中国科学院
西双版纳热带植物园

INFLUENCE OF LAND-USE CHANGES ON SOIL MICROBIAL COMPOSITION AND FUNCTION IN THE NILE RIVER WATERSHED OF UGANDA
(PhD Research Proposal)

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(2010-2013)

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Presentation

- Introduction & background
- Objectives
- Research questions/hypothesis
- Recent developments
- Research problem
- Materials & methods
- Expected output

Introduction

- In all ecosystems, soil microbes play important roles in decomposition of organic matter, nutrient cycling, and plant nutrient availability (Paul and Clark, 1989)
- Microbial activities shape the biogeochemistry of the planet and macro organism health (Sinsabarough, 2008).
- The activity and biomass of microbes respond to soil management, organic matter, and the abiotic environment, and are influenced by plant litter and rhizosphere effects (Zaady et al., 1996; Hooper and Vitousek, 1998; Jones, 1998; Caldero´n et al., 2000; Chen and Stark, 2000).

Microbial Diversity

- There have been several reports of remarkable microbial diversity (Torsvik, Goksoyr & Daae 1990; Tiedje et al. 1999; Torsvik, Ovreas & Thingstad 2002).
- Estimates suggest anything up to 10^4 bacterial species per g of soil, of which at least half (and perhaps as many as 95%) are as yet unculturable (Sait, Hugenholtz & Janssen 2002; Joseph et al. 2003).
- Loreau, 2009 elucidated the relationship between biodiversity and ecosystem function in a simple ecosystem model
- The model predicts that microbial diversity has a positive effect on nutrient cycling efficiency, and contributes to increased ecosystem processes.

Objectives

To relate land-use changes in the Nile River watershed of Uganda to the soil microbial community and functional diversity as an indicator of the stability of the ecosystems in the region.

Specific Objectives

- To determine by GIS the land-use changes that have occurred on tropical rainforests, savannah rangelands and wetland ecosystems of the Nile river watershed of Uganda, in the past 3 decades
- To compare the richness and distribution of microbial communities in tropical rainforests, savannah rangelands and wetland ecosystems with converted areas
- To determine the microbial functional diversity and changes occurring in the Nile river watershed of Uganda
- To determine resilience of soil microbial communities to conversion in the 3 ecosystem types in the watershed

Microbial Cosmopolitanism

'everything is everywhere'

"There is no biogeography for anything smaller than 1 millimeter", Bland Finlay quoted in Whitfield (2005).

Mechanisms:

1. Large population sizes and short generation times resulting in high dispersal rates (Fenchel and Finlay, 2004; Coleman, 2002; Finlay and Clarke, 1999)
   - increased probability of chance dispersal (e.g. via an accidental vector such as a bird or mammal)
2. Capacity to disperse over long distances
   - the small size of microbes can facilitate long-distance passive dispersal, and microbes such as Bacillus can form dormant life stages that enable them to survive long-distance transport and harsh environmental conditions (Plomp, et al., 2005).
3. Low extinction and speciation rates limit local diversification
   - based on the assumption that microbes have large population sizes, making stochastic extinction events less likely (Fenchel and Finlay, 2004). It has also been argued that microbes develop hardy life stages (e.g. spores) that can reduce the probability of local extinction following catastrophic environmental conditions.

Microbial biogeography

- Nacke et al., 2011 compared soil bacterial composition and diversity between forests and grassland sites found a significant difference and that; bacterial community structure was largely driven by tree species and soil pH.
- Bisset *et al*., 2011 in Australia showed that soil microbial communities in different agricultural treatments consistent with degrees of disturbance showed a remarkable difference from relatively undisturbed non-agricultural sites.
- Jesus et al., 2009 in western Amazon revealed that the main differences in bacterial community structure were related to changes in soil attributes that, in turn, were correlated to land use.
- Similarity between primary and secondary forest communities indicated the recovery of bacterial community structure during succession
- Fierer and Jackson, 2006 also studied the relationships between soil properties and the relative abundances of six dominant bacterial phyla (Acidobacteria, Bacteroidetes, Firmicutes, Actinobacteria, a-Proteobacteria, and the b-Proteobacteria). they found that net carbon (C) mineralization rate (an index of C availability) was the best predictor of phylum-level abundances.
- These studies reveal that soil microbes like other organisms have habitat preferences that are related to the specific needs of each population.

