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Effect of Shifting Cultivations on
Bacterial Communities in Hurulu
Forest of Sri Lanka: A
Metagenomic Approach for
Diversity Estimation



Background

Forested ecosystems in Sri Lanka:

- Constantly under pressure
- Forest cover in Sri Lanka: 26 % of the land area
- Dry forested ecosystems: 22 % of the land area

Soil biodiversity → Least studied!!!

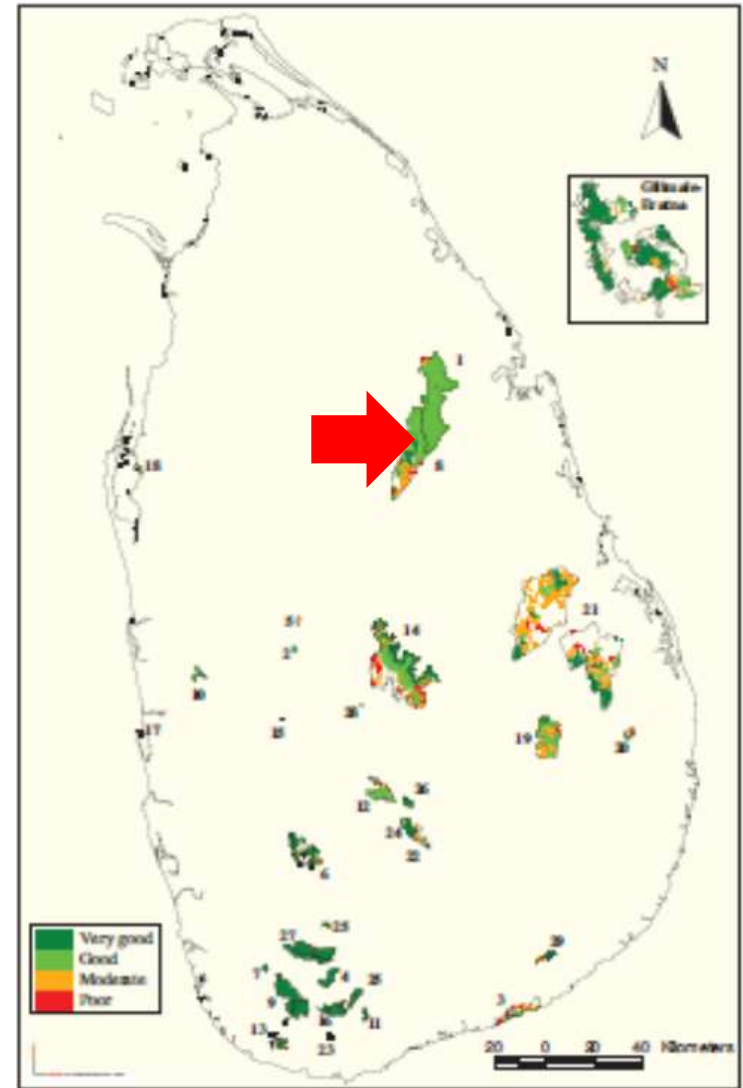


Background

Targeted study area:

- Hurulu International Biosphere reserve
- Under high pressure of socio-economic stresses and experiencing a range of forest disturbances

(Jayasuriya et al., 2011; Iqbal et al., 2012)



Background

- Forest disturbances affect soil microbial diversity in evergreen forests (Dale et al., 2001)
- Understanding factors governing soil biodiversity in forested ecosystems is important for formulating biodiversity conservation measures



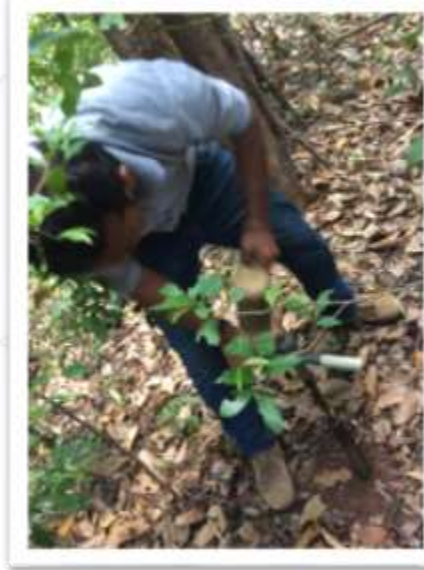
Objectives

- To assess the effect of forest disturbances on diversity of soil bacterial communities inhabiting Hurulu International Biosphere Reserve in the Dry Zone of Sri Lanka

