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Digging into the soil metagenome

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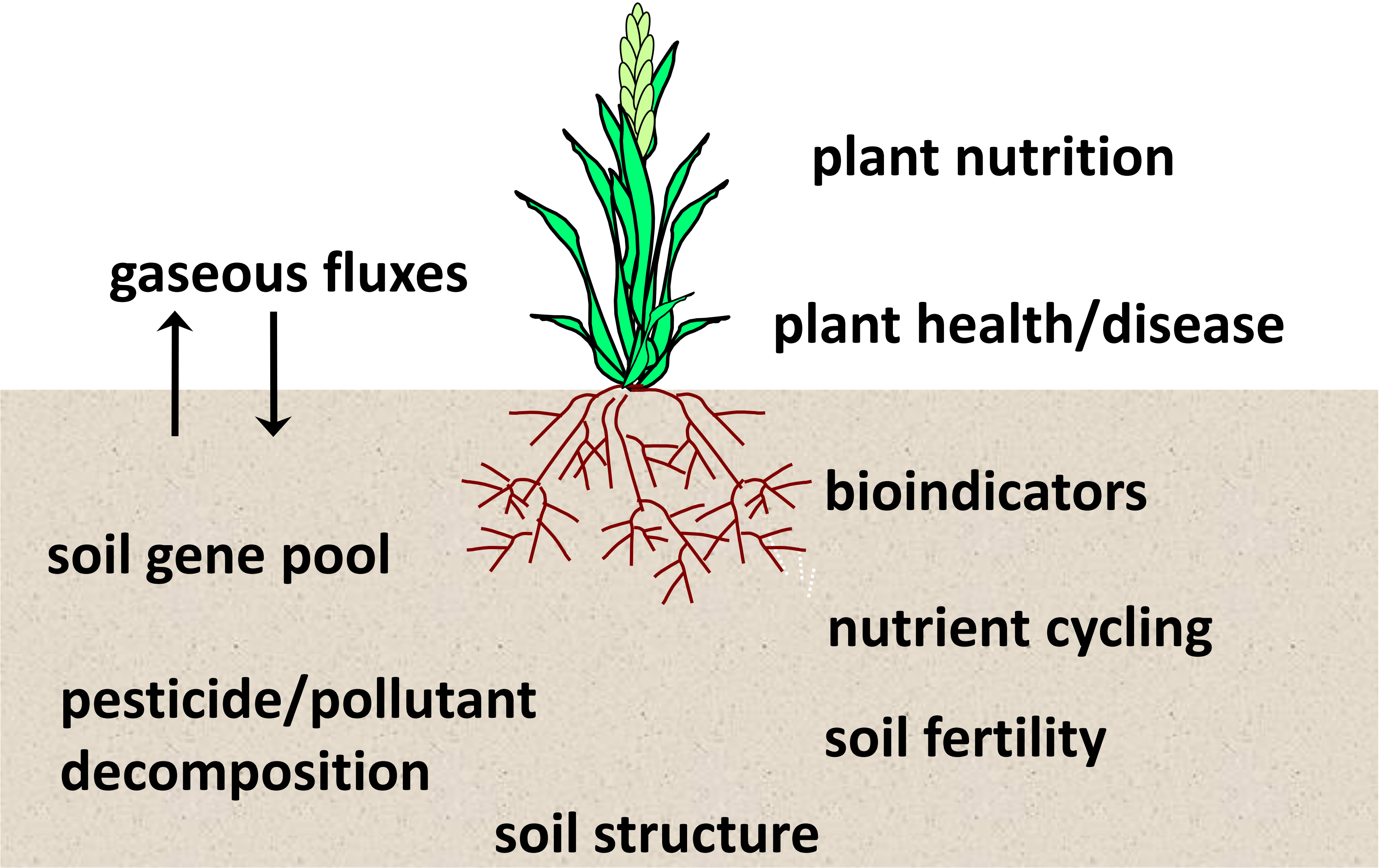
Why soil?



plant nutrition

plant health/disease

gaseous fluxes

bioindicators

soil gene pool

nutrient cycling

pesticide/pollutant decomposition

soil fertility

soil structure

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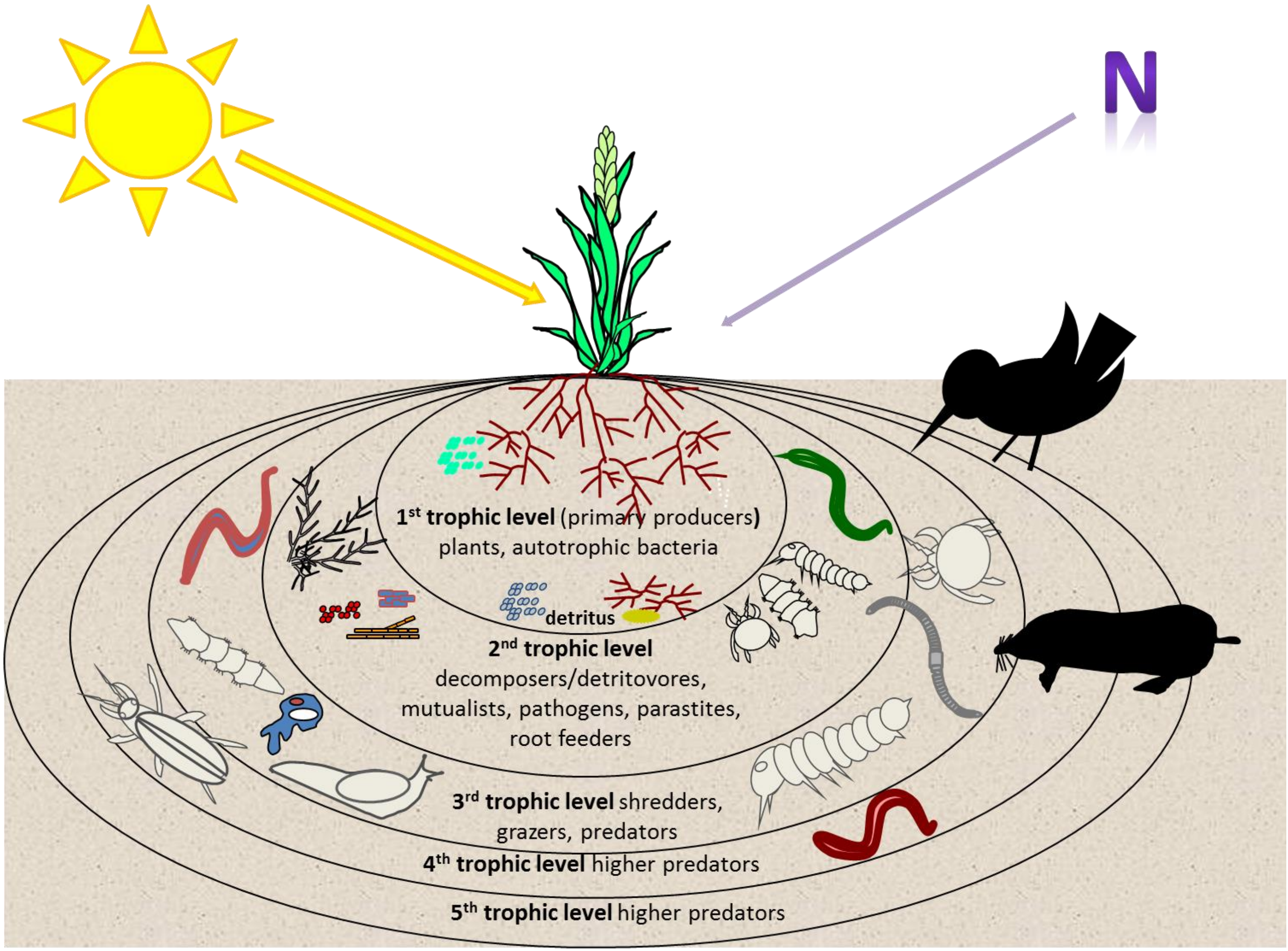
The soil food web is complex…..



1st trophic level (primary producers)
plants, autotrophic bacteria

2nd trophic level
decomposers/detritovores,
mutualists, pathogens, parastites,
root feeders

3rd trophic level shredders,
grazers, predators

4th trophic level higher predators

5th trophic level higher predators

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…subject to different perurbations…

Cultivation

Fertilizers

Livestock

Harvesting

……varying at different scales

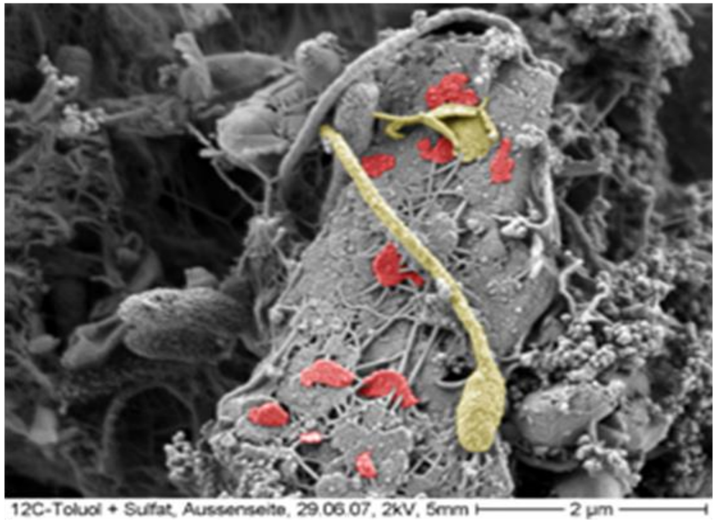

Photo: Burkhard Schmidt-Brücken, Institute of Material science/TU Dresden
Colored by: Christian Schurig/ UFZ CC BY 3.0

Why metagenomics?