Research problem

The Nile River watershed

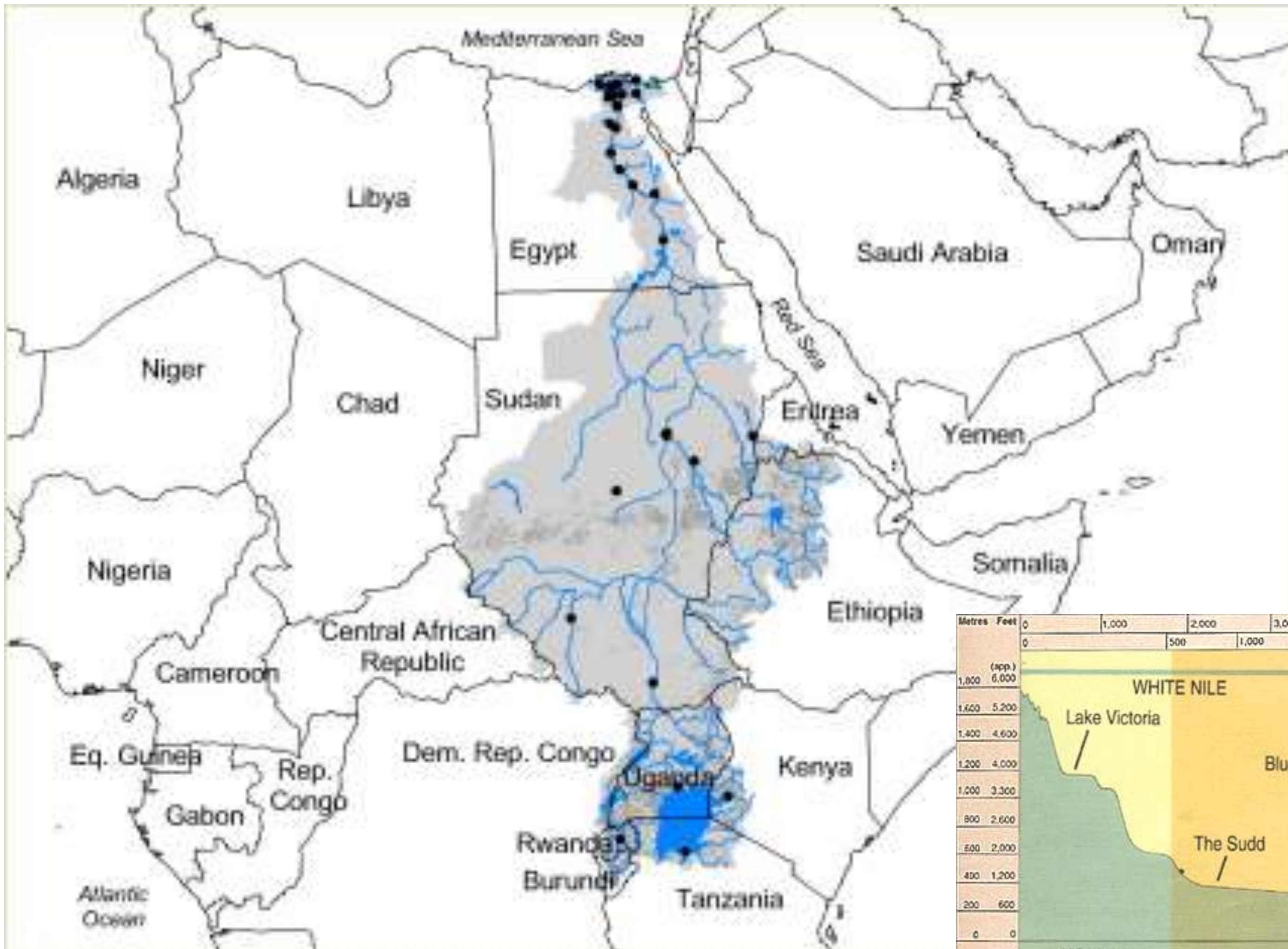
- 3.3% annual population growth rate in Uganda; 80% dependant on agriculture
- Unsustainable utilization of resources

