Opening the black box of soil microbial communities

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Soil is…..

- A very thin skin over the land;
- A mix of minerals, organic matter, **organisms**, water and air;
- A precious resource for producing crops (provides air, water, nutrients and physical support to plants).
Soil is alive!

- Soil contains one of the most diverse groups of living organisms on Earth (a quarter of total biodiversity).
- A single gram of soil may contain billions (not millions) of bacteria and thousands of bacterial species.
- Bacterial biomass can be 1-2 tons per hectare in the surface soil.
Compositions of soil organisms

(a) Ectomycorrhizal fungi
(b) Decomposer fungi
(c) Bacteria
(d) Nematode
(e) Tardigrade
(f) Collembola
(g) Mite
(h) Enchytraeid worm
(i) Millipede
(j) Centipede
(k) Earthworm
(l) Ants
(m) Woodlouse
(n) Flatworm
(o) Mole
Human Population Growth

- Population Growth over Time

SOIL BIODIVERSITY IN NUMBERS

- 50 kilometers of mycelium 500’s species
- 100 billion 10,000 species
- 500 metres of roots 10’s species
- few species (e.g. mole)
- 100’s species
- 100 thousand 100’s species
- 10 thousand 50’s species
- 5 thousand 100’s species

### Comparison of microbial and plant biomass and nutrient elements (Unit: Pg)

<table>
<thead>
<tr>
<th></th>
<th>Microbes</th>
<th>Plants</th>
<th>M / P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total C</strong></td>
<td>350 – 545</td>
<td>562</td>
<td>≈ 1:1</td>
</tr>
<tr>
<td><strong>Total N</strong></td>
<td>85 – 130</td>
<td>10</td>
<td>&gt; 10:1</td>
</tr>
<tr>
<td><strong>Total P</strong></td>
<td>9 – 14</td>
<td>1.05</td>
<td>&gt; 10:1</td>
</tr>
</tbody>
</table>

- The total amount of prokaryotic carbon is 60–100% of the estimated total carbon in plants;
- The earth’s prokaryotic N and P are about 10-fold more of these nutrients than do plants, and represent the largest pool of these nutrients in living organisms.

Data source: Whitman et al., PNAS, 1998
➢ Soil microbial community has long been treated as a **black box**.
➢ Only ~ 1% soil microbes are culturable.
➢ Advances in molecular biology have made it possible to develop culture-independent techniques to investigate the structure, diversity and function of soil microbial communities.

*Internal behavior of the code is unknown*
Methods for microbial community analysis

- Culture-dependent methods
- Microbial biomass
- BIOLOG plate
- PLFA (Phospholipid Fatty Acid)
- Molecular methods
Culture-dependent methods:
- Isolation and enumeration of microbial cells on specific nutrient agars

Limitations:
- The exact growth-conditions are unknown:
  - Vitamins
  - Redox potential
- Bacteria grow very slow
- ‘Dormant cells’ do not multiply
- Some organisms cannot be cultivated → e.g. symbiosis

<table>
<thead>
<tr>
<th>Habitats</th>
<th>Cultivable (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water</td>
<td>0.25</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>1-15</td>
</tr>
<tr>
<td>Sediments/soils</td>
<td>0.25-0.3</td>
</tr>
</tbody>
</table>

Diagram:
- Enrichment culture or natural sample
- Dilution
- Growth
- No growth
Methods: 16S rRNA (amoA) gene analysis of microbial communities

1. Lysis, DNA/RNA extraction
2. DNA/RNA purification
3. PCR amplification
4. Cloning
5. Sequencing
6. Quantitation qPCR
7. Fingerprinting: DGGE, TGGE, RFLP, t-RFLP, SSCP
8. Identification
9. Phylogenetic analysis

Additional methods: SIP, Metagenomics, Transcriptomics, Proteomics... (Multi-omics)
➢ Who are there?
➢ How many are there?
➢ Why they are there?
➢ What are they doing?
➢ How will they change?
➢ ......
FastPrep® - Instrument for the lysis of microbial cell to isolate DNA & RNA from soil
Experimental site and treatments in Yarraman, QLD
**DNA Extraction. Pre-lysis washing procedure:** Washing the samples with the buffer solution to remove those easily co-extracting soil components

Humic substances amount (g kg\(^{-1}\) soil) (mean ± SD, \(n = 4\)) extracted by pre-lysis washing buffers from three soil samples of Yarraman natural forest (YNF) and the first (Y1R) and second (Y2R) rotation of hoop pine plantations

<table>
<thead>
<tr>
<th>Washing buffer</th>
<th>YNF</th>
<th>Y1R</th>
<th>Y2R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% sodium hexametaphosphate</td>
<td>0.93±0.03</td>
<td>0.95±0.05</td>
<td>0.90±0.03</td>
</tr>
<tr>
<td>0.1 M sodium phosphate</td>
<td>1.10±0.03</td>
<td>1.10±0.04</td>
<td>1.08±0.04</td>
</tr>
<tr>
<td>20 mM EDTA</td>
<td>1.28±0.08</td>
<td>1.31±0.10</td>
<td>1.25±0.06</td>
</tr>
</tbody>
</table>
PCR: Polymerase Chain Reaction

The exponential amplification of the gene in PCR

What are they doing?