≤1% soil bacteria grow on agar

Each g soil contains $10^{9\text{-}10}$ bacterial cells ($10^{7\text{-}8}$ culturable; 10^{4} species)

The soil metagenome is vast

Greater biodiversity in soil than any other habitat on earth:

- **>1,000,000 Gbp per g soil** (>10^9 bacterial cells, ~ 5 Mb genome)
- Human genome – 3 Gbp
- Sargasso sea – 6 Gbp sequenced

Rothamsted Research

- Oldest agricultural research station in the world
  - founded 1843 by John Bennet Lawes, amateur chemist, owner of the Rothamsted Estate
- Investigating impacts on soil quality
  - land management, fertilizer and other agrochemical inputs , climate change and the influence of plants
- Well documented
  - “typical” northern European arable and pasture management, many long-term field experiments

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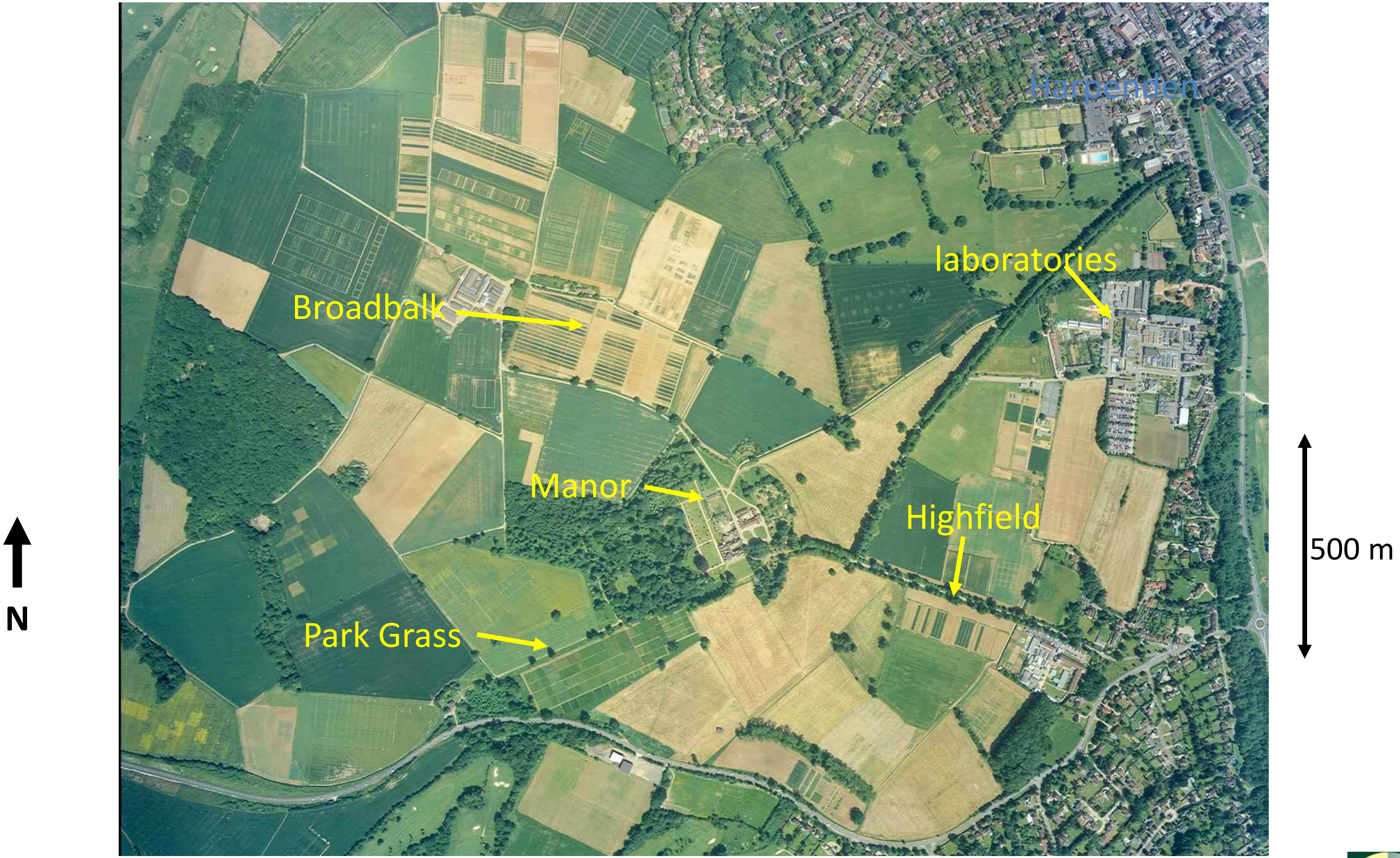
Rothamsted Research



Satellite photograph courtesy of the ECN

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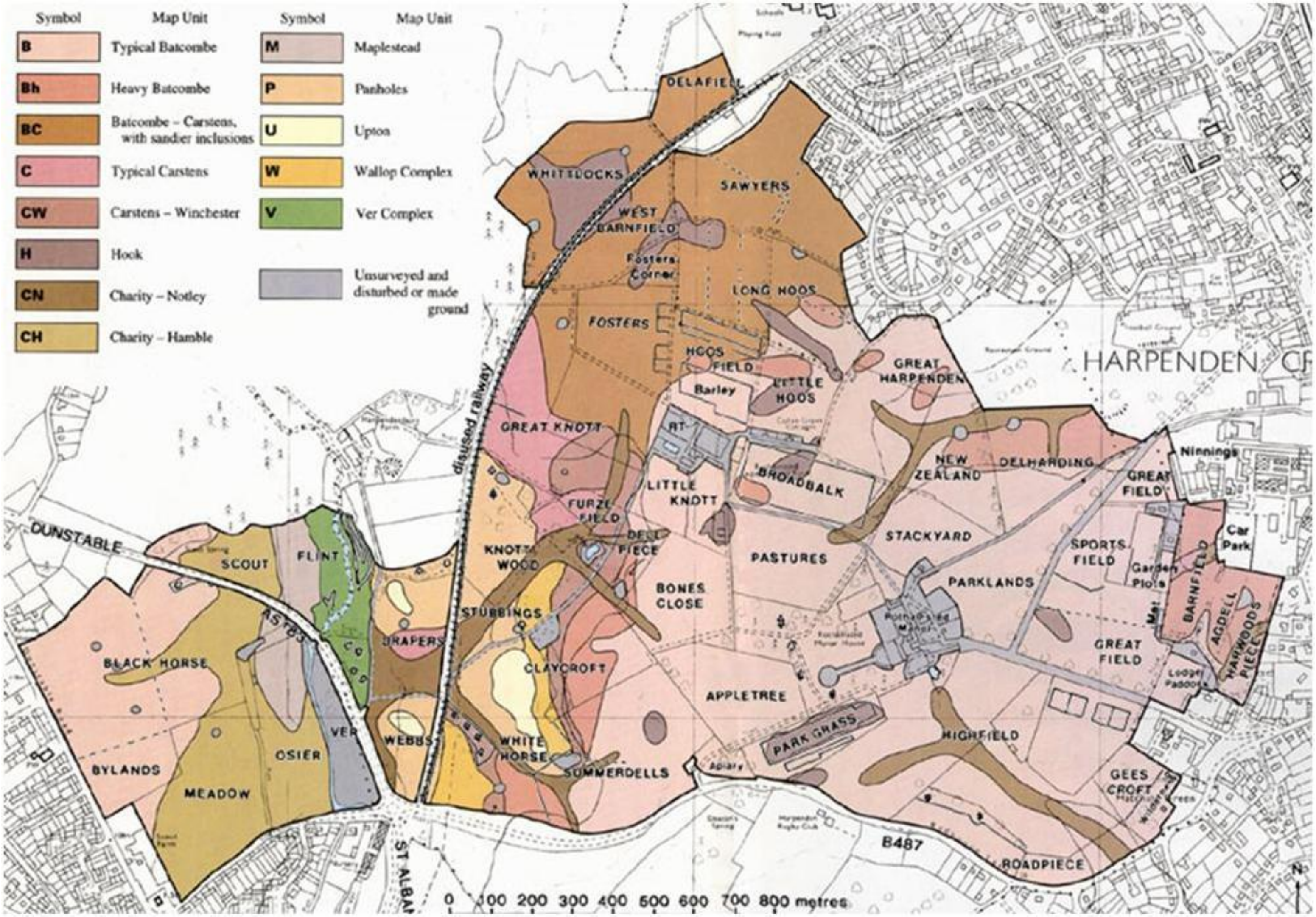
Rothamsted soil map



(Avery, B.W. and Catt, J.A., The soil at Rothamsted, 1995)

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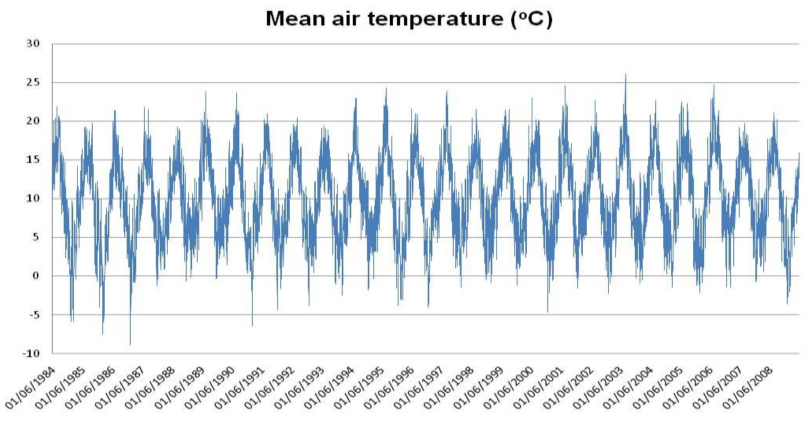
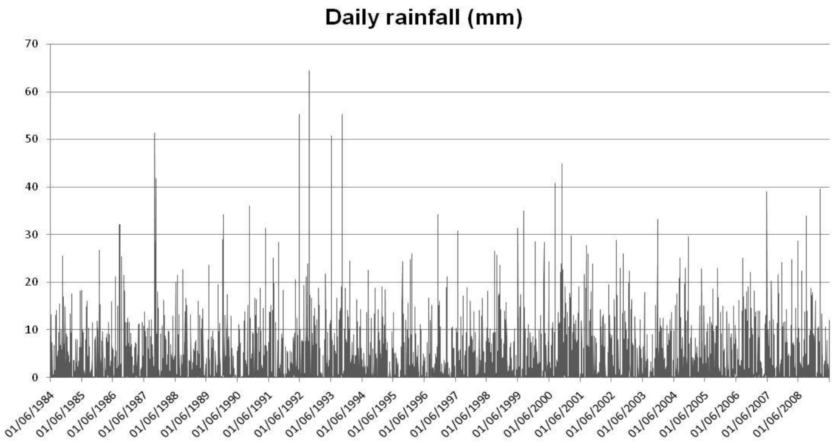
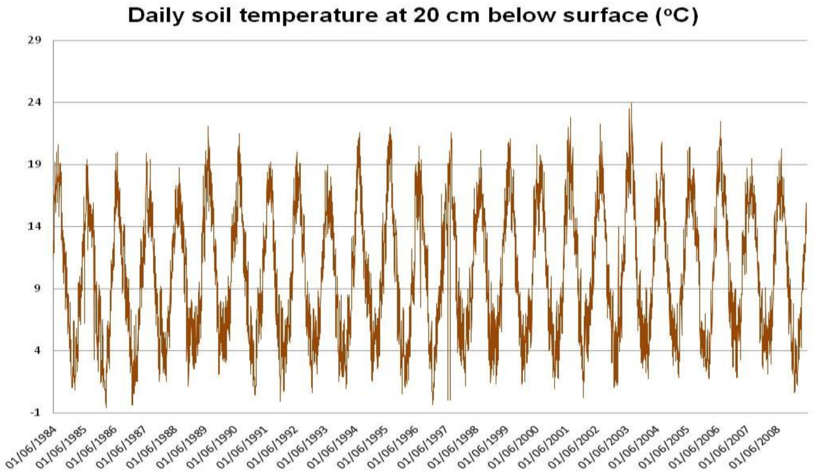
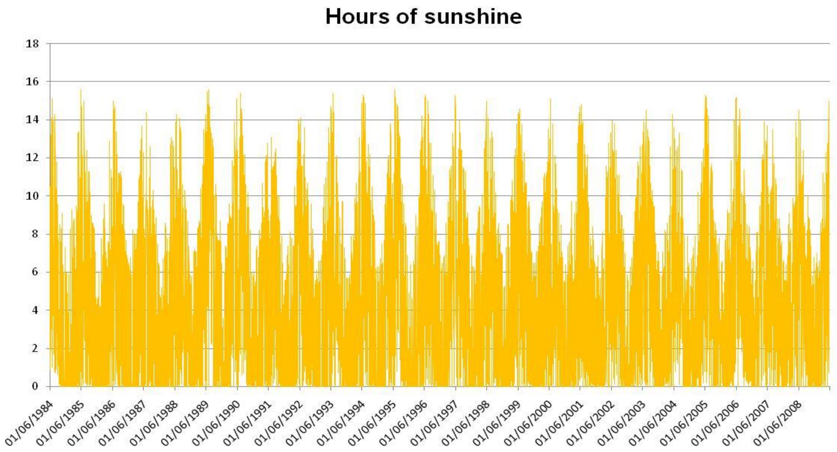
Metadata collected since 1843