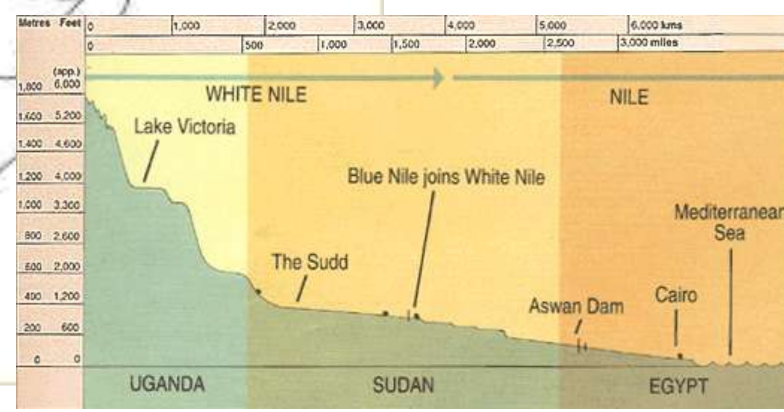
Land use requirement of a fast growing population results in land use changes

- Conversion of natural habitats and species population and decline
- Historically forest cover has declined from 70% to 17%

MATERIALS & METHODS

Site Location





Sampling design & analysis

- I will therefore take 5 sites (replicates) for this study for each ecosystem type (at p-value of 0.05) (since 0.5^5=0.03125 and 0.5^4=0.0625)
- There will be 5 plots(of $1000m^2$ each) from each site for the natural ecosystem paired with 5 plots (of $1000m^2$ each) for the converted ecosystem; hence 50 samples per ecosystem type.
- Top soil (0-15cm) samples will be collected from 10 subplots within each plot.
- The samples will be mixed together and a 100g composite sample collected from the mixture, sieved and packed for DNA extraction and soil physicochemical analysis.
- For each site, the basal area, crown/canopy cover, slope, aspect, elevation, and position in topographic sequence will be recorded.
- I will also determine the ground cover and vegetation cover rating for the sites. Also digital images of the sites for the ground surface, horizontal image of the site and the canopy will be taken where applicable.
- To compare the 2 treatments in the matched-pair method, I will use the sign test.
- I will then do correlation and principle component analysis to determine the relationship between the 2 treatments (natural and converted ecosystems).

Treatments

Savannah/woodland Ecosystem
5 sites (L. Mburo N P, Queen Elizabeth N P, Murchison falls N P, Budongo forest reserve, and Katonga nature reserve)

A pair of natural **(5plots)** and converted/grazed **(5plots)** land within 5km of each other

Treatments

Tropical Rainforest Ecosystem
5 sites (Budongo forest, Mabira Forest, Bwindi impenetrable forest, Maramagambo forest, and Murchison Falls N.P)

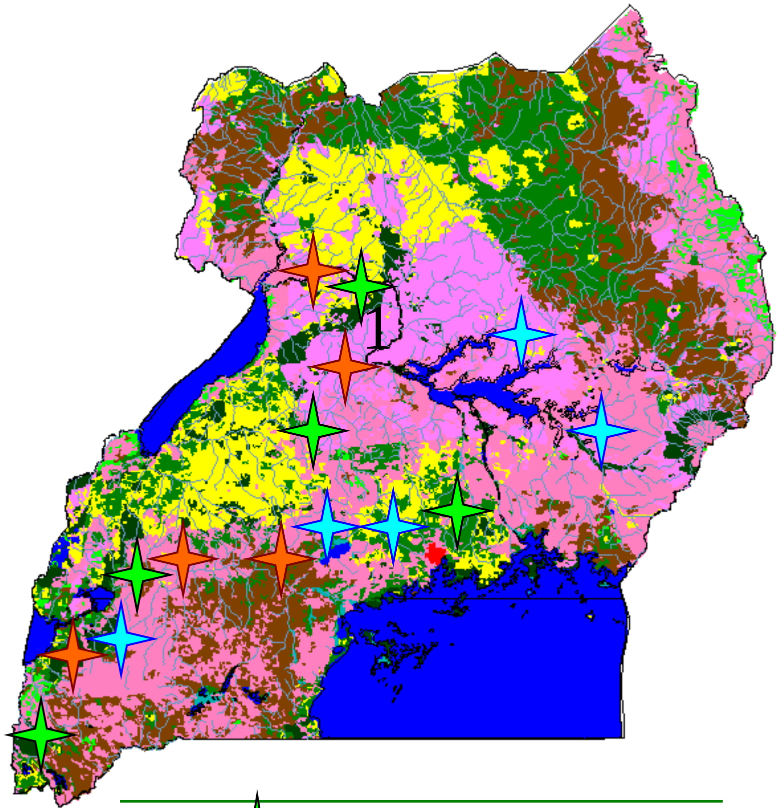
A pair of natural **(5plots)** and converted/ cultivated **(5plots)** land within 5km of @ other