Pentachlorophenol (PCP) is an organochlorine compound used as a pesticide and a disinfectant.
PCP degradation
RNA-SIP-DGGE analysis

Day 63

(courtesy Prof. Jim Prosser)
Case study example 1

➢ Soil microbial distribution pattern (biogeography) and temporal succession

Microbes on map?

Microbial biogeography: putting microorganisms on the map

Jennifer B. Hughes Martiny*, Brendan J.M. Bohannan†, James H. Brown§,
Matthew Kane**, Jennifer Adams Krumins††, Cheryl R. Kuske**, Peter J. Morin††,
Shahid Naeem***, Lise Øvreås****, Anna-Louise Reysenbach******, Val H. Smith†††,
and James T. Staley†††

Martiny et al. Nature Reviews Microbiology, 2006

➢ What is the distribution pattern of soil microbes? What factors are driving (governing) the pattern (ecological theories)?
➢ How do they respond to environmental factors (modelling and prediction)?
Soil microbial biogeography at national scales

The bacterial biogeography of British soils
Griffith et al., 2011, EM

Distribution and diversity of archaeal communities in select Chinese soils
Pei Cao, Zhi-Bin Zhang, Jian-Ping Shen, Guo-Xin Zhang, Hong-Ji Gao, & Jia-Qing He

Plateforme GénoSol

Exploring the microbial world to better protect our environment
Research strategy

Cold-temperate
Temperate
Warm-temperate
Subtropics
Tropics

Boreal forest
Temperate mixed coniferous forest
Temperate deciduous forest
Evergreen broadleaf forest
Rainforest

5×60 400m² plots and totally 300 soil samples
Community inventory on aboveground trees and herbs

Plotting

Identification

Census

<table>
<thead>
<tr>
<th>Species</th>
<th>plot1</th>
<th>plot2</th>
<th>plot3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species1</td>
<td>12</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Species2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Species3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Tree/herb community composition
Soil sampling and high-throughput sequencing on microbes

1. Surface soil sampling
2. Sieved to 1 mm
3. 4 °C transportation
4. DNA extraction

<table>
<thead>
<tr>
<th></th>
<th>plot1</th>
<th>plot2</th>
<th>plot3</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU1</td>
<td>925</td>
<td>1182</td>
<td>351</td>
</tr>
<tr>
<td>OTU2</td>
<td>204</td>
<td>226</td>
<td>488</td>
</tr>
<tr>
<td>OTU3</td>
<td>3762</td>
<td>160</td>
<td>956</td>
</tr>
</tbody>
</table>

Soil microbial community composition

Bioinformatics
Miseq sequencing
16S rDNA Amplification
Understory herbaceous plant composition (genus)

- Tropical forest
- Subtropical forest
- Temp. decid. forest
- Temp. mixed conif. forest
- Boreal forest
Soil microbial community composition—phylum level

(Wang et al, unpublished)
Latitudinal richness patterns of tree, herb and microbes (species counts)

Tree-Linear fit  $R^2 = 0.60, P < 0.001$

Herb-Quadratic fit  $R^2 = 0.27, P < 0.001$

Microbe-Quadratic fit  $R^2 = 0.21, P < 0.001$

Wang et al., 2015
Climate drives the plant distribution but not microbes; soil pH drives herb and microbial diversity.
Palaeoclimate explains a unique proportion of the global variation in soil bacterial communities

- **Bacterial composition**
  - Global drylands
  - Americas
  - Australia
  - New South Wales

- **Bacterial richness**
  - Palaeoclimate
  - Current climate
  - Environmental drivers
  - Palaeoclimate + environmental drivers
  - Other shared variance

**Variation explained (%)**
Ecosystems and biomes across China

Mt Daxinganling  Mt Changbai  Mt Shengnongjia

Tibetan Plateau  Tian Shan

Hulunbeier
Sino Soil Biome (SSB): Archives

Soil sample Archive

Genomic DNA Archive

260/280
The different symbols represent different species of Eucalyptus based on the database of AVH.