Mean air temperature (oC)


Daily rainfall (mm)


Daily soil temperature at 20 cm below surface (oC)


Hours of sunshine

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Rothamsted long-term experiments

Sample & data archive

- Began 1843
- Material from Classical and other experiments
- Air-dried soil & crop samples
- Metrological, crop yield & disease data

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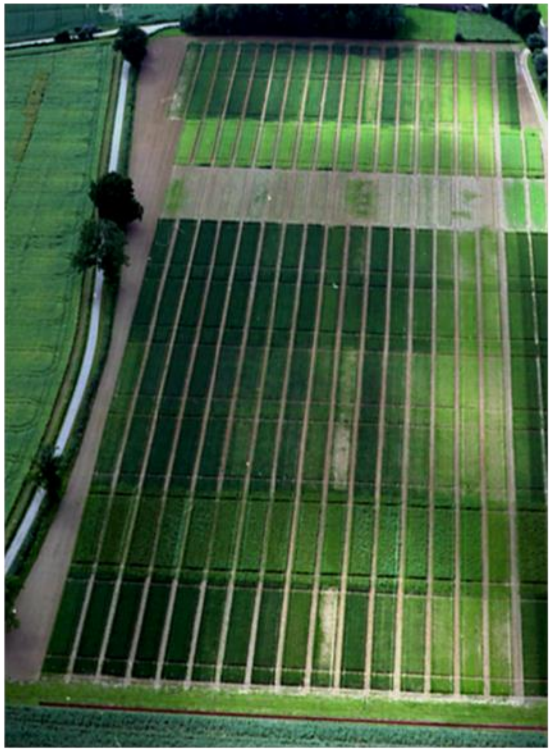
Rothamsted field sites



Broadbalk
wheat & fertilizers

Park Grass
grass & fertilizers

Highfield
plants & tillage

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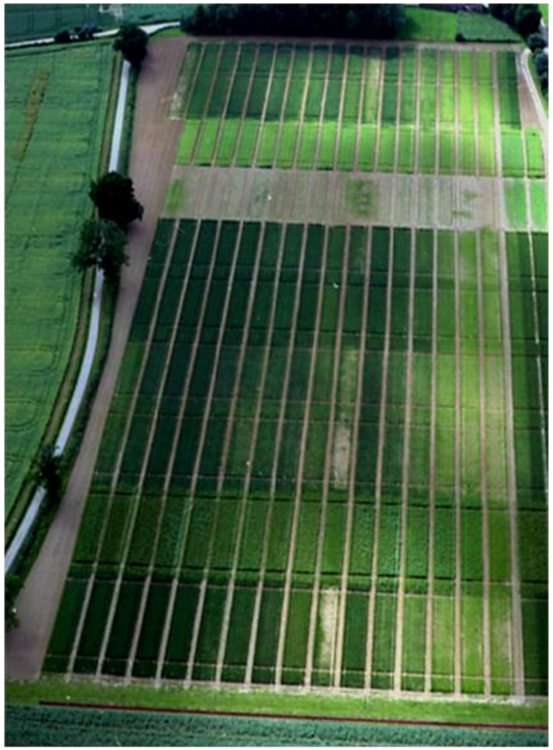
Rothamsted field sites



Broadbalk

- 70,000 16SrRNA amplicons (NSF, Eric Triplett, U Florida)
- Zhalnina. et al. 2013 Frontiers in Microbiology 4:104.

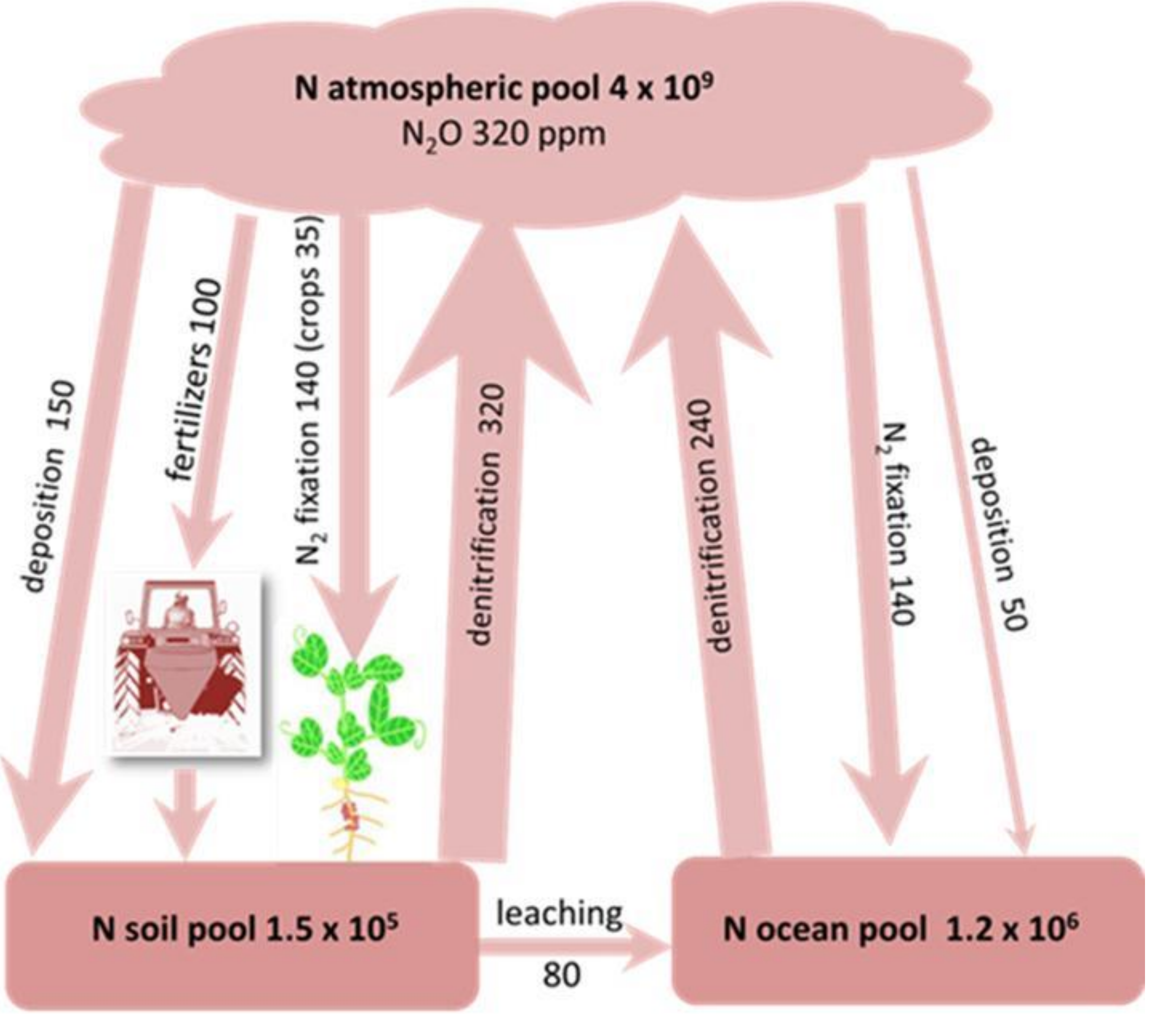
Park Grass

- 20 million 454 sequences (ANR MetaSoil, Tim Vogel, U Lyon)
- Delmont et al. 2012 ISME Journal 6: 1677-1687

Highfield

- 2.7 billion RNA, 2.7 billion DNA, 1.6 million amplicons
- Janet Jansson, Folker Meyer, Illumina, TGAC