Treatments

Wetland Ecosystems
5 sites (Olweny, Nakivubo, Pallisa, Bushenyi, Nabugabo wetlands)

A pair of Natural wetland**(5plots)** and cultivated/disturbed wetlands **(5plots)** per site within 5 km of each other

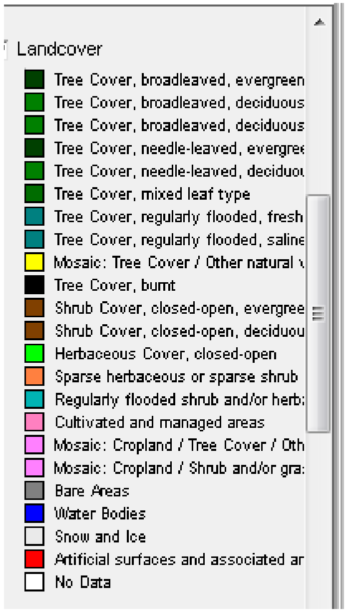
Site locations



Tropical Rainforest sites

Wetland sites

Savannah/rangeland sites



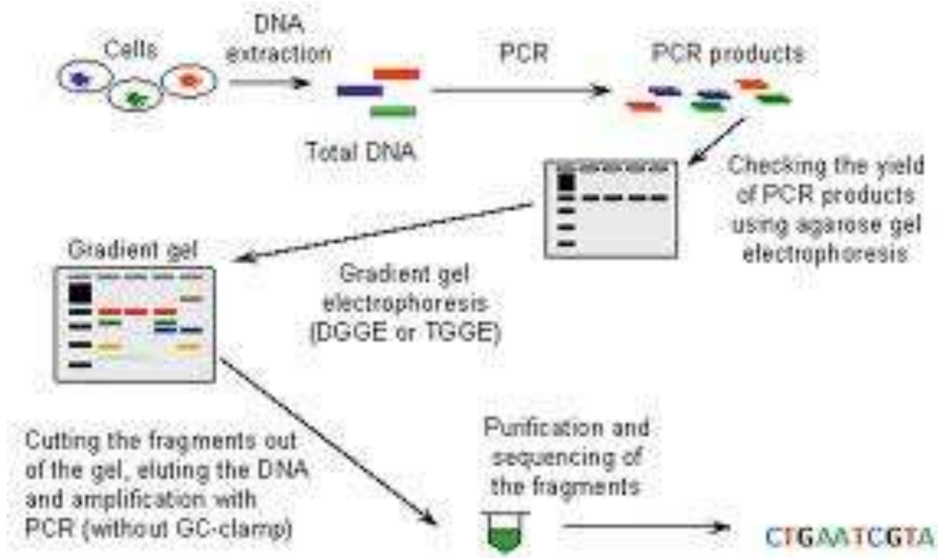
Analysis

GIS

- will be used to establish land-use patterns throughout the study area and the changes the land cover has undergone in the past 3decades
- I will use satellite images and existing national datasets as my main source of data and information on land cover mapping.
- Acquire relevant satellite images, do image processing, and image interpretation.
- Landsat images can be used for land cover mapping and stratification. Image interpretation can be done using GeoVIS and the Land Cover Classification System (LCCS) also being used by FAO AFRICOVER.

