Microbial assemblages of post-mining soils on Christmas Island: beneficial microbes for agricultural production

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Outline

- Why agriculture following mining?
- Christmas Island
- Research questions
- Two studies in brief
  - Methods
  - Results & Conclusions
- Related research
- Next steps
- Take home messages

Transitioning from mining to agriculture

Globally, the use of degraded habitats for agricultural production:

- increasingly practical
- economically viable
- alternative to clearing pristine environments
- alternative target for rehabilitation (non-analogue systems)

Advantages for agriculture following mining

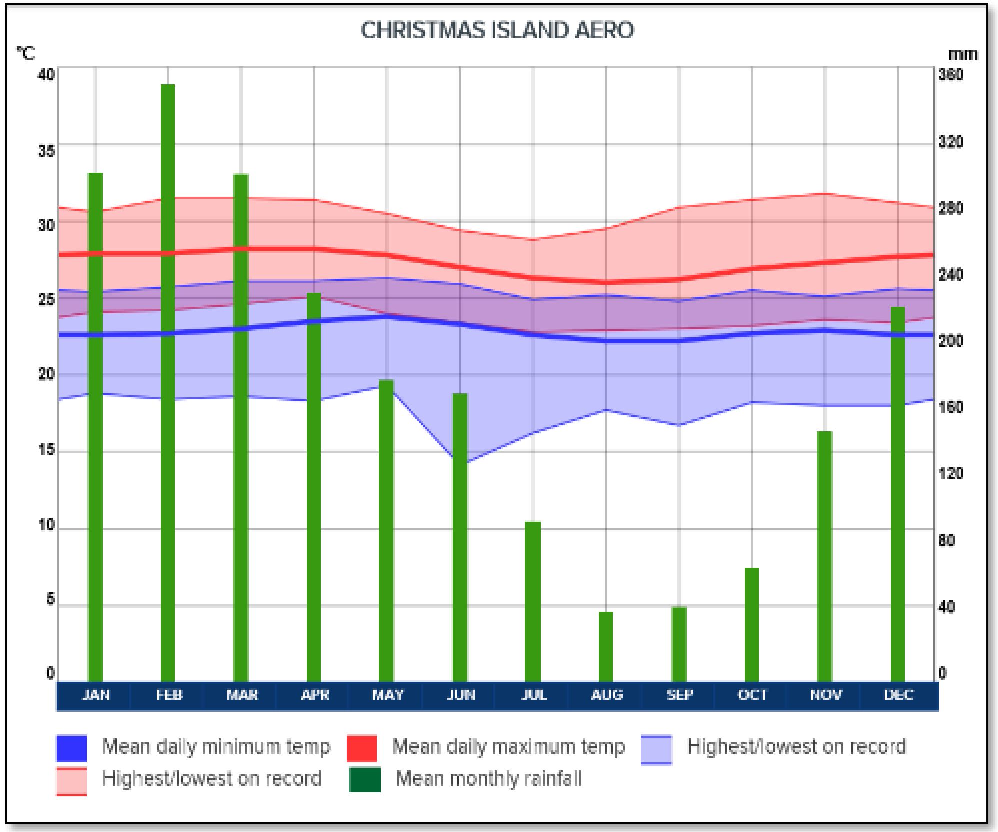
- availability of labour and machinery
- need for food and animal feed beyond mine closure
- need for employment beyond mine closure
- social responsibility

These industries could collaborate more often…

Christmas Island

- Unique opportunity to research transition from mining to agriculture
- Leading industry & main employer: rock phosphate mining (deplete by ~2030)
- No history of large-scale agriculture
- There is critical need to:
    - Provide on-going employment for the community
    - Find alternative industries
    - Increase food security - heavy reliance on airfreighted produce

About Christmas Island



- ~10º S, 2600km NW of Perth, 360km S of Indonesia
- 135 km^2
- Phosphate rich volcanic soils over limestone
- ~15% phosphate mining
- ~60% National Park
- Tropical climate: 80-90% humidity, 22-28ºC, ppt ~2000mm/yr (2016:~5000mm)
- 1897: CI Phosphate Company
- ~2000 people

Christmas Island Red Crab
Gecarcoidea natalis

- Vegetation: rainforest, scrub forest
- High conservation value
- High level of endemism
- Many threatened species
- Major seabird colonies

Challenges for post-mining agriculture

Post-mining substrates often have abiotic and biotic challenges for plant growth, including:

- soil erosion
- altered hydrology
- poor fertility (e.g. low OC, K, N)
- heavy metals
- lack of beneficial microbes

Microbial diversity of post-phosphate mining sites on Christmas Island

- What microbes are present in mined soils?
- Are root-associated microbes available to potential crop plants?
  - Will endemic rhizobia form associations with crop legumes?