Global nitrogen cycle



Fluxes in Tg y^{-1}; pools in Tg

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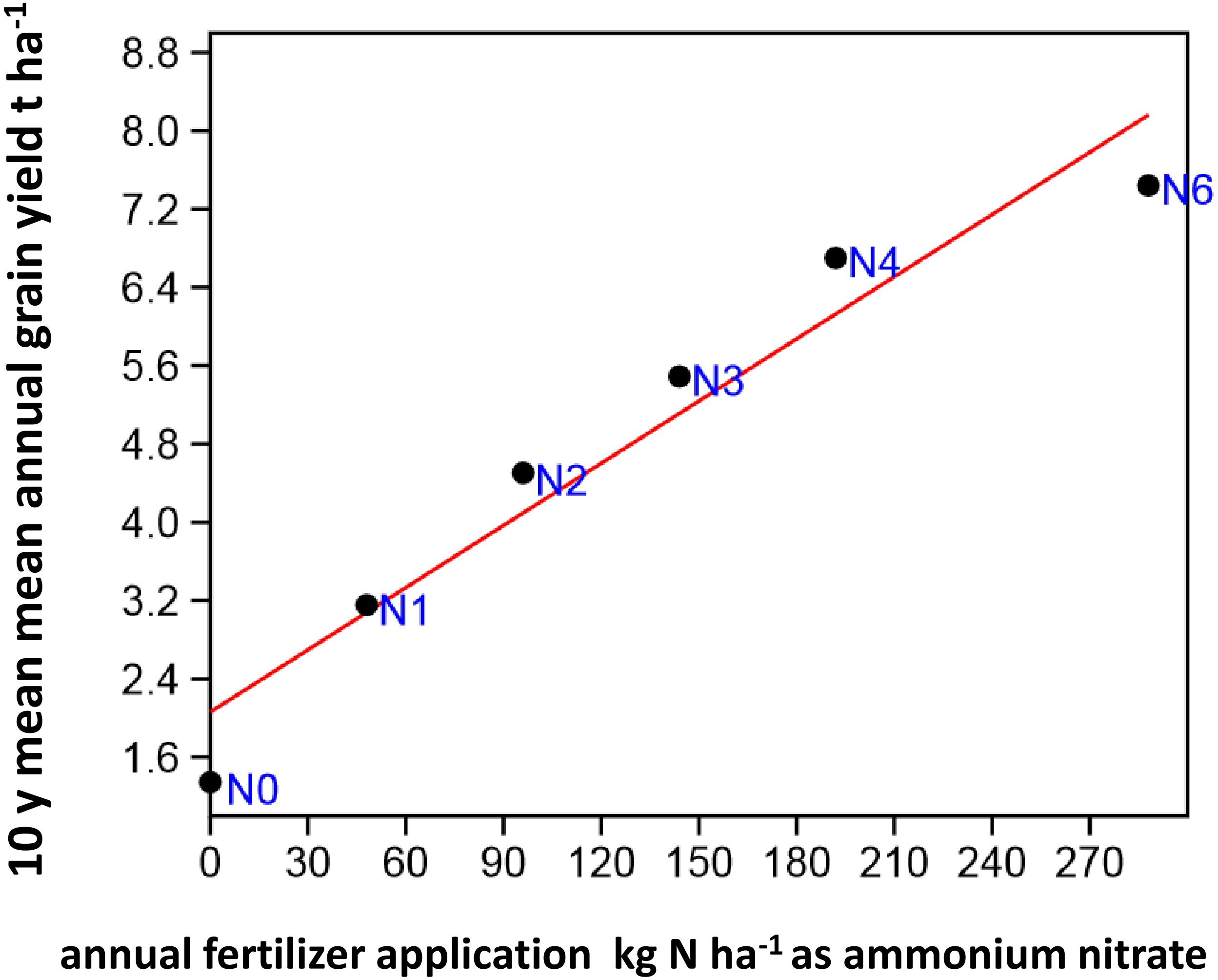
Why work on nitrogen in soil?

Broadbalk winter wheat yields increase with N inputs



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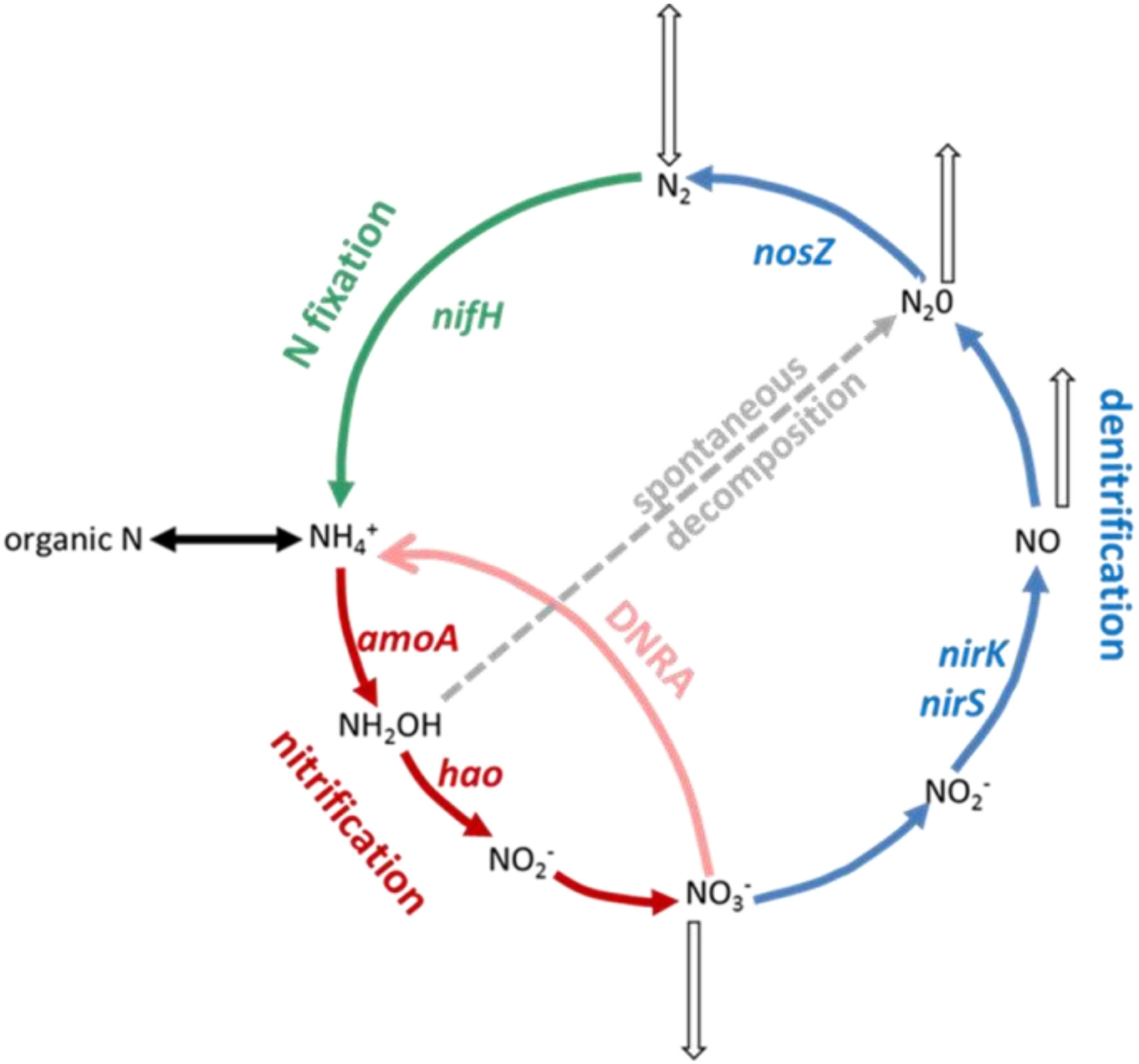
The Nitrogen cycle



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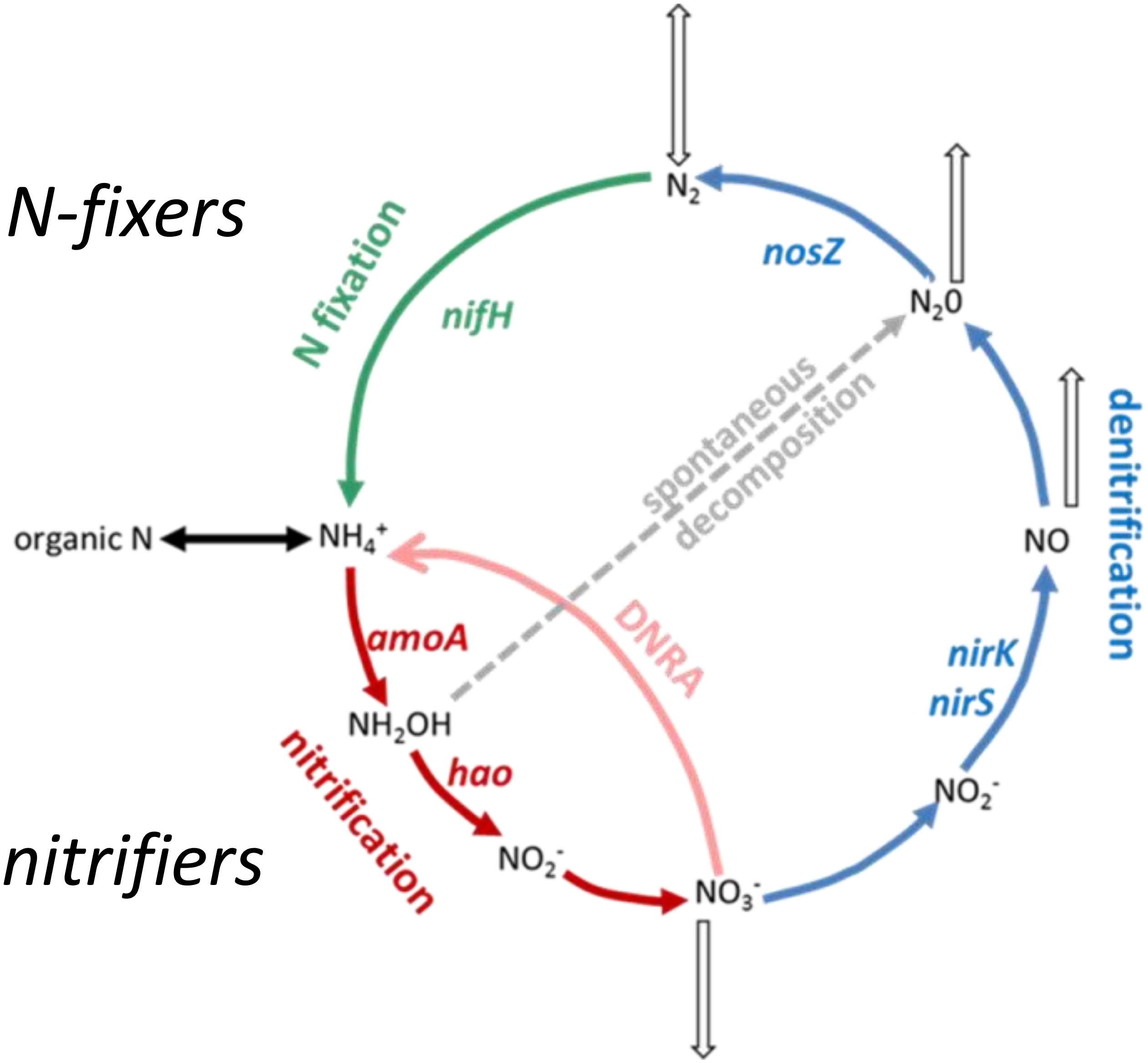
The Nitrogen cycle