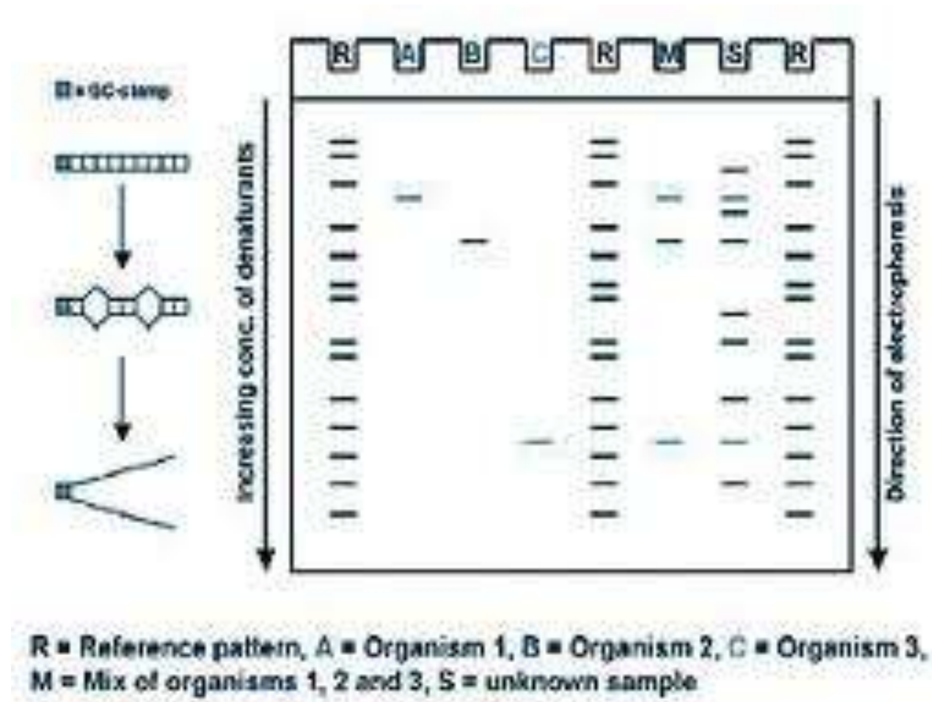
Sample analysis

- An in-depth analysis of soil physicochemical properties will be done.
- The analysis will include the pH, OM, N, P, Ca, Na, Mg, K, and soil texture (% of Sand, Silt and Clay). This is to control for all other factors other than changes in land-use systems that could affect microbial activity.
- DNA extraction will be done at Makerere University genetics lab using the Soil DNA isolation kit (MO-BIO) according to the manufacturer’s instructions
- Polymerase Chain Reaction (PCR) at XTBG lab in China