Materials and Methods

Selection of sites

- Based on a survey on forest disturbance
- Soil samples collected:
 - 0 - 5 and 5 - 10 cm depths



Relatively undisturbed

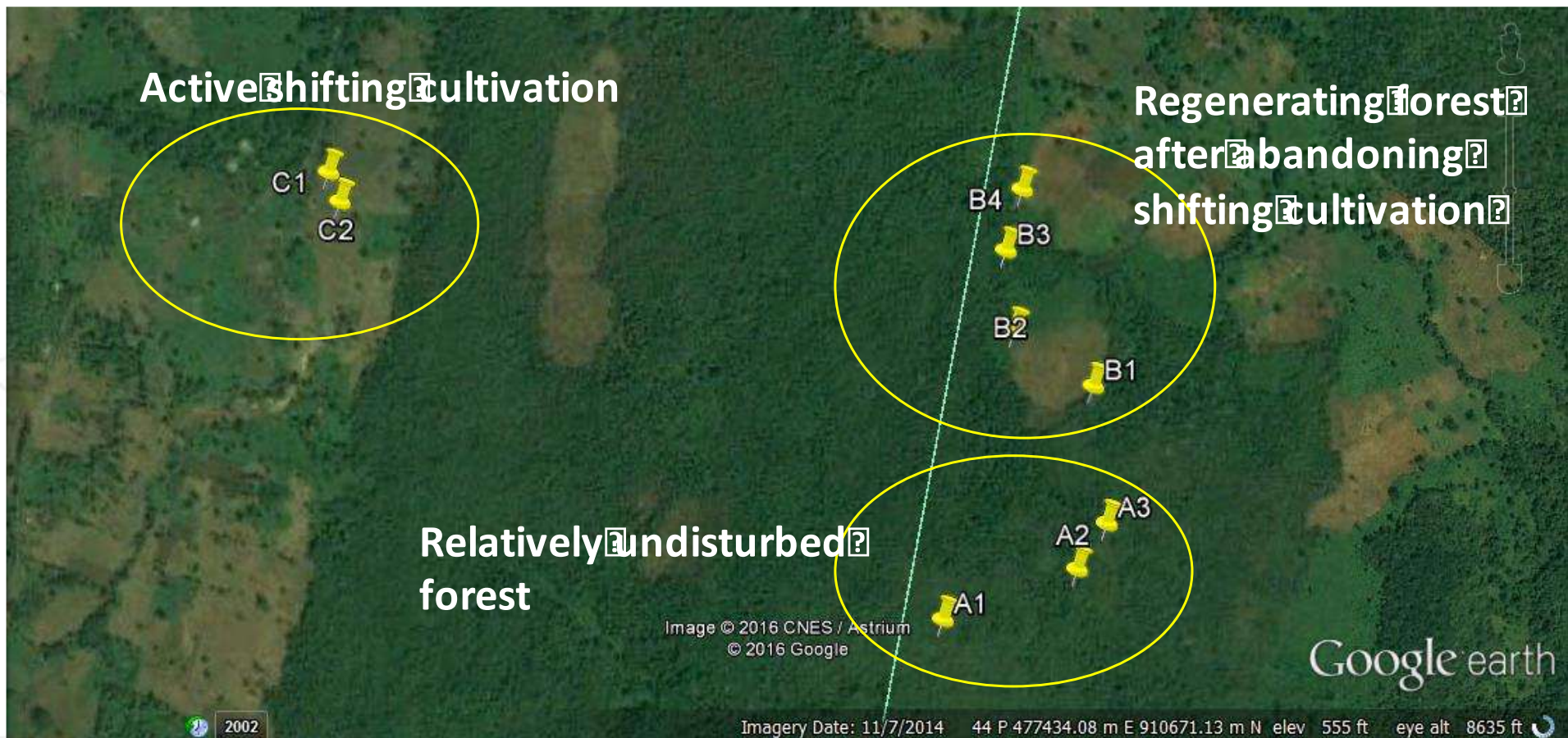


Regenerating