N-fixers

denitrifiers

nitrifiers



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Biological N fixation (BNF)

- N is limiting in many natural environments
  BNF is a very common property of bacteria and archaea

- Energy-intensive
  BNF only occurs where carbon is not limiting

- Repressed by presence of fixed N sources
  BNF switches off - don't fertilize legumes

- Many BNF bacteria also produce phytohormones
  e.g. *Azospirillum* increases yields via root growth not BNF

N_2 - - - - - - → NH_4

nitrogen gas **ammonia**

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The rhizobia-legume symbiosis

Pisum sativum

Root nodules contain the nitrogen-fixing bacterial species *Rhizobium leguminosarum*

Biological N fixation (BNF)

- Which bacteria & archaea are active in BNF?
- How significant is BNF by different groups in fertilized fields?
- Does long-term use of N fertilizer reduce abundance of BNF-capable organisms?

$$\underset{\text{nitrogen gas}}{N_2} \dashrightarrow \underset{\text{ammonia}}{NH_4}$$

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Nitrifiers in soil

- ubiquitous function in soil
- bacterial & archaeal NH_4 oxidizers; bacterial NO_2^- oxidizers slow growing, few cultured in lab
- archaeal & bacterial ammonia monooxygenase (AMO) & gene (*amoA*) distinctly different
- ammonia oxidizers also reduce nitrite
- enzyme inhibitors can reduce nitrification, bacteria & archaea differ in susceptibility

Nitrification

$$NH_4 \longrightarrow NO_2^- \longrightarrow NO_3^-$$

ammonia **nitrite** **nitrate**

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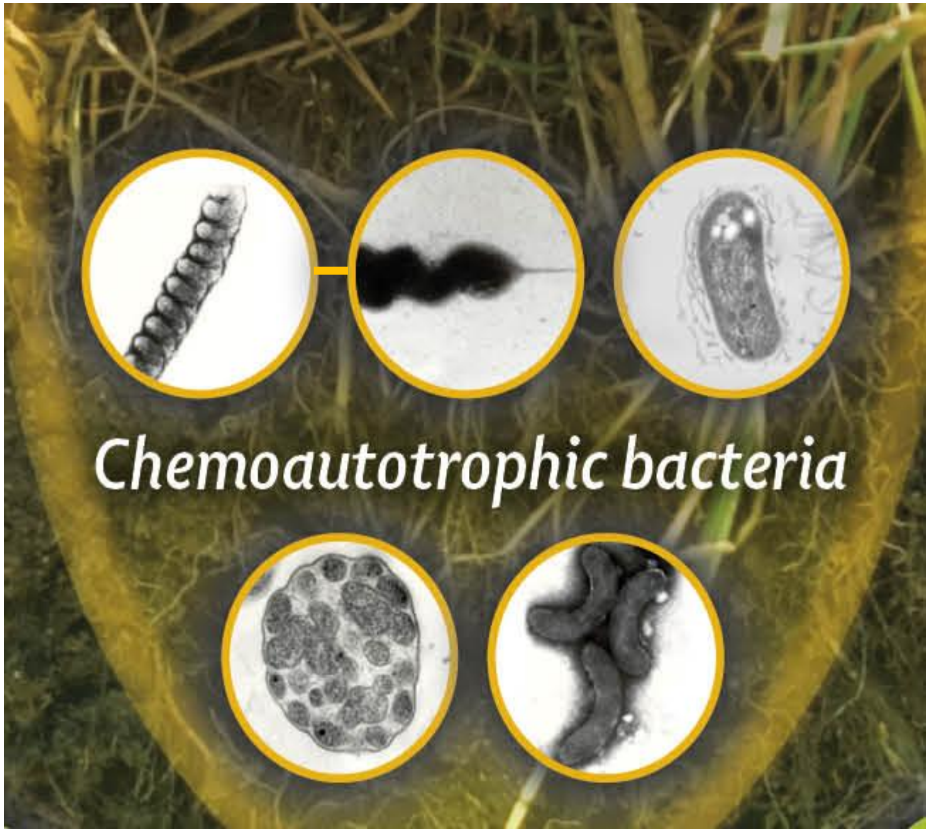
Nitrifying bacteria in soil



Nitrosospira sp.
ammonia-oxidizer

Nitrobacter winogradskyi
nitrite oxidizer

Nitrosolobus sp.
ammonia oxidizer

Nitrosovibrio sp.
ammonia oxidizer

(these ammonia oxidizers are β-proteobacteria, nitrite oxidizers are α-proteobacteria)

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Ammonia oxidizers in soil

Bacteria:

- *amoA* less abundant
- AMO low affinity for NH_4
- strict autotrophs (fix CO_2)

Archaea:

- *amoA* more abundant
- AMO high affinity for NH_4
- some isolates are oligotrophic, using organic C

Nitrification

$NH_4 \longrightarrow NO_2^- \longrightarrow NO_3^-$

ammonia **nitrite** **nitrate**

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Ammonia oxidizers in soil

- How does the contribution of Bacteria and Archaea to observed nitrification compare?

- Can soil management influence their relative abundance?

- Is there differential susceptibility of bacterial and archaeal AMO to nitrification inhibitors?

Nitrification

$$\underset{\text{ammonia}}{NH_4} \longrightarrow \underset{\text{nitrite}}{NO_2^-} \longrightarrow \underset{\text{nitrate}}{NO_3^-}$$

Denitrification

- Dissimilatory reduction of nitrate confined to Bacteria & Archaea
- Genes found in representatives of all known soil phyla - either *nirS* or *nirK* but not always *nosZ*
- Activity is ubiquitous when stimulated by anaerobic conditions and substrate

Denitrification

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

nitric oxide

nitrous oxide

nitrogen gas

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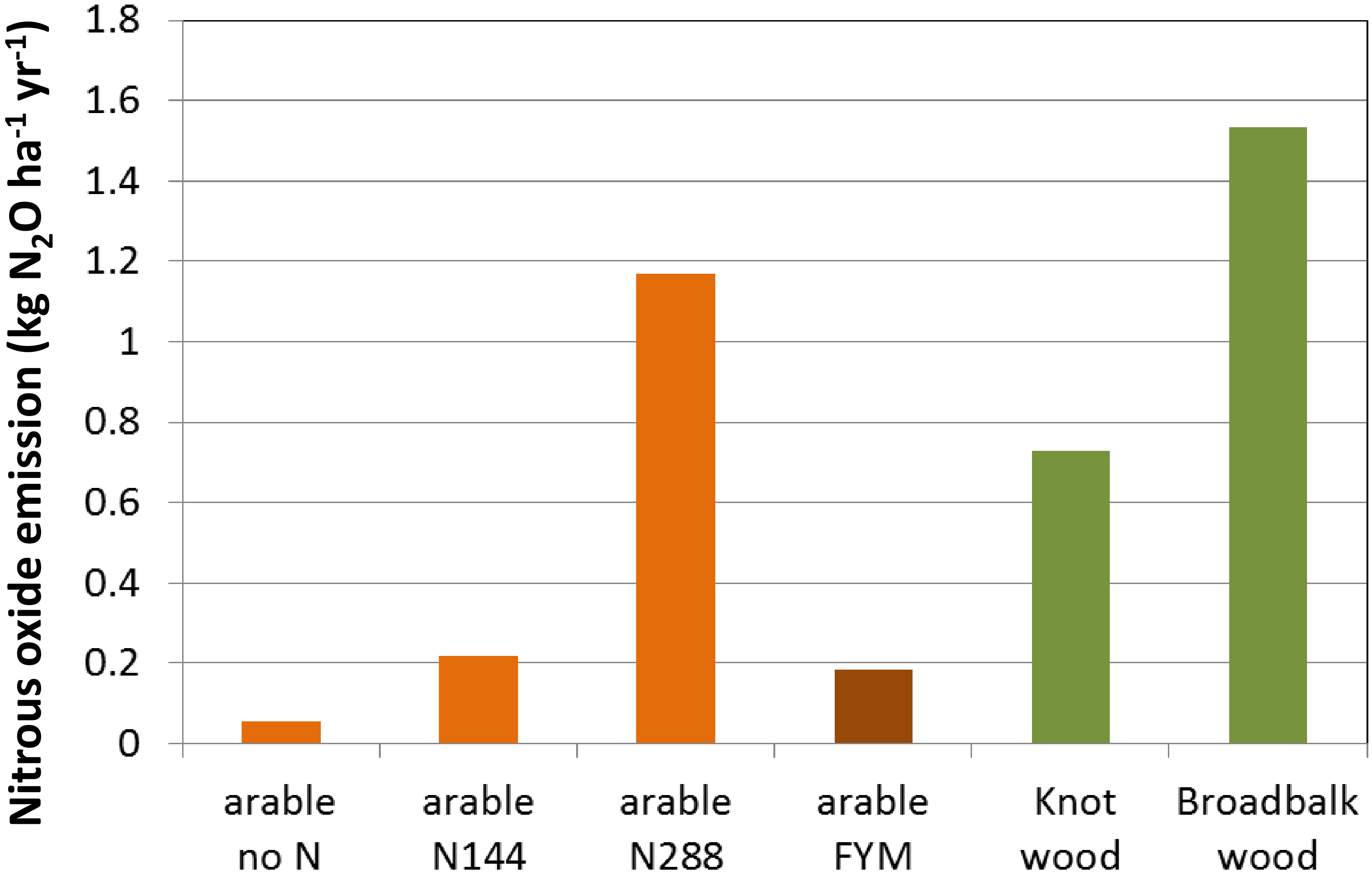
Mean annual N_2O emissions



c 250 kg total N ha^{-1} yr^{-1} applied in FYM

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Denitrification

- functional significance of alternative nitrite reductases: *nirK* (Cu); *nirS* (cyt cd_1 heme)?
- nitrous oxide reductase *nosZ* not present in all denitrifiers & not always expressed – why?
- Can land management optimise *nosZ* presence/expression?