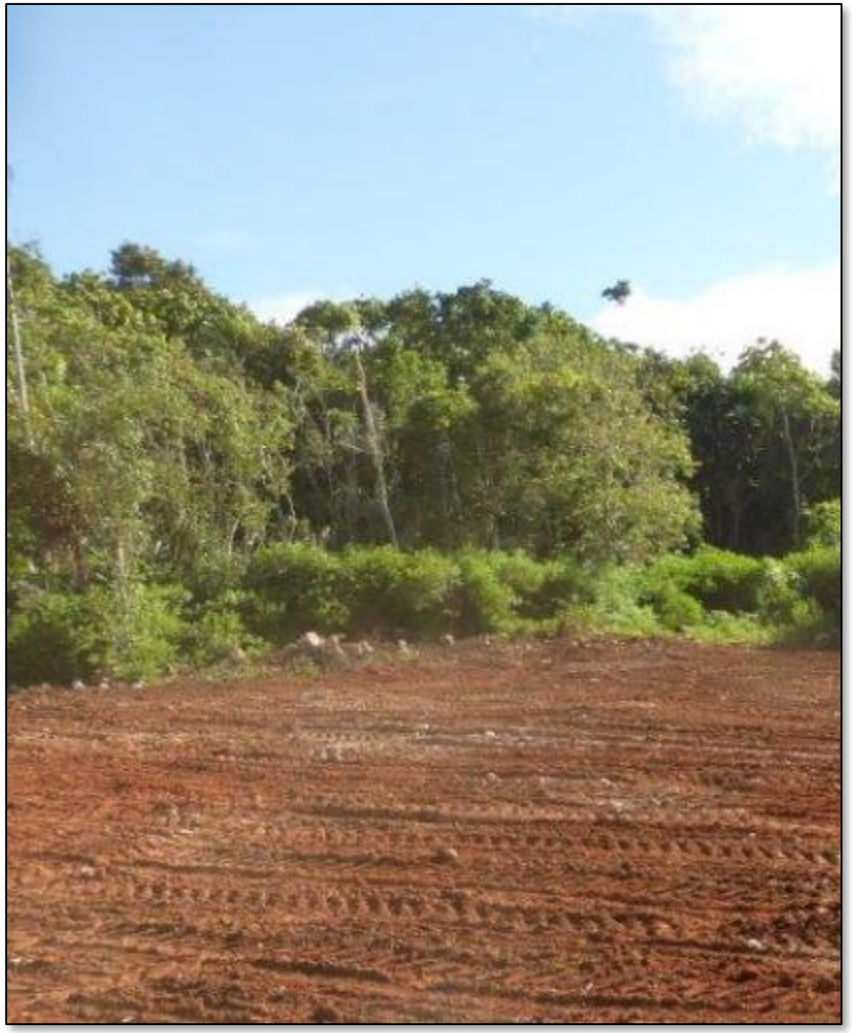
Study approach

1. What microbes are present in mine soils?

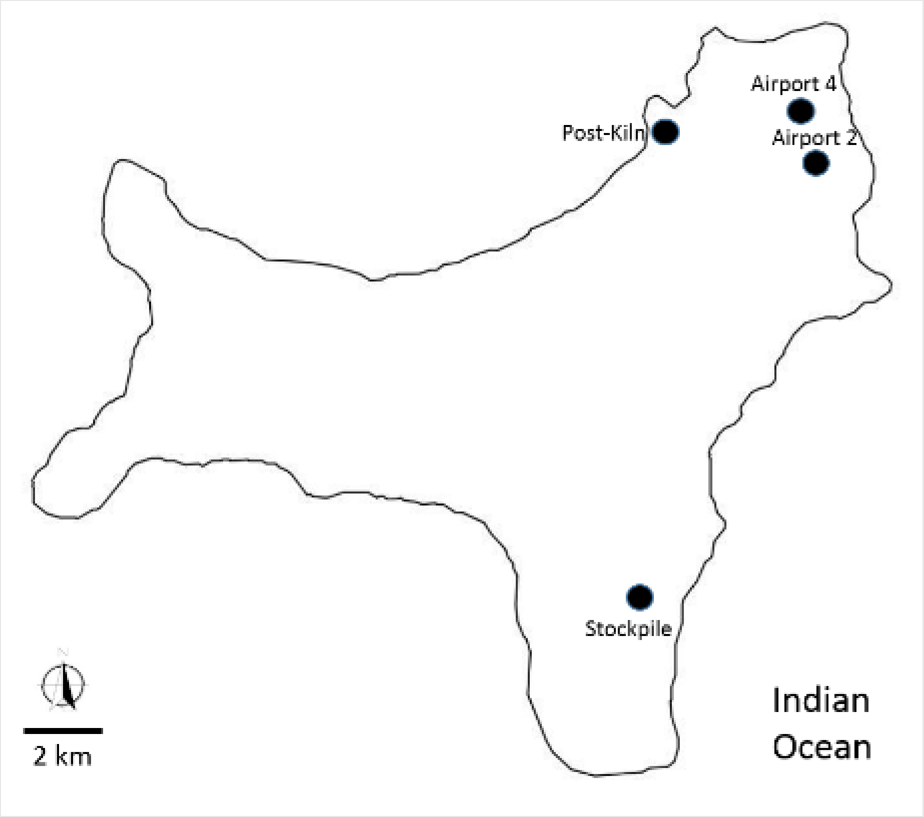
   Rhizomicrobiome
   - Isolate microbes (bacteria, archaea, fungi) from the rhizosphere of 'bait' crop plants

2. Will endemic rhizobia form associations with crop and introduced legumes

   Rhizobia
   - Collect root nodules from uninoculated legume crops on the island, isolate & culture rhizobial bacteria
   - Assess capability of these bacteria to enter symbiosis with crop legumes



1. Rhizomicrobiome Soil sampling sites



Site	Mine status	Land use
Airport2	Post-mine	Arable
Airport4	Not mined	Arable
Airport4 centre	Not mined	Arable
Airport4 edge	Not mined	Arable
Post kiln	Post-mine	Not arable
Stockpile1	Post-mine	Not arable
Stockpile2	Post-mine	Not arable

Mined = removal of 1–5 m of rock phosphate soil, return of low grade soil to 1 m

Arable = contoured, ripped, planted with trial crop plants

1. Rhizomicrobiome

Methods

Glasshouse: trapping root-associated microbes

- Christmas Island soil layered between sterilised washed river sand
- **Corn** (*Zea mays*) & **Siratro** (*Macroptilium atropurpureum)*
- Fine roots and adhering soil sampled at 12 weeks

- Isolated **bacterial (16S)**, **archaeal (18S)** and **fungal (ITS)**
- Illumina MiSeq

- Sequence quality control & 97% OTU clustering – USEARCH (Edgar 2010)
- OTU community richness & composition – R ‘vegan’ (Oksanen et al. 2019) , PAST (Hammer et al 2001)