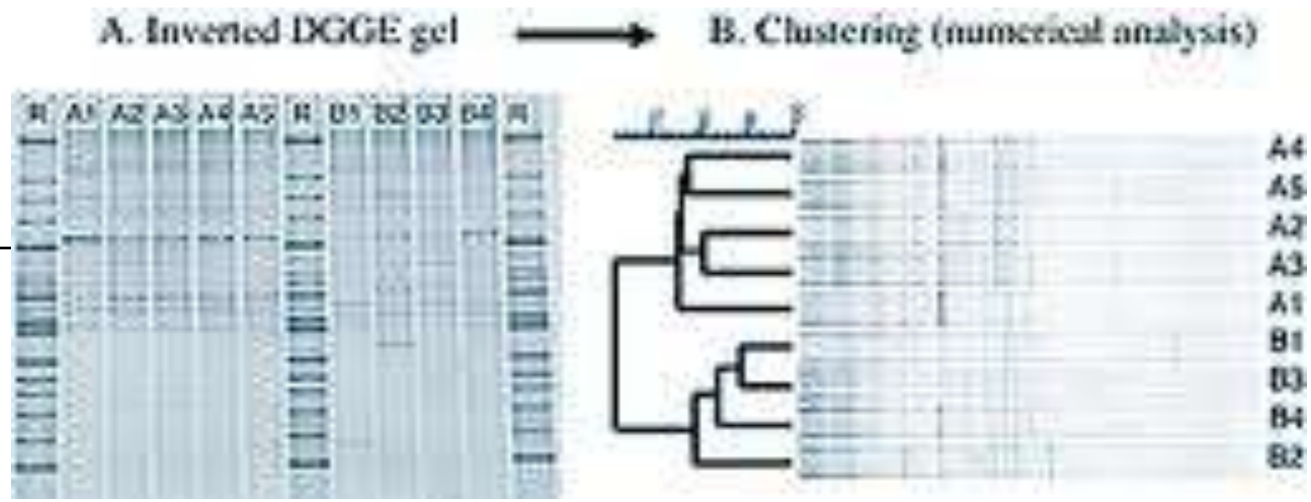


DGGE

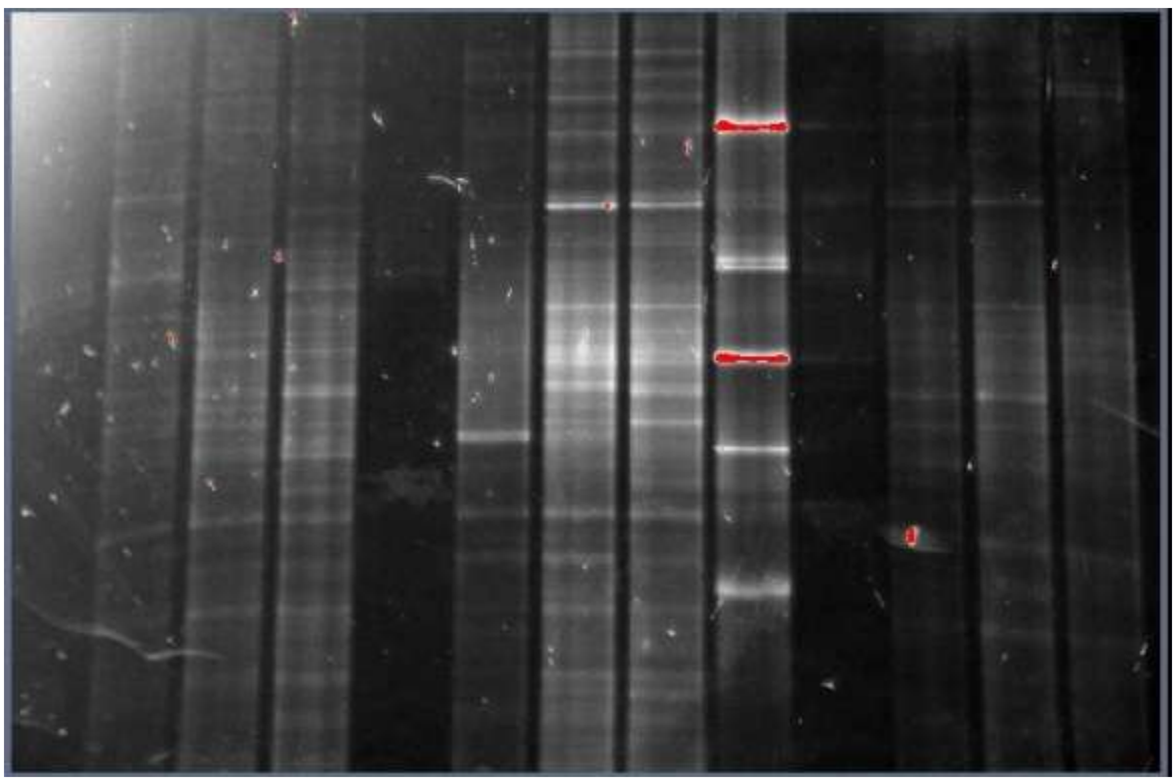
- DNA is subjected to constant heat of about 60degrees & increasing concentration of denaturing chemicals
- DNA (negatively charged) is attracted by the positive electrode & is forced to migrate thru the polyacrylamide gel.
- Once it reaches concentration of denaturing agents it unwinds – said to have melted
- Based on electrical charge, shape & size of molecules