Denitrification

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

		nitric oxide	nitrous oxide	nitrogen gas
NO_3^-	NO_2^-	NO	N_2O	N_2

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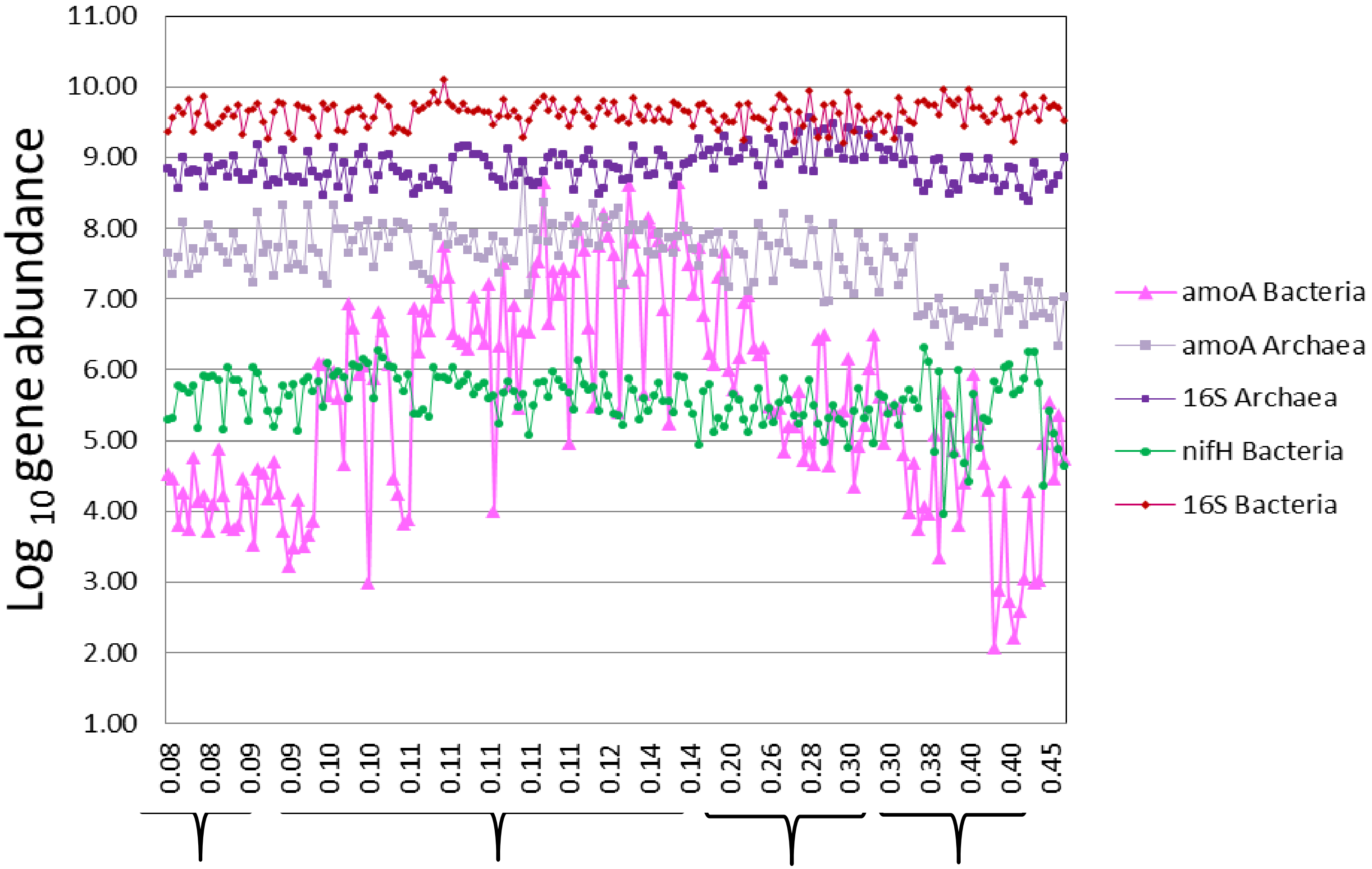
qPCR indicates differential N effects



arable
no added N

arable →
increasing N

arable
FYM

uncultivated
wood & grass

% soil N

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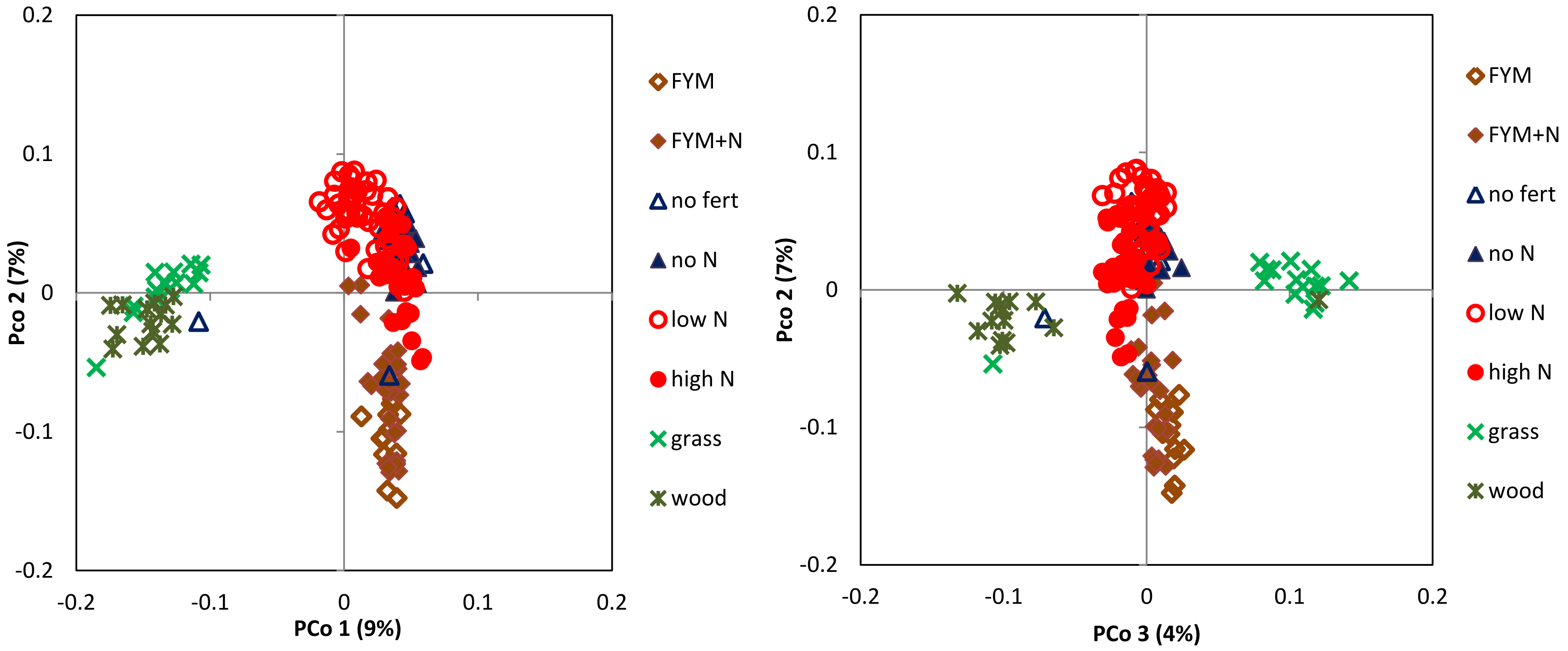
Broadbalk diversity PCoA



Based on amplicons assigned to 2739 species using PANGEA/Tax Collector

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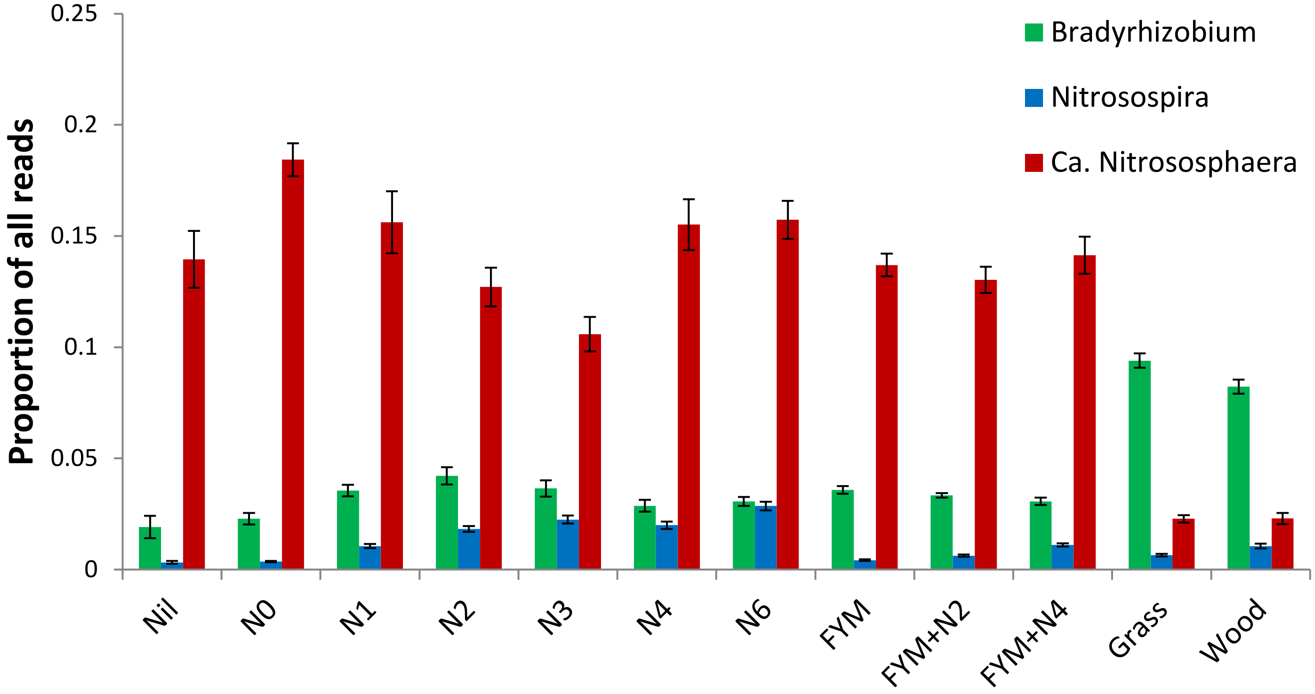
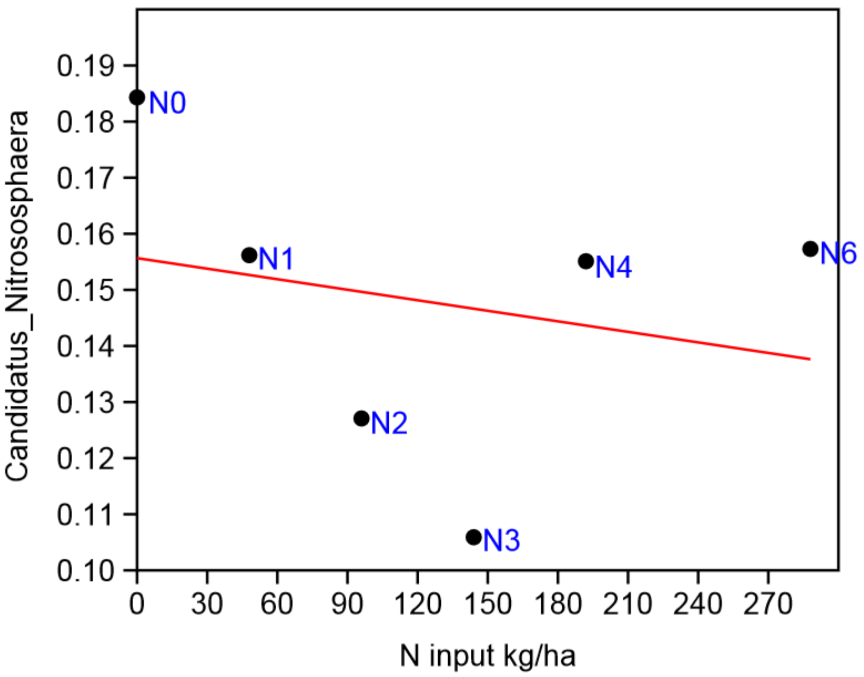
Broadbalk amplicon data