1. Rhizomicrobiome

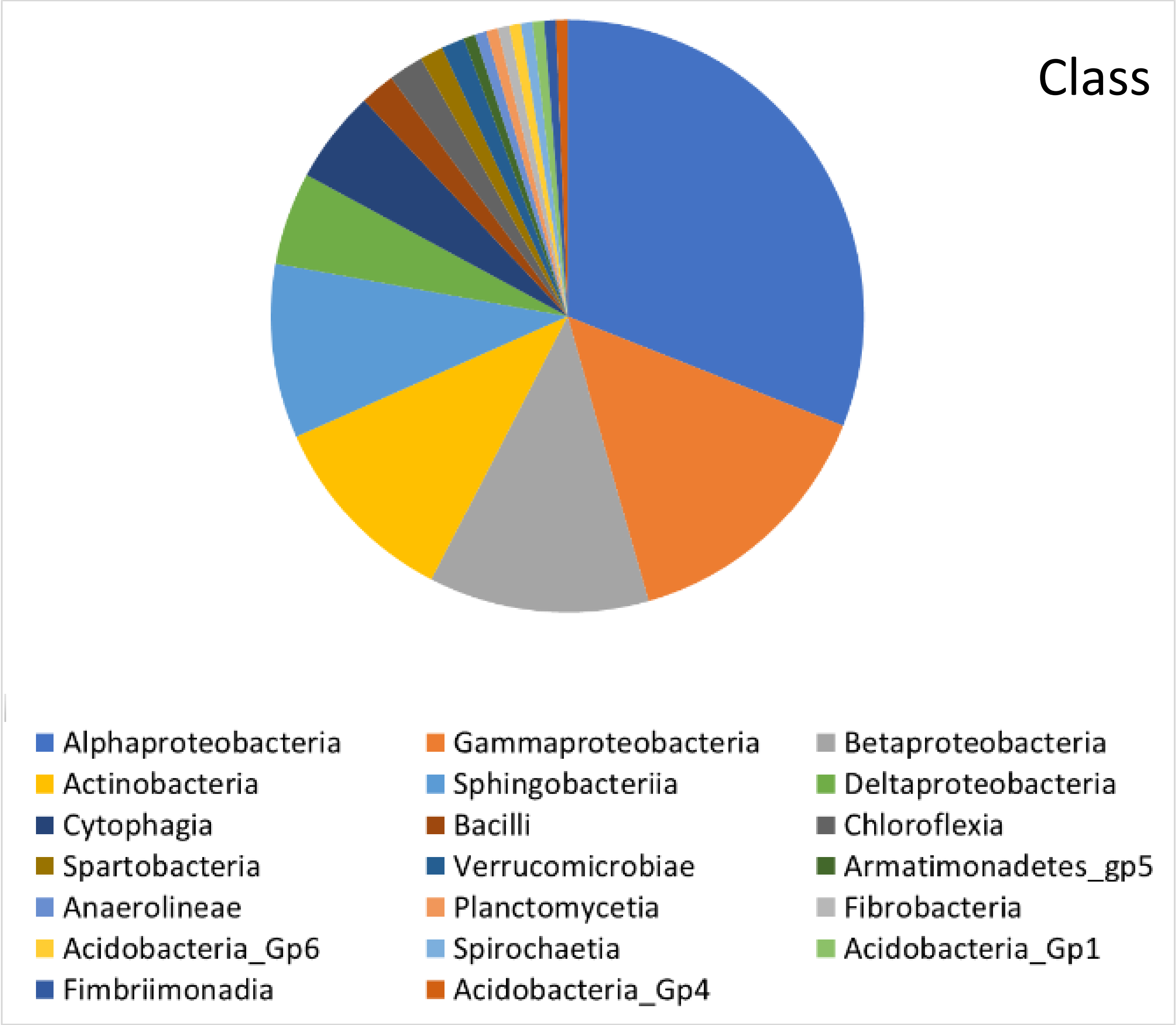
Results

OTUs – overall richness

Corn

16S

24 Samples
276 OTUs



1. Rhizomicrobiome

Results

Community richness & composition

- OTU Richness

ANOVA – Site, Mining status, Land use
NSD at α 0.05

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Results

Community richness & composition

- OTU Richness

ANOVA – Site, Mining status, Land use