Sampling
12-31 May 2019
by Hangwei Hu, Qinglin Chen, Zhenzhen Yan, Chaoyu Li
➢ Soil microbial (resources) distribution patterns and driving factors
➢ Sync/co-evolution of soil and plant microbiomes (antibiotic resistance genes), indigenous plants (crops) rhizosphere and bulk soil microbiomes/survival mechanisms—breeding, synthetic microbial ecology, soil remediation.
Welcome to the ISME18 website!

ISME18 is the 18th edition of our symposium which takes place every two years. The conference is the front runner in the field of microbial ecology, with an average of around 1,750 international scientists that attend the conference.

Session Chair and Invited Keynote Speaker:
Global and Continental Biogeography, ISME 18

Invited Keynote Speaker: The 9th International Conference on Geochemistry in the Tropics & Sub-Tropics, 28-31 July 2019, Gold Coast, QLD

Grants and Paper reviewers in the field of microbial biogeography

Impact Factor: 9.520
Journal Rank: 3/160 Ecology; 9/126 Microbiology
The nitrogen cycle contains four main steps, i.e. biological nitrogen fixation, ammonification, nitrification and denitrification, all of which are mainly driven by microorganisms.
**Nitrification**

- Central role in global nitrogen cycle
- Loss of ammonia-based fertilisers
- Nitrate pollution
- N removal in wastewater treatment
- Production of greenhouse gases, N2O
- ….
For over a century, ammonia oxidation was long thought to be performed exclusively by bacterial ammonia oxidizers (AOB). However, acidic soil was generally considered detrimental to AOB. It is not known how is ammonia oxidized in the acidic soil.
Soil nitrification rates

Nitrofication potential
0.051 ~ 0.804 mg N kg\(^{-1}\) soil day\(^{-1}\)

Soil pH: 4.3 ~ 5.5

Booth et al. (2005) Ecol Monogr 75

Zhang J et al. (2011) Plant Soil 342
Ammonia oxidation is the first step in nitrification, a key process in the global nitrogen cycle that results in the formation of nitrate through microbial activity\(^1\). The increase in nitrate availability in soils is important for plant nutrition, but it also has considerable impact on groundwater pollution owing to leaching. Here we show that archaeal ammonia oxidizers are more abundant in soils than their well-known bacterial counterparts. We investigated the abundance of the gene encoding a subunit of the key enzyme

Ammonia oxidizing archaea (AOA)

Isolation of an autotrophic ammonia-oxidizing marine archaeon

Martin Könneke\(^{1*}\), Anne E. Bernhard\(^{1*}\), José R. de la Torre\(^{1*}\), Christopher B. Walker\(^1\), John B. Waterbury\(^2\) & David A. Stahl\(^1\)

LETTERS

Archaea predominate among ammonia-oxidizing prokaryotes in soils

S. Leininger\(^3\), T. Urich\(^1\), M. Schlote\(^1\), L. Schwark\(^3\), J. Qi\(^4\), G. W. Nicol\(^5\), J. I. Prosser\(^5\), S. C. Schuster\(^4\) & C. Schleper\(^1\)
Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices

Ji-zheng He,¹† Ju-pei Shen,¹,²† Li-mei Zhang,¹† Yong-guan Zhu,¹ Yuan-ming Zheng,¹ Ming-gang Xu³ and Hongjie Di⁴

➔Quantification of target DNA by measuring fluorescence in the log-linear phase (kinetic quantification).
Table 1. Chemical properties and potential nitrification rates (PNR) of a Chinese upland red soil under different fertilizer treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH (H₂O)</th>
<th>Organic matter (g kg⁻¹)</th>
<th>NO₃⁻-N (mg kg⁻¹)</th>
<th>PNR μg NO₃⁻-N g⁻¹ dry soil h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK₀⁶</td>
<td>5.78 ± 0.16dᵇ</td>
<td>13.7 ± 5.5a</td>
<td>0.67 ± 0.42a</td>
<td>2.07 ± 0.28b</td>
</tr>
<tr>
<td>CK</td>
<td>5.47 ± 0.18b</td>
<td>13.6 ± 0.6a</td>
<td>5.05 ± 1.54a</td>
<td>1.49 ± 0.05ab</td>
</tr>
<tr>
<td>N</td>
<td>3.71 ± 0.15a</td>
<td>15.2 ± 2.9a</td>
<td>43.6 ± 7.7d</td>
<td>0.47 ± 0.05a</td>
</tr>
<tr>
<td>NP</td>
<td>3.95 ± 0.11a</td>
<td>16.4 ± 0.9a</td>
<td>21.5 ± 4.1b</td>
<td>0.43 ± 0.03a</td>
</tr>
<tr>
<td>NK</td>
<td>3.76 ± 0.12a</td>
<td>14.3 ± 0.5a</td>
<td>35.2 ± 4.0c</td>
<td>0.99 ± 0.06a</td>
</tr>
<tr>
<td>PK</td>
<td>5.03 ± 0.14c</td>
<td>14.6 ± 0.7a</td>
<td>6.03 ± 1.19a</td>
<td>0.30 ± 0.03a</td>
</tr>
<tr>
<td>NPK</td>
<td>4.04 ± 0.06a</td>
<td>16.9 ± 0.8a</td>
<td>8.66 ± 2.70a</td>
<td>0.26 ± 0.02a</td>
</tr>
<tr>
<td>NPK + OM</td>
<td>5.81 ± 0.15d</td>
<td>21.3 ± 0.8b</td>
<td>18.7 ± 4.0b</td>
<td>4.41 ± 0.29c</td>
</tr>
</tbody>
</table>