Zhalnina K., de Quadros P.D., Gano K.A., Davis-Richardson A., Fagen J.R., Brown C.T., Giongo A., Drew J.C., Sayavedra-Soto L.A., Arp D.J., Camargo F.A.O., Daroub S.H., Clark I.M., McGrath S.P., **Hirsch P.R**. & Triplett E.W. (2013)
***Ca*. Nitrososphaera and *Bradyrhizobium* are inversely correlated and related to agricultural practices in long-term field experiments**. *Frontiers in Microbiology* **4:**104. doi: 10.3389/fmicb.2013.00104.

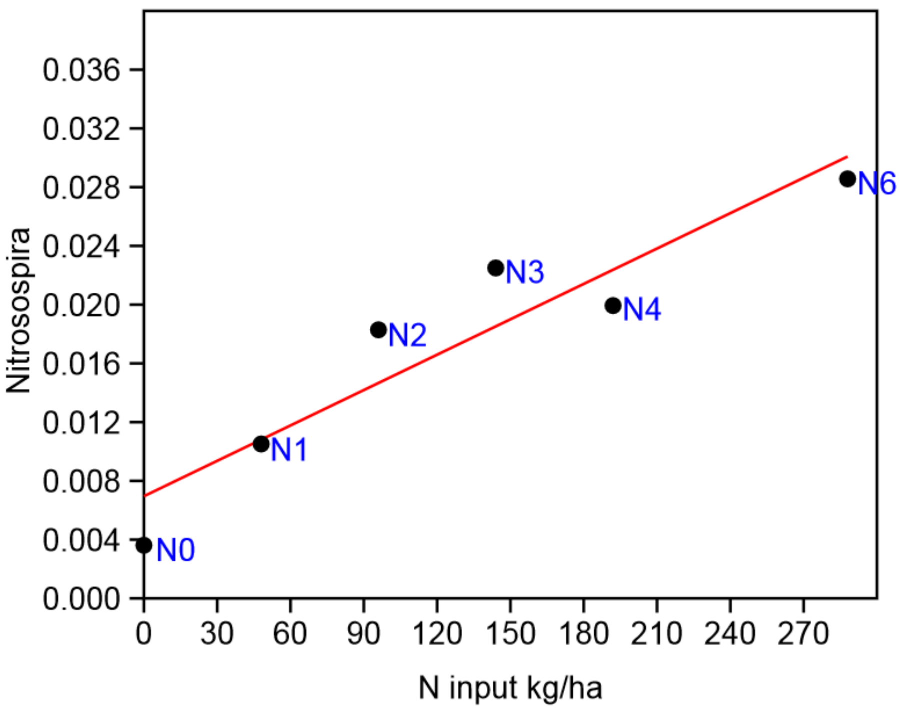
Broadbalk nitrifier responses to N

Principal archaeal nitrifier



r=-0.24, p=0.65

Principal bacterial nitrifier



r=0.94, *p*=0.005

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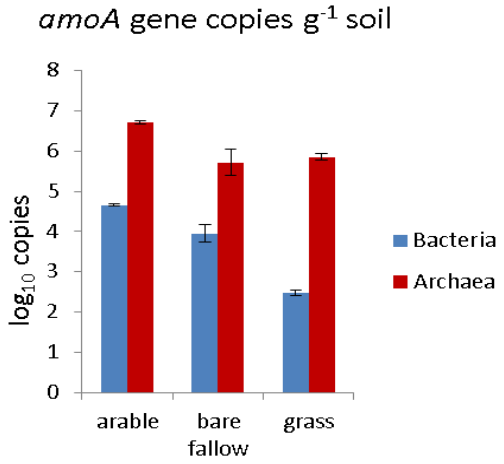
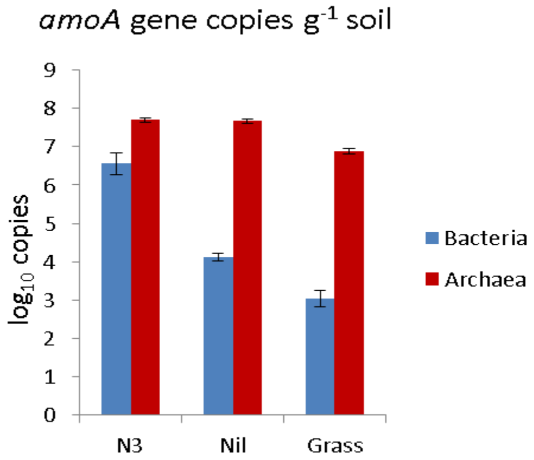
Rothamsted nitrifier communities

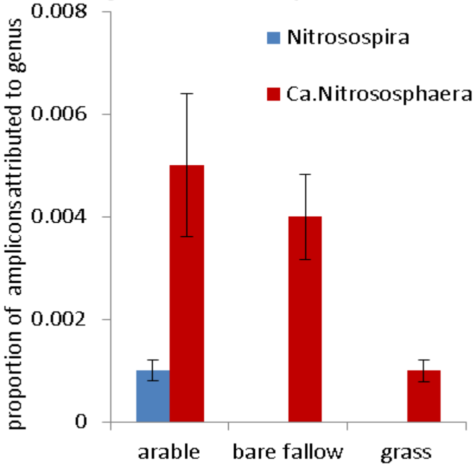
Highfield qPCR



Broadbalk qPCR

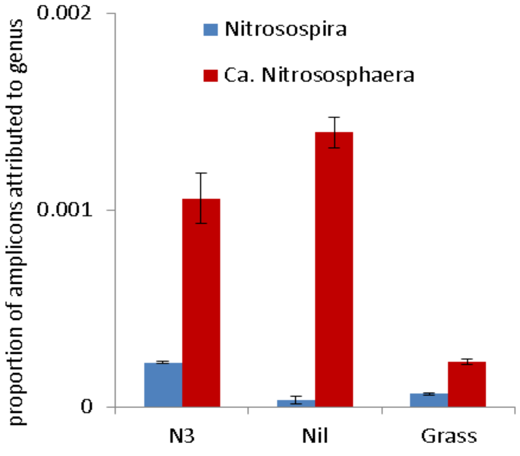


Highfield amplicons



analysed using QIIME

Broadbalk amplicons



analysed using Tax Collector/PANGEA

Functional gene analysis pipeline

Align reference proteins

Retrieve full-length DNA sequences – **reference tree**

Align backtranslated sequences

BLAST against metagenome/transcriptome

Place retrieved sequences on phylogenetic **reference tree**

Create blobs showing relative abundance

Functional gene analysis pipeline

Align reference proteins

Hidden Markov Model

Retrieve full-length DNA sequences – **reference tree**

Align backtranslated sequences

BLAST against metagenome/transcriptome

pplacer

Place retrieved sequences on phylogenetic **reference tree**

Create blobs showing relative abundance

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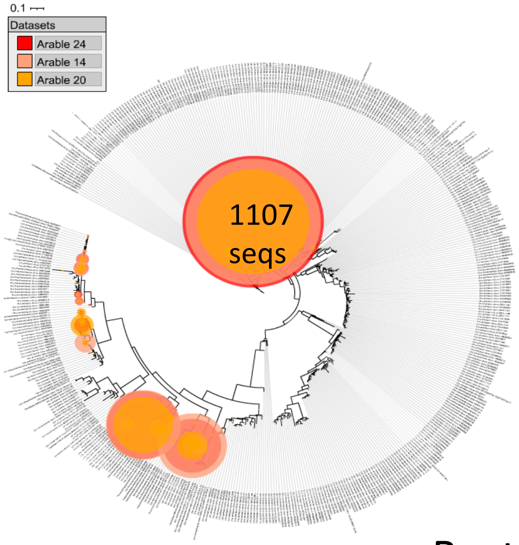
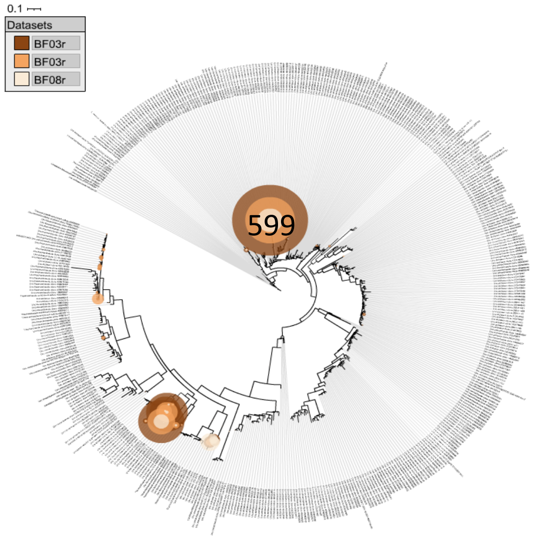
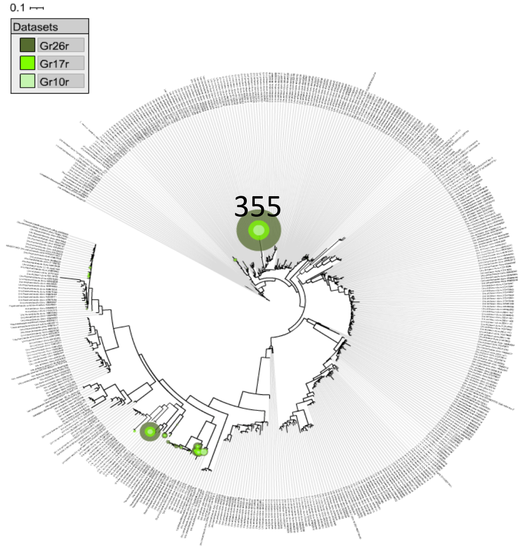
Output from pipeline

