Root endophyte communities differ between sodic and non-sodic soils in a catena ecosystem of the Kruger National Park, South Africa

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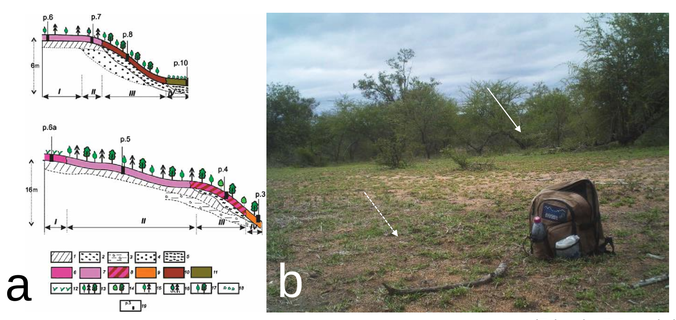
Marieka Gryzenhout[1], Brooke Bailey[1], Antonie Kloppers[1], Errol D. Cason[2], Tonjock Rosemary Kinge[1,3]

[1]Department of Genetics, University of the Free State, Bloemfontein, P. O. Box 339, Bloemfontein 9300, Republic of South Africa; [2]Department of Microbial Biochemical and Food Biotechnology, University of the Free State, Bloemfontein
[3]Department of Biological Sciences, Faculty of Science, The University of Bamenda, P.O. Box 39, Bambili, North West Region, Cameroon.

INTRODUCTION

- Fungal communities play an important role in the functionality of any ecosystem.
- Next Generation Sequencing (NGS) technologies allow for the rapid characterization of communities with a level of identification that adds insight to interactions.
- Using this more rapid approach, the possible usefulness of fungal communities as indicators can be studied.
- A catena (Fig. 1a) is a sequence of soil types down a hill slope because of precipitation, infiltration and runoff (Taleqani, 2008).
- These create diverse ecotypes, soils and hydrological processes.
- AIM: Study the effect of sodic vs. non-sodic soils in a catena system on endophytes associated with plant roots.
- An indicator plant present in both soil types were chosen to negate bias based on plant species.



https://www.researchgate.net/figure/258342996_fig7_Fig-1-The-studied-soil-catenas-catena-1-above-catena-2-below

(Taken by B Janecke)

Figure 1. (a) Representation of catena by means of terrain morphological units. (b) Adjacent sodic (dotted arrow) and non-sodic (arrow) sites in a Kruger Park catena system.

MATERIALS AND METHODS

PREPEPARTION FOR ILLUMINA SEQUENCING

- Selected plant: *Sida cordifolia* (Malvaceae), flannel weed, invasive.
- 20 samples were collected from both the sodic and non-sodic site within a catena at the Southern Granite Supersite, near Skukuza in the Kruger National Park.
- These sites were adjacent to each other (Fig. 1b) and the total area of sampling were c. 50 $m^{2.}$
- Roots were surface sterilized in a water-3% bleach-70% ethanol-sterile, distilled water series.
- The Nucleospin® Plant II Kit (Machery-Nagel) was used to extract gDNA.
- The Internal Transcribed Spacer 2 region was amplified with ITS3 and ITS4 primers fitted with overhang Illumina adapters. Amplicons were pooled and sequenced with an Illumina MiSeq at the Next Generation Sequencing facility at the Department of Health Sciences, University of the Free State.

ITS2 DATA ANALYSIS

- Fastqc (Babraham Bioinformatics) was used to assess sequence quantities and quality of the sequences.
- Quality control was performed using Prinseq-lite v0.20.4 to obtain a sequence length of 240-251 bases and a mean quality score of ≤20 using a 7 nt window with a (?) nt step.
- Reads were merged with PEAR 0.9.6, and quality filtering was run in QIIME to obtain a FASTA output file.
- Identification of chimeric sequences was performed using usearch 6.1.544 against the RDP "Gold" database.
- QIIME was used to filter out all chimeras using the identify_chimeric_seqs.py and filter_fasta.py commands.
- OTU clusters and taxonomy were assigned using the pick_open_reference_otus.py scripts at a 97% sequence similarity against the UNITE database 7.0 (Kõljalg, 2013).
- Species level identities were investigated with Maximum Likelihood analyses in MEGA v. 6, with the most relevant sequences from Genbank included.