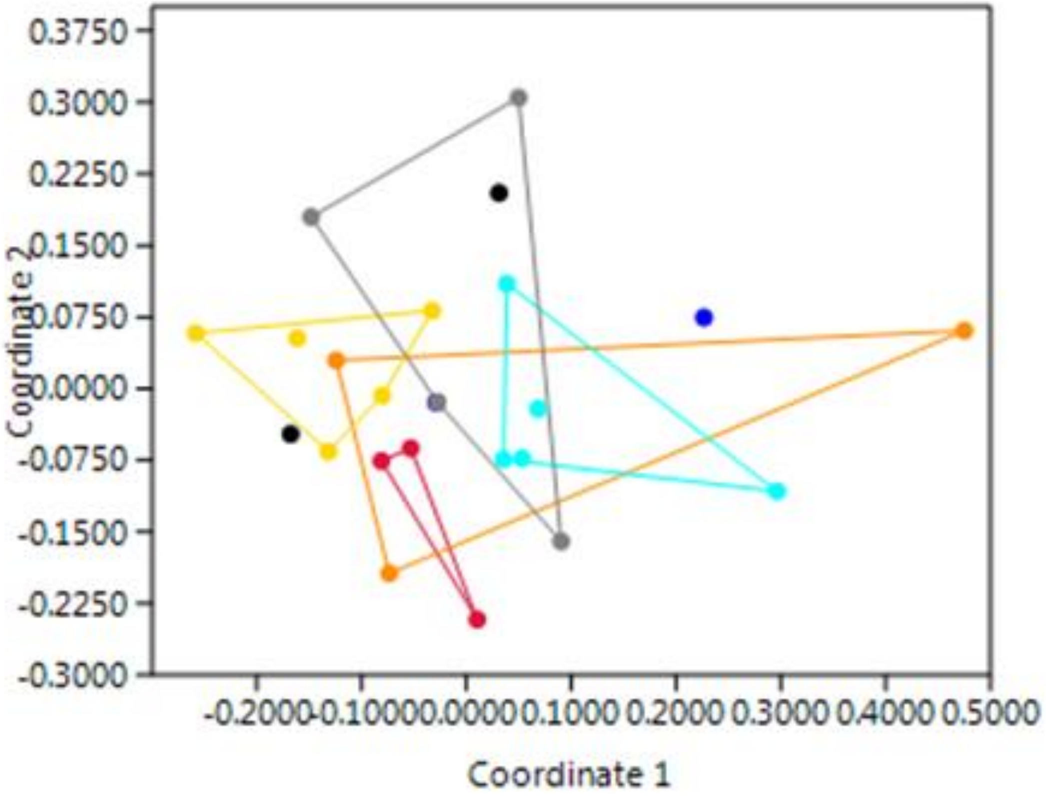
NSD at α 0.05

- Community composition (presence/absence)

PERMANOVA

Site: pseudo $F = 1.135$, $p = 0.0612$

nMDS - Site



1. Rhizomicrobiome

Results

Community richness & composition

- OTU Richness

ANOVA – Site, Mining status, Land use

NSD at α 0.05

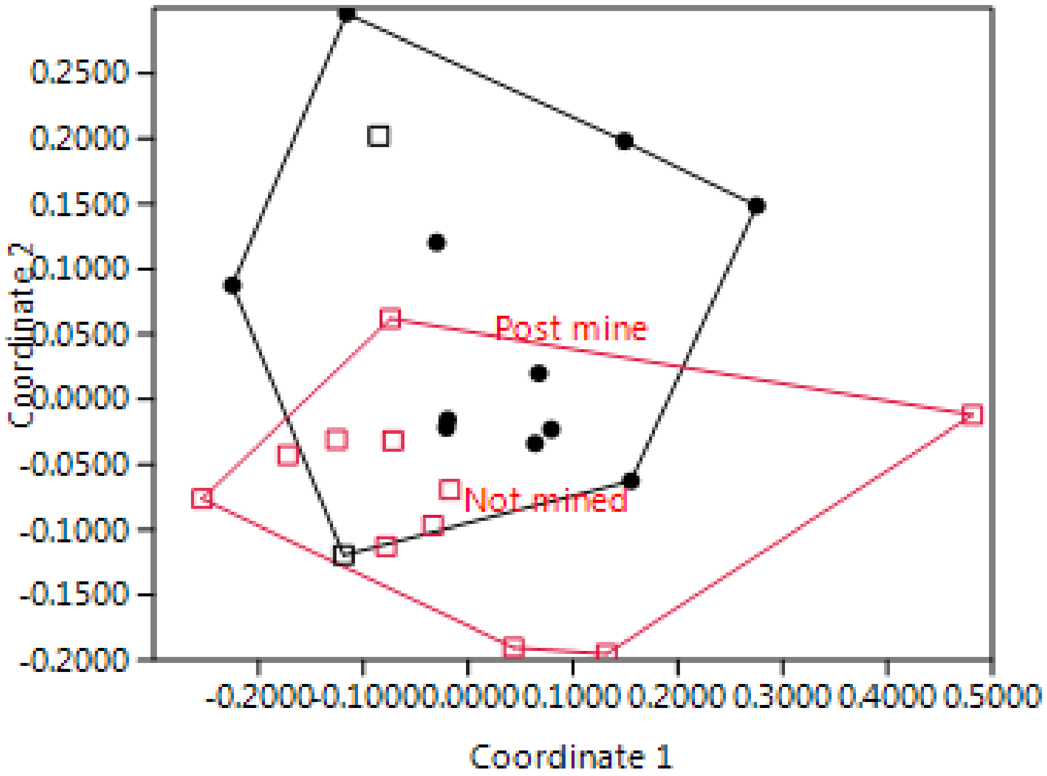
- Community composition (presence/absence)

