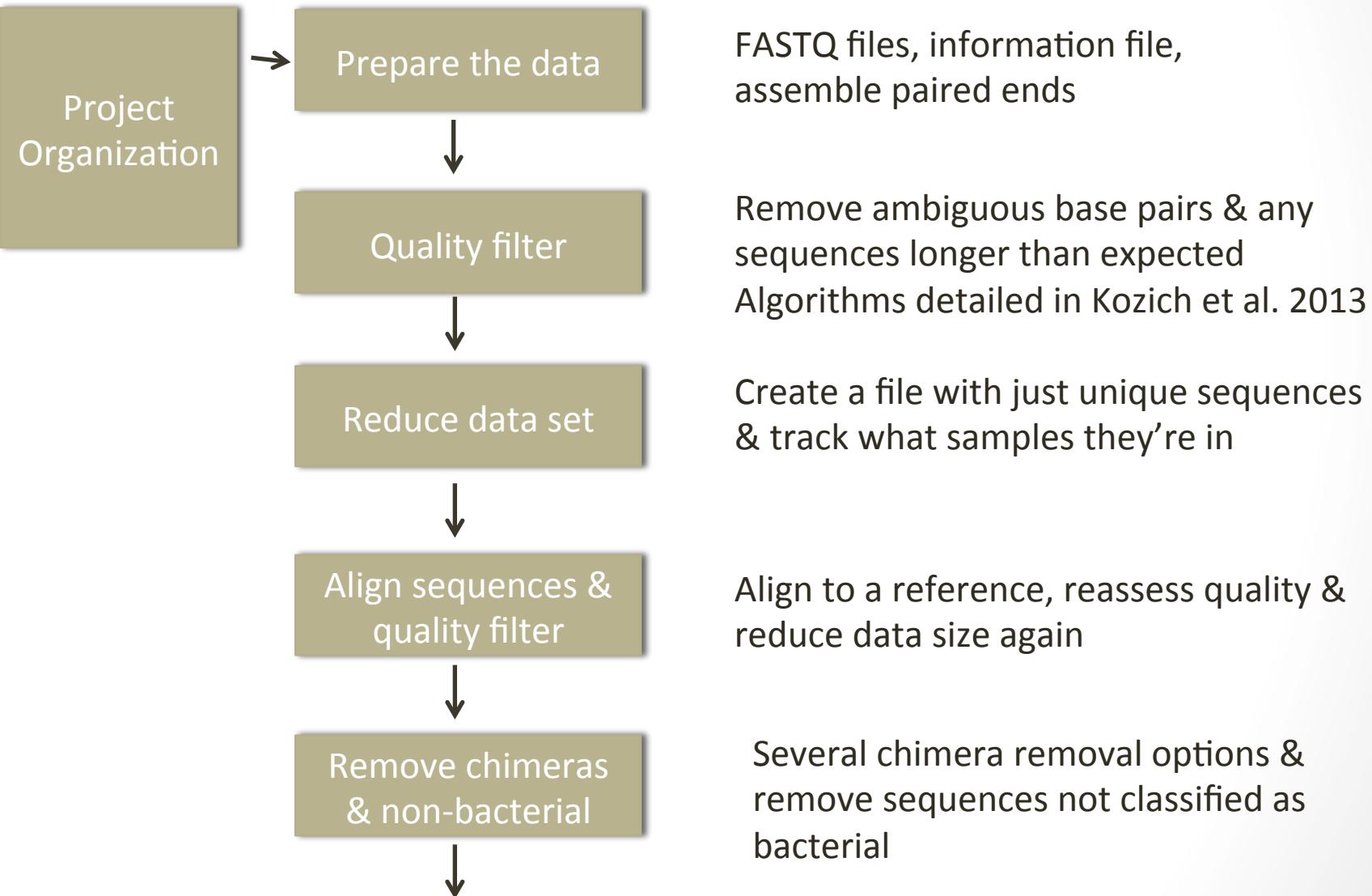
- Case study – microbial communities in soil
- mothur workflow
- mothur MiSeq SOP