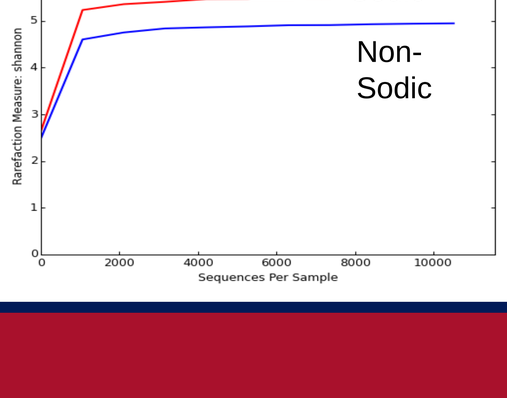


Fig.2. Shannon Diversity Index for each plant tissue.

RESULTS

- A total of 104 474 and 102 066 ITS2 sequences where generated for the non-sodic and sodic site, respectively.
- Alpha Diversity rarefaction plots of Shannon indices (5.51 for sodic soil and 4.95 for non-sodic soil) were significant.
- A total of 57 molecular operational taxonomic units (MOTU) were detected (Fig. 3).
- Less than 10% of sequences from both sites contained sequences that did not cluster with MOTUs on the UNITE database.
- The majority of the MOTU's belonged to the Ascomycota (47 of 54) with the rest in the Basidiomycota.
- A number of the OTUs found in the non-sodic soil were not identified in the sodic soil, while others found in the sodic soil w (e.g. Botryosphaeriaceae) were not present in the non-sodic soil.
- The most abundant MOTU for non-sodic soil belonged to the Botryosphaeriaceae, making up 32% of the sequence data while only making up 0,012% of the genera of the sodic site.
- The most abundant OTU in sodic soil was that of an unclassified taxon in the Dothideomycetes, making up 26% of the sequence data, while only making up 4% of the non-sodic MOTU's.
- Some genera were showed to represent various species based on phylogenetic analyses (Fig. 4)

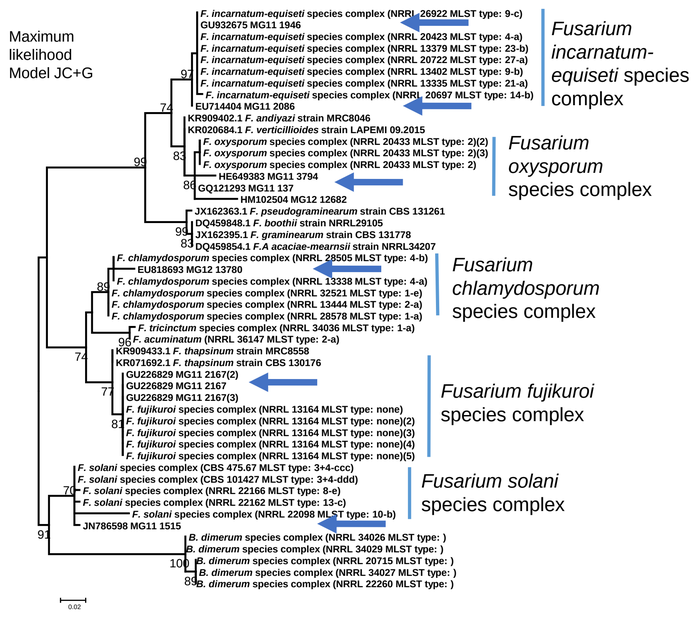


Fig. 4. Maximum Likelihood phylogram based on Internal Transcribed Spacer 2 sequences of representatives of *Fusarium* and *Bisifusarium* with bootstrap support values. The reads from this study are indicated with arrows. Analyses were done with Mega v. 7.

DISCUSSION

- The obtained metagenome sequencing data of the ITS2 rDNA yielded data useful to analyse fungal community composition and differences in based on soil pH in a catena.
- Rarefaction plots revealed that the samples contained enough sequences to represent the fungal community present in the roots in both sites.
- Differences existed between the endophytic communities in the roots of the sodic and non-sodic sites.
  - For instance the most prevalent OTUs identified in either site were often less frequent in the other site.
  - Some taxa were missing between sites.
- Using an environmental approach, it was shown that fungal communities within plant roots of the same plant species differ within an certain locality based only on soil conditions.
- For conservation purposes results are significant because our approach indicated that despite the wide spread occurrence of a plant species, differences on the microbial levels can exist that should be incorporated in conservation planning.
- Fungi can thus be useful as bioindicators.