a. Treatment: Fallow (CK₀), Control without fertilizers (CK), and with fertilizers N, NP, NK, PK, NPK, NPK + OM.
b. Mean ± SD (n = 4). Values within the same column followed by the same letter do not differ at P < 0.05.
Abundances of archaeal and bacterial amoA genes

Ratio of AOA to AOB

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CK0</th>
<th>CK</th>
<th>N</th>
<th>NP</th>
<th>NK</th>
<th>PK</th>
<th>NPK</th>
<th>NPK+OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.47</td>
</tr>
<tr>
<td>AOA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.87</td>
<td>1.42</td>
</tr>
</tbody>
</table>

r=0.835**
Potential ammonia oxidation (nmol N g$^{-1}$ dry soil h$^{-1}$)

PNR with AOB  $r = 0.91^{**}$
PNR with AOA   $r = 0.87^{**}$
Both AOA and AOB may have contributed to soil nitrification in these acidic soils. AOA community structure changed dramatically under different treatments.
**Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam**

Ju-pei Shen,1,2 Li-mei Zhang,1 Yong-guan Zhu,1 Jia-bao Zhang,3 Ji-zheng He1*

was observed among different treatments. Phylogenetic analysis showed a dominance of *Nitrosospira*-
Correlations of AOB (AOA) abundance and potential nitrification rates

AOB:  \( r = 0.773, n=7, P <0.05 \) *

AOA:  \( r = 0.331, n=7, P >0.05 \)

AOB play predominant role in these alkaline sandy loams, although AOA are more abundant than AOB.
Establishing SIP method for AOA and AOB investigation

$^{13}$C- Stable-isotope probing (SIP)

$^{12}$CO$_2$  $^{13}$CO$_2$

DNA extraction

CsCl density centrifugation

$^{12}$C-DNA/RNA

$^{13}$C-DNA/RNA

Molecular analysis: TRFLP, PCR-DGGE, Sequencing
Groundwater pollution
Fertiliser loss

Decomposing organic matter
Animal excreta
Fertiliser N

Ammonia oxidisers

\[ \text{NH}_3 + \text{O}_2 + 2 \, e^- + 2 \, \text{H}^+ \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O} \]

\[ \text{NH}_2\text{OH} + \text{H}_2\text{O} + \frac{1}{2} \, \text{O}_2 \rightarrow \text{NO}_2^- + 2 \, \text{H}_2\text{O} + \text{H}^+ \]

Sum: \[ \text{NH}_3 + \frac{1}{2} \, \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \]

\[ \Delta G^0 = -275 \, \text{kJ/reaction} \]

Nitrosomonas europaea

Nitrite oxidisers

\[ \text{NO}_2^- \rightarrow \text{NO}_3^- \]

Nitrobacter winogradskyi

Nitrooxidisers

\[ \text{NO}_3^- \rightarrow \text{CO}_2 \]

Leaching
Denitrification

Groundwater pollution
Fertiliser loss
Abundance of amoA gene in CsCl density gradients after $^{12}$C- /$^{13}$C-CO$_2$ incubation

AOA

$^{12}$C incubation
$^{13}$C incubation

AOB

$^{12}$C incubation
$^{13}$C incubation
AOA/AOB amoA DGGE patterns under CsCl densities

AOA could grow by fixing $^{13}$C-CO$_2$ and metabolizing NH$_3$, driving nitrification in the soil; for the first time, this study provided direct evidence of AOA autotrophic ammonia oxidation.