How to get your data in, get your data out
(an OTU table), and get help

- Some preliminary statistics

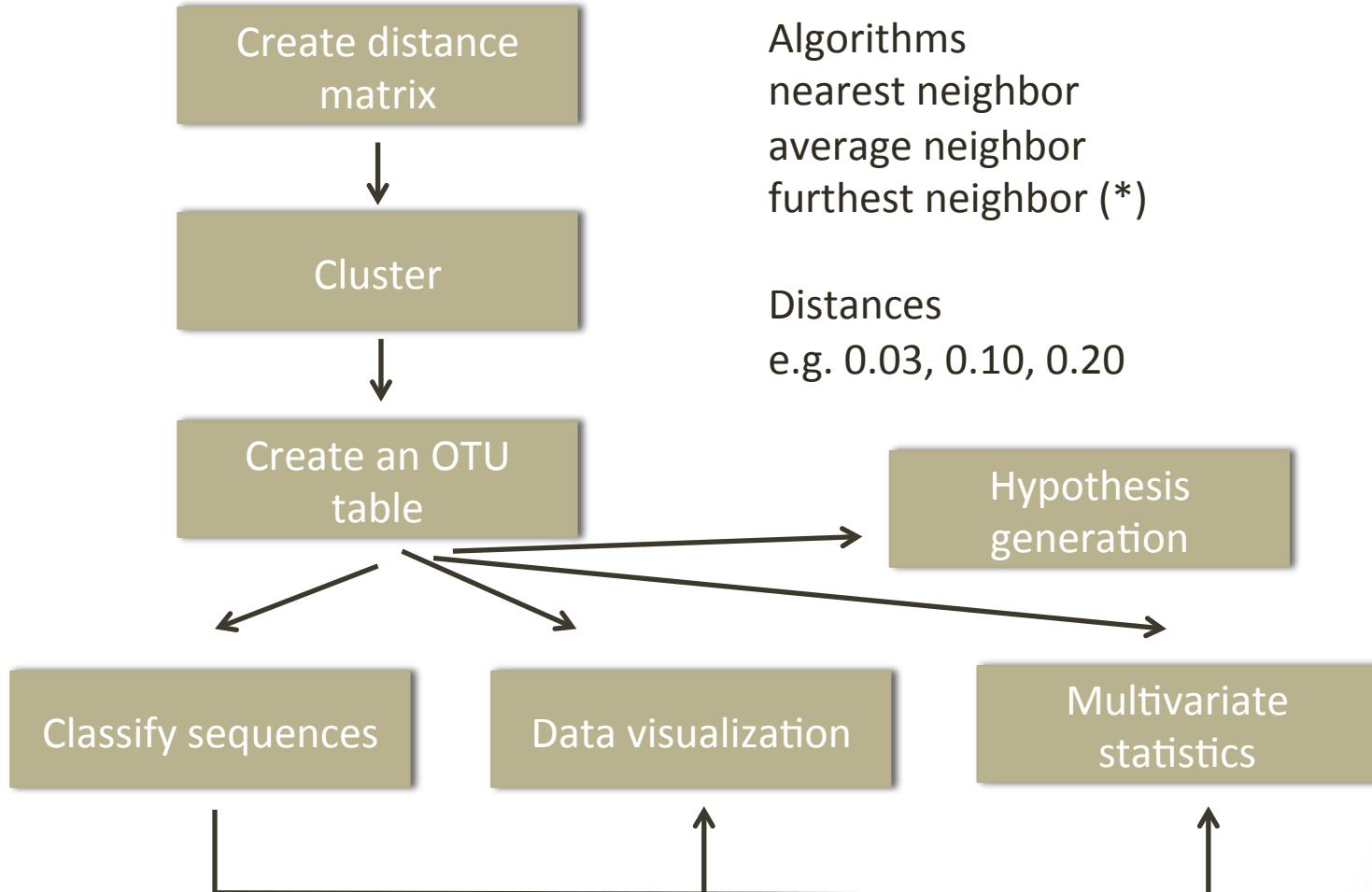
mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)



mothur workflow

(Schloss 2009; Schloss 2010; Schloss 2013; Pruesse et al. 2007, doi:10.1093/nar/gkm864; Pruesse et al. 2012, doi:10.1093/bioinformatics/bts252)



Working in mothur

Learn how to:

- Get in (get data in)
- Get out (Come home with an OTU table or on it)
- Get help

<https://github.com/tracykteal/tutorials/tree/master/mothur>

http://www.mothur.org/wiki/MiSeq_SOP

Options for running mothur

- http://www.mothur.org/wiki/Download_mothur
- Windows, Mac, Linux
 - Interactive
 - Batch
 - GUI
- In my test runs, I used 2 processors
 - Both 454 & Illumina tutorials took ~ 1 – 1.5 hrs
 - 454, start with trim.seqs (the output files have been provided)
 - You will not do the “using quality scores” approach
- You can copy & paste all commands but, as Sue correctly noted with R, you won’t learn it this way

Specific file types worth noting

Illumina

- .files
- .count_table



mothur tutorial

- Is your sample coverage sufficient for meaningful analyses?
- Do mouse fecal microbiota differ between weanling and adult mice?
 - alpha, beta diversity
 - OTUs responsible?
- Is variation in fecal microbiota greater among weanlings than adults?



Use both graphical and statistical tools to answer each question.

Ignore the obvious pseudoreplication and simultaneous lack of replication.