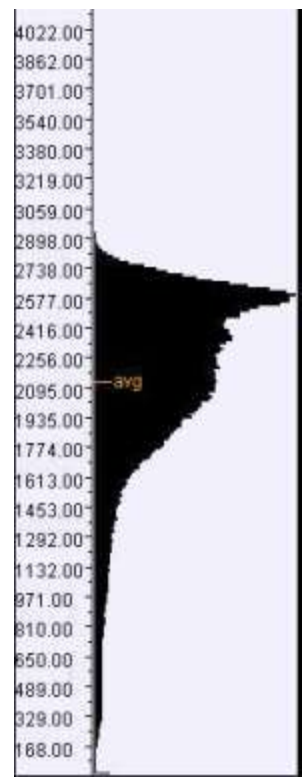


DGGE



A. Inverted DGGE gel
B. Clustering (numerical analysis)
R A1 A2 A3 A4 A5 R B1 B2 B3 B4 R
A4
A5
A2
A3
A1
B1
B3
B4
B2





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2738.00
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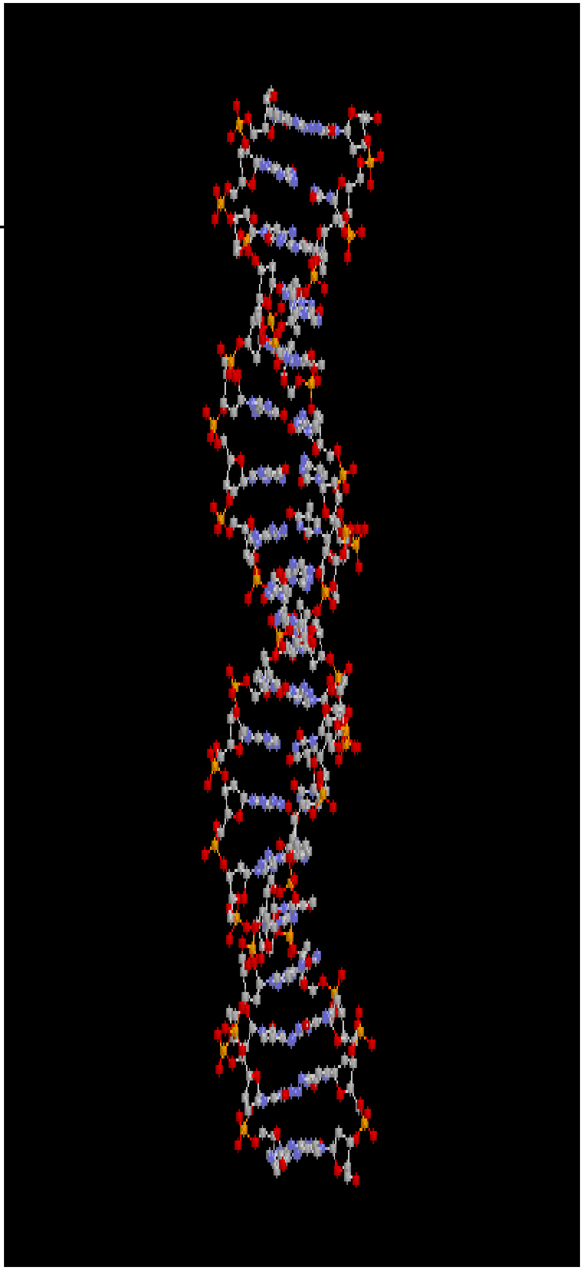
2256.00
2095.00
1935.00
1774.00
1613.00
1453.00
1292.00
1132.00
971.00
810.00
650.00
489.00
329.00
168.00
avg

Illumina sequencing

- Illumina's sequencing by synthesis (SBS) supports massively parallel sequencing using a terminator-based method that enables detection of single bases as they are incorporated into growing DNA strands.
- A fluorescently-labeled terminator is imaged as each dNTP is added and then cleaved to allow incorporation of the next base.
- Since all 4 reversible terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias.
- The end result is true base-by-base sequencing that enables accurate data for a broad range of applications.



Expected outputs

The tropical rainforest, wetlands and savannah rangelands comprise the bulk of natural ecosystems in the Nile river watershed and could cover up to 65%of the total land area of Uganda.

Study will provide an insight into the stability of these ecosystems from a soil microbial perspective

How do these microbial species adapt to conversion?

Do they migrate/immigrate or evolve?

How about at the community level?

What are the patterns at the dominant subsystems perspective (metabolic reactions that sustain a species)?

THANK YOU

FOR

LISTENING