Comparing the identity, diversity, and community structure of nitrogen-fixing bacteria associated with wild and cultivated populations of South African rooibos tea (Aspalathus linearis)

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Introduction

Fabaceae family

Second largest family: 760 species, in 37 genera belonging to 18 tribes (Goldblatt et al., 2005)
Introduction

Morphology of Rooibos

- Distinct ecotypes and diverse life history traits: adapted to fire regimes of the fynbos

- Erect ecotype
- Tree ecotype
- Salignus
- Prostrate
- Shrub
Introduction

Morphology of Rooibos

Ecotypes restricted to geographic regions

- differ genetically
- vary in life history traits

Map (Hawkins et al. 2010)
Introduction

*Aspalathus linearis* (Rooibos)
Introduction

Rooibos

- Symbiotic mutualism
- Biological Nitrogen Fixation (BNF) provides sustainable availability of N for plant productivity
Background

Legume-rhizobia symbiosis: BNF process

1. Host legume releases plant flavonoids

2. Stimulation of bacterial nodulation genes (Nod genes)

3. Root hair infection by rhizobia and the formation of functional nodules

(Fisher & Long 1992; Downie 1998, Zhang et al., 2009)
Background and rationale

Commercial monotypic cultivation

Organic/ wild harvesters
Background

**Hypotheses**

1. Monotypic cultivation alters diversity of soil microbes

2. Reduced diversity will translate into altered compositions of soil microbes

3. Changes in soil composition will influence that of rhizobia symbionts isolated directly from host-plants
Methods

1. Sample collections

Aim: Characterise soil NF bacteria and nodulating bacteria existing in wild and cultivated areas

- **Soil** (3 replicates per status in 6 paired sites = 18 samples)
- **nodules** (5 nodules harvested from ten plants per status; from 6 paired sites = 300 nodules)
Methods

2. DNA Isolation

- **Soil DNA**: PowerSoil® DNA extraction kit (MO BIO laboratories Inc., Carlsbad, CA, USA)

- **Nodule DNA**: extracted using the DNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA)

- **Quality check**: NanoDrop ND-1000 UV-Vis Spectrophotometer and ran samples on 2% agarose gel.
Methods

2. Next generation sequencing

- Soil DNA (*nodC* gene)
- Nodule DNA, *nodC* and *nifH* gene

- **Primers**
  
  *NodC*: nodCFI2F & nodCRI2R  
  *NifH*: nifH1F & nifH2R
Methods

3. Bioinformatics

- Removal of sequence errors and chimeric sequences: PyroNoise (Quince et al., 2011)
- Operational Taxonomic Units (OTUs): alignment of sequences, creation of a distance matrix, and clustering of sequences at 98% DNA similarity level (Tedersoo et al. 2010).

- Programs
  - Morthur version 1.22.0 (Schloss et al., 2009)
Methods

4. Statistical Analyses

Hypothesis 1

**Diversity of microbes between statuses**

- Diversity metrics: \( S, H \) and \( J \) (Shannon & Weaver 1949)
- Two-way ANOVA with Tukey HSD post-hoc for soil
- Paired T-test for cultivated vs wild for nodules
- Rényi’s diversity ordering (Rényi’s 1961)
Methods

4. Statistical Analyses

Hypothesis

Compositional differences between statuses

- nMDS based on Bray-Curtis dissimilarity distances
- PERMANOVA for soil
Methods

4. Statistical Analyses

Hypothesis 3

Mantel tests

- Is compositional change in soil NF bacteria also linked to compositional change in nodule occupant bacteria?

Program:

R (version R 3.2.2.) [http://CRAN.R-project.org/package=vegan](http://CRAN.R-project.org/package=vegan).
Results

- **SOIL NF Bacteria diversity and richness (Hyp 1)**

Diversity

Richness
Results

• SOIL NF Bacteria diversity (Hyp 1)
Results

- Compositional structure for soil NF bacteria (*nodC*) (Hyp 2)

PERMANOVA (999 permutations)

- Region x status (P-value=0.01)
- Status: (P-value =0.09)

*Figure 4: nMDS for soil microbial (*nodC*) diversity*
Results

• Implications for compositional structure (Hyp 2)

Some rhizobia species were structured according to region corresponding to occurrence of their host-plants (Bontemps et al. 2010).
Results

• Diversity and richness for nodules (Hyp 1)

  Paired T-Test showed no significant differences between statuses

  • Diversity (H): $P$-value=0.72
  • Evenness (J): $P$-value=0.52
  • Richness (S): $P$-value =0.77
Results

• Diversity for nodules (Hyp 1)
Results

- Compositional structure for Nodules (Hyp 2)

**Figure 5:** nMDS clustering for nodules data: *nodC* (A) and *nifH* (B).
Results

• Soil and Nodules compositional structure (Hyp 3)

Does change in composition of soil NF bacteria correspond to rhizobia isolated from host-plants?

NO, (Mantel Tests, R= -0.31)
Results

• Implications for soil and nodules compositional structure (Hyp 3)

There are multiple factors that need to be considered such as soil chemistry.
Conclusion

• There were no differences in soil and nodule microbial populations despite cultivation status.

• Most soil NF and rhizobia symbionts showed compositional structure according to region.

• Hence a high beta diversity turnover of NF bacteria over shorter range.

• Considering status only on NF bacteria community structuring is misleading; there are multiple factors that affect diversity, composition and structure of microbial e.g. soil nutrients.

• Future studies should incorporate multiple factors and assess plant productivity vs NF communities.
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