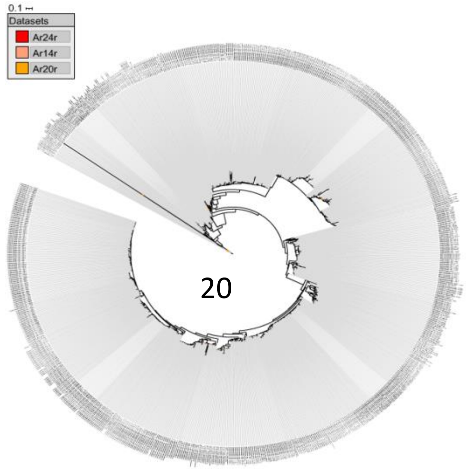
Archaeal transcripts for *amoA* 100 million transcripts per rep (3 reps x 3 treatments)

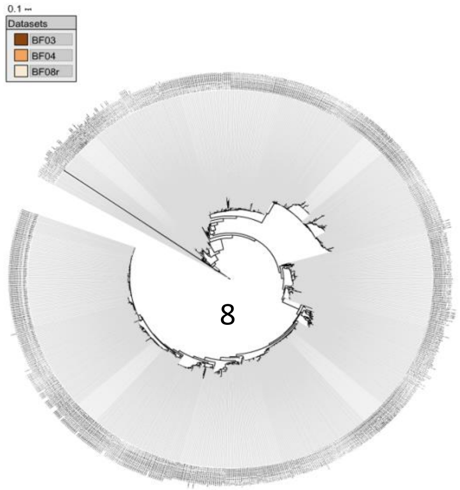


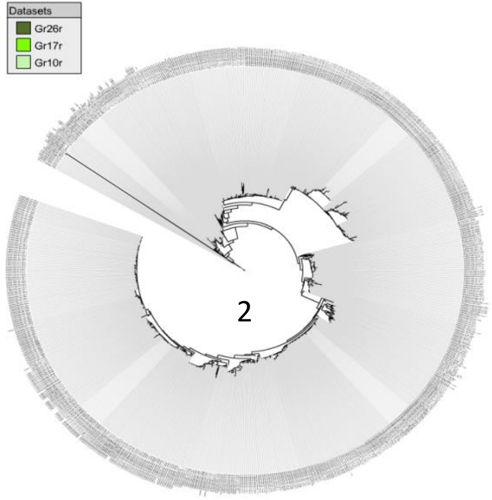




Bacterial transcripts for *amoA* 100 million transcripts per rep (3 reps x 3 treatments)







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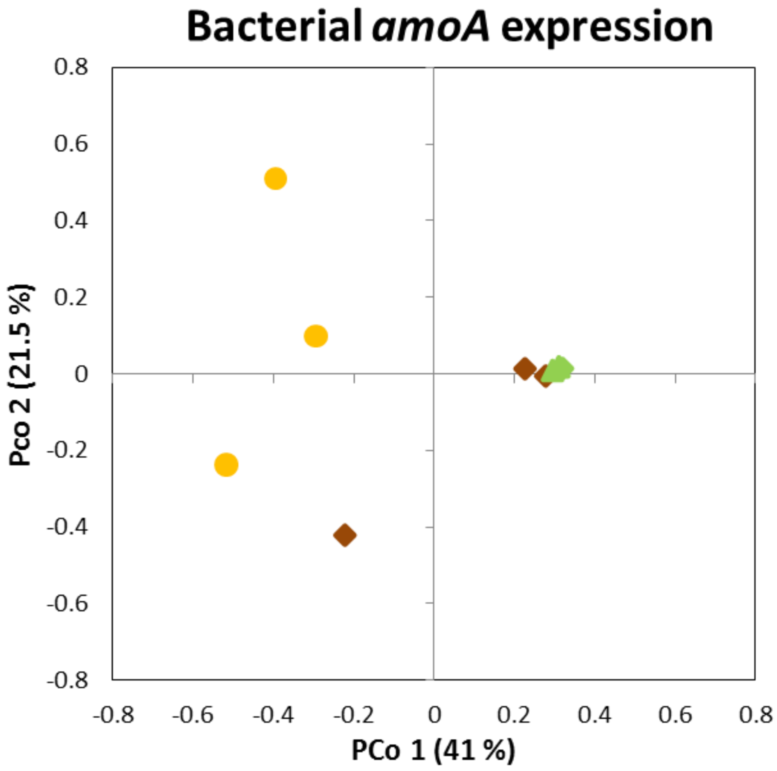
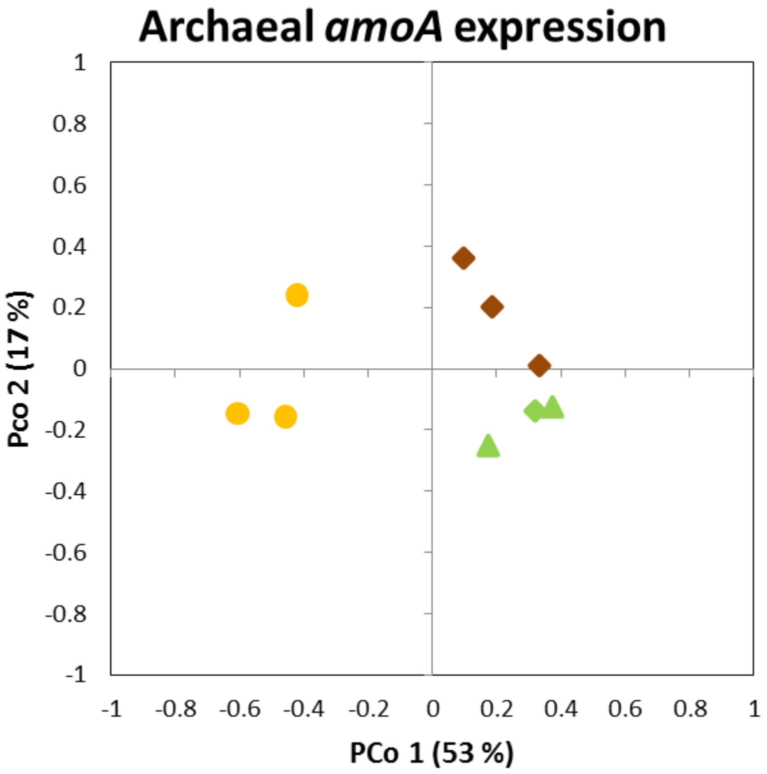
Nitrifier diversity on Highfield


Bacterial *amoA* expression


Archaeal *amoA* expression

Principal Coordinate Analysis similarity matrix of *amoA* "blobs" based on Euclidean distances using GenStat

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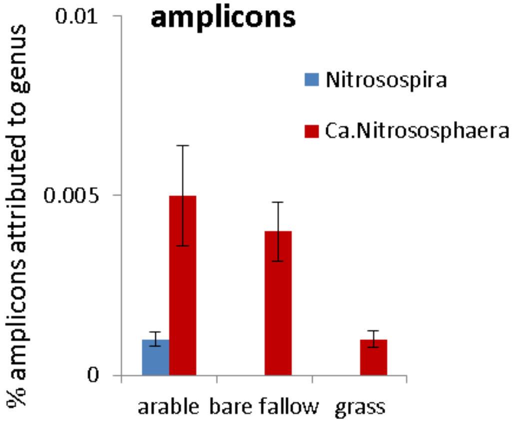
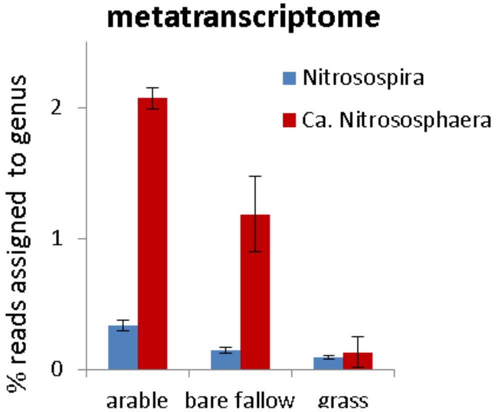
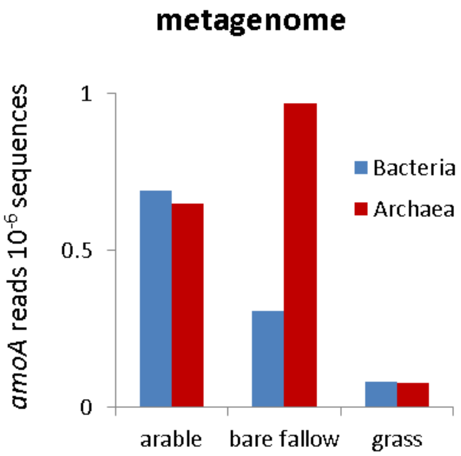
Highfield nitrifier communities

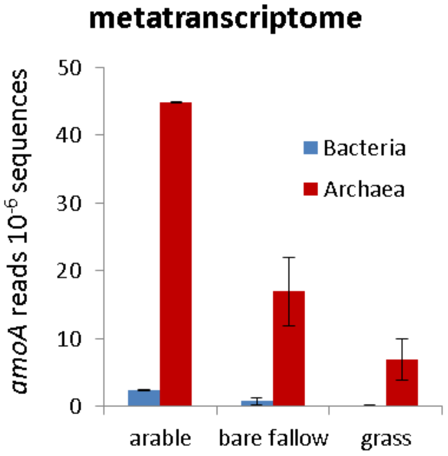


analysed using QIIME



analysed using MEGAN5 (David Swarbreck, TGAC)





analysed using new pipeline with bacterial and archaeal ammonia monooxygenase (*amoA*) reference tree

Thanks for listening!