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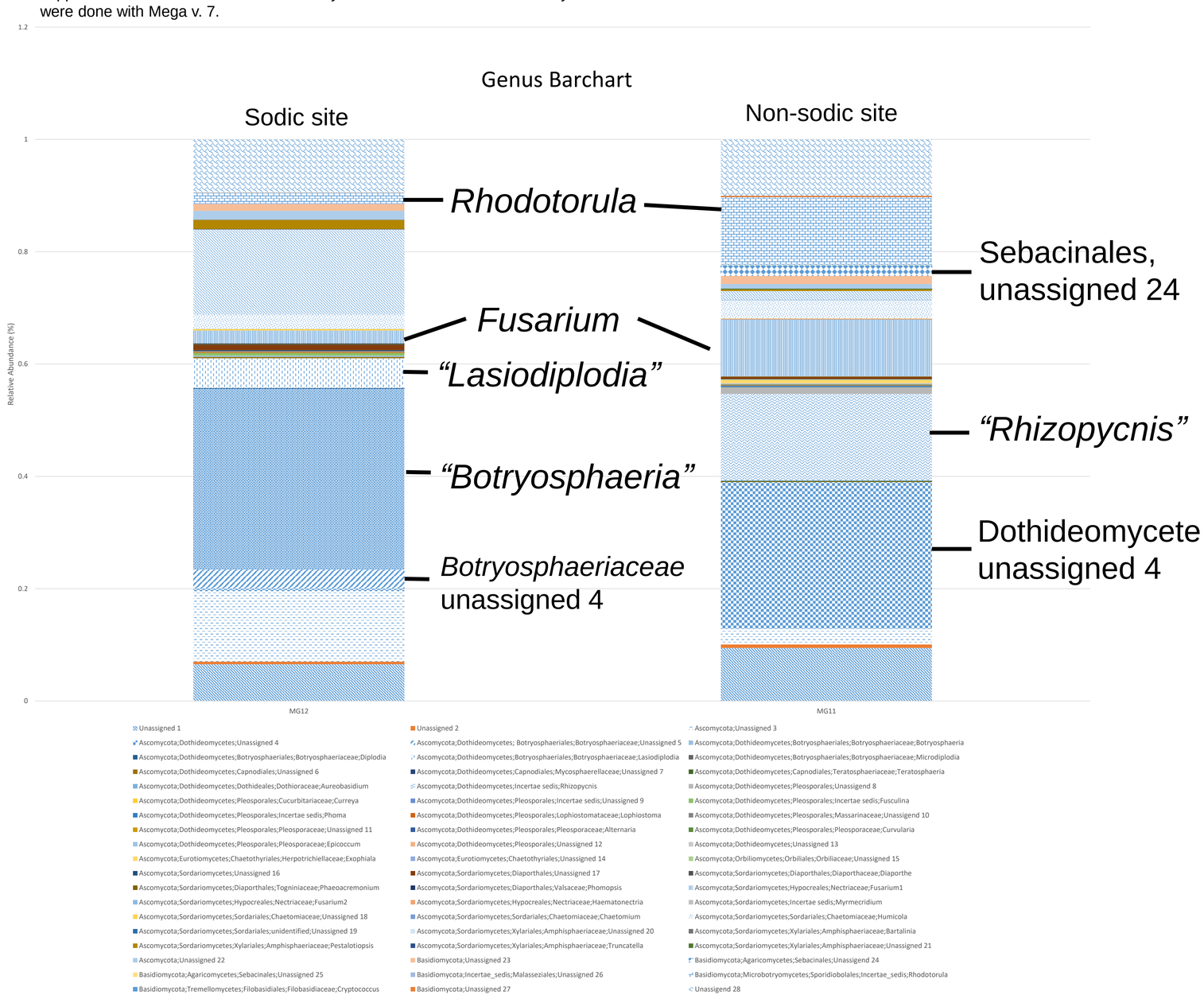

Fig. 3. Graph comparing the identified MOTU clusters on genus level with a similarity of 97% to the UNITE v7.0 database as well as proportion of sequences within each cluster.