Zhang et al. 2010, PNAS, 107, 17240-17245.
<table>
<thead>
<tr>
<th>Location</th>
<th>pH (H₂O)</th>
<th>pH (KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HZ</td>
<td>4.20</td>
<td>3.29</td>
</tr>
<tr>
<td>YH</td>
<td>4.21</td>
<td>3.34</td>
</tr>
<tr>
<td>QJ</td>
<td>4.36</td>
<td>3.66</td>
</tr>
<tr>
<td>QY</td>
<td>4.43</td>
<td>3.87</td>
</tr>
<tr>
<td>TY</td>
<td>4.47</td>
<td>3.89</td>
</tr>
</tbody>
</table>
Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils

Li-Mei Zhang¹,³, Hang-Wei Hu¹,²,³, Ju-Pei Shen¹ and Ji-Zheng He¹

¹State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China and ²Graduate School, Chinese Academy of Sciences, Beijing, China

Increasing evidence demonstrated the involvement of ammonia-oxidizing archaea (AOA) in the global nitrogen cycle, but the relative contributions of AOA and ammonia-oxidizing bacteria (AOB) to ammonia oxidation are still in debate. Previous studies suggest that AOA would be more adapted to ammonia-limited oligotrophic conditions, which seems to be favored by protonation of ammonia, turning into ammonium in low-pH environments. Here, we investigated the autotrophic nitrification activity of AOA and AOB in five strongly acidic soils (pH < 4.50) during microcosm incubation for 30 days. Significantly positive correlations between nitrate concentration and amoA gene
Abundances of amoA genes from comammox Nitrospira, AOA, and AOB, and nxrB gene of NOB in 300 forest soil samples with soil pH ranging from 4.0~8.6. Quantitative PCR analysis of comammox amoA clade A and clade B was performed using the primer sets and thermal-cycling conditions as described previously.

Hu & He, 2017
**Title**
Comammox *Nitrospira* play an active role in nitrification of eutrophic agricultural soils

**Manuscript Type**
Biological Sciences - Letter

**Corresponding Author**
Ji-Zheng He (The University of Melbourne)

**Contributing Authors**
Chaoyu Li, Hang-Wei Hu, Deli Chen

**Abstract**
The recent discovery of complete ammonia oxidizers (comammox *Nitrospira*) challenged the paradigm of the stepwise nitrification processes mediated by two distinct groups of nitrifiers, and raised fundamental questions regarding their niche specialization and relative contribution to nitrification in agricultural soils which are often nitrogen rich. Physiological studies suggest that comammox *Nitrospira* have an oligotrophic lifestyle and would outcompete canonical ammonia oxidizers (ammonia-oxidising bacteria and ammonia-oxidising archaea) under ammonia-limited conditions. We used $^{13}$CO$_2$-DNA-stable isotope probing and molecular approaches demonstrated, for the first time, that comammox *Nitrospira* clade A were significantly more abundant than canonical ammonia oxidizers and $^{13}$CO$_2$ was incorporated into comammox *Nitrospira* clade A, evidently showing the active contribution of comammox *Nitrospira* to the soil nitrification. Phylogenetic analysis of comammox amoA gene revealed that active $^{13}$CO$_2$-labeled comammox *Nitrospira* clade A belonged to *Nitrospira inopinata*-related cluster and a new cluster separated from the known comammox isolates. These results challenged the perceived oligotrophic lifestyle of comammox *Nitrospira* and demonstrated their important role in autotrophic ammonia oxidation in agricultural soils amended with nitrogen fertilizers. There is a substantial contribution of comammox *Nitrospira* to soil nitrification, which calls re-evaluation of the microbial nitrogen cycling processes and the subsequent impacts upon agriculture and the environment.

**Subject Terms**
Earth and environmental sciences/Biogeochemistry/Element cycles
Biological sciences/Microbiology/Applied microbiology
➢ >50% applied N lost to environment;
➢ Nitrification process accelerates soil acidification;
➢ We need to develop measures to manipulate nitrification microbes

ARC DP & LP working on the processes


A single gram of soil may contain billions of bacteria & archaea. However, only ~ 1% soil microbes are culturable under laboratory conditions.

Molecular biological methods based on microbial DNA/RNA play a key role in understanding the soil microbial diversity and functions.

The basic procedures of molecular soil microbial analyses include microbial DNA extraction, PCR amplification, finger-printing profiling and sequencing etc.

Soil microbial ecology study plays an essential role in understanding soil processes and soil sustainable management.
Acknowledgements

➢ Funding agencies: UoM, Australian funding agencies (ARC, ACIAR, ACJRF, CRCp), Chinese funding agencies (NSFC, MoST, CAS), and industry partners.

➢ My collaborators and students.