PERMANOVA

Site: pseudo F = 1.135, p = 0.0612

Mining status: pseudo F = 1.513, p = 0.0074*

nMDS – Mining status



1. Rhizomicrobiome

Results

Community richness & composition

- OTU Richness

ANOVA – Site, Mining status, Land use

NSD at α 0.05

- Community composition (presence/absence)

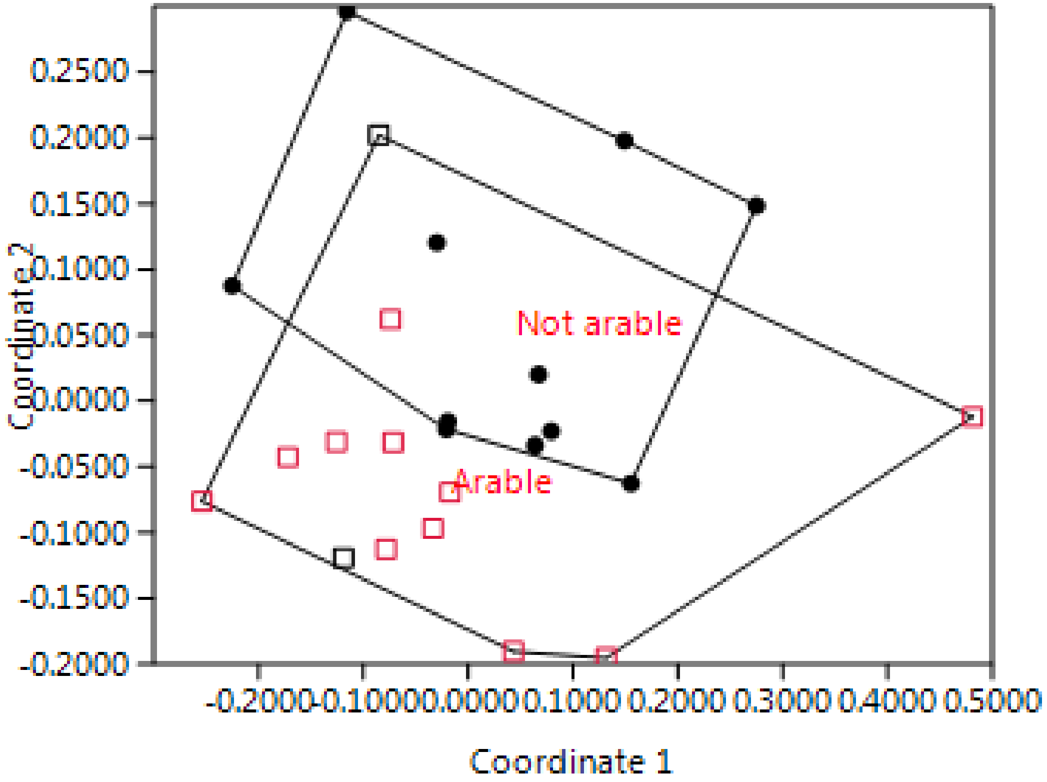
PERMANOVA

Site: pseudo F = 1.135, p = 0.0612

Mining status: pseudo F = 1.513, p = 0.0074*

Land use: pseudo F = 1.436, p = 0.0155*

nMDS – Land use



1. Rhizomicrobiome

Results

OTUs of interest

Potential plant growth promoting rhizobacteria (PGPR):

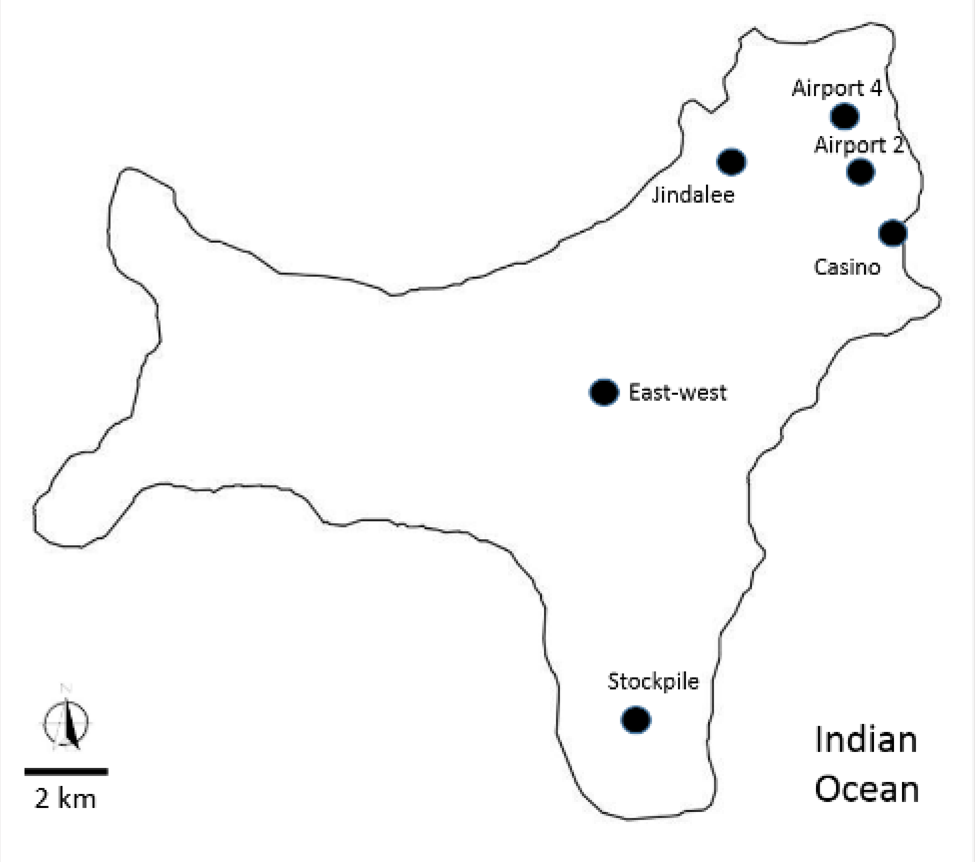
	Phosphate solubilizing	N fixing symbionts	N fixing Free living	Disease resistance-inducing
Rhizobium	x			
Cupriavidus	x	x		
Pseudomonas	x			
Methylobacterium		x		
Devosia			x	
Bacillus				x
Massillia				x
Bosea		x		

Post Kiln and Stockpile

Potential pathogens:

Rickettsia

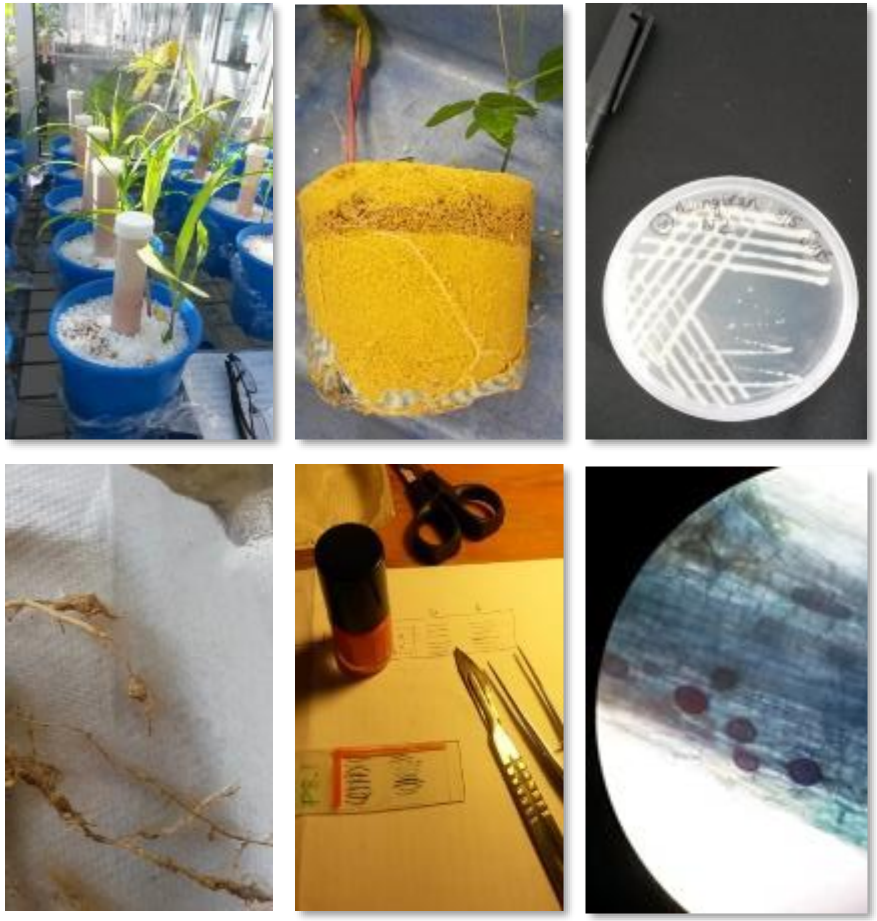
2. Rhizobia



Root nodule sampling & analysis:

- Nodules from 9 different host plants
  - Invasive legumes - *Indigofera hirsuta, Leucaena leucocephala*, *Mimosa pudica*, *M. invisa* and siratro (*M. atropurpureum*).
  - Introduced legume (uninoculated crop) - cowpea (*Vigna unguiculata*), mungbean (*V. radiata*), lablab (*L. purpureus*), and peanut (*A. hypogaea*)
- Surface-sterilized
- Isolate & culture bacteria
- Sequence 16S, recA, nodA, nodC, nifH
- Gene phylogenies

Methods



Glasshouse:
Authentication experiments

- Christmas Island soil layered between sterilised washed river sand
- **cowpea**, **siratro** and ***Mimosa pudica***
- Root nodules sampled at 8 weeks
- Presence/absence & type of nodules

2. Rhizobia

Results & Conclusions

- Diverse naturalised rhizobia, *Agrobacterium, Bradyrhizobium, Cupriavidus, Ensifer, Methylobacterium* and *Rhizobium*
- Rhizobia present & available to nodulate with cow pea, mung bean, peanut lablab
- Bean, soybean, chickpea failed to nodulate - inoculation of crops will be necessary
- Evidence of recent colonisation by rhizobia from nearby regions & evolution on the island – niches created by mining

(De Meyer et al 2018)

Related research

- Nutrient trials – cereal and legume crops (Ruthrof et al 2018; Ruthrof et al 2018)
  - K is critical for legumes, N is critical for cereals
  - K addition ↑ legume biomass and ↓ heavy metal (leaf) concentrations
  - N will need to be added until legumes (rotational crop) can add enough to the system

- Developing new higher-value PO_4 fertiliser product using existing mine resources
  - Pelletise phosphate rock dust (mine waste), add microbes, polymer coating

➔slow release fertiliser pellets

Next steps/Future directions

- Further investigate taxonomy & functional groups of bacterial, archaeal and fungal OTUs detected in mine soils
- Which taxa are driving differences between post-mine/not mined and arable/not arable?
- Identify microbes suitable for crop plant trials as microbial inoculum or ‘biofertilizer’ – reduce fertilizer requirement, improve plant growth

Transition to sustainable agriculture on Christmas Island

Food products for local & export markets

- Broadacre crop trials
  - Vegetables (pumpkin, peanut)
  - Cereals (dryland rice, sorghum, millet, maize/corn, chia, quinoa)
  - Legumes (mungbean, cow pea, lablab, chickpea, soybean)

Higher-value crops / adding value to crops

- Coffee
- Hemp
- Edible mushrooms
- Cereals for craft beer and spirit production




Take home messages

- First look at Christmas Island soil microbes
- Root-associated bacteria present in various post-phosphate mining soils, including some of the harshest soil environments on the island
- Many putative beneficial to crop plants
- Diverse rhizobia available to nodulate with crop legumes
- Transition from mining to agriculture:
  - food security
  - employment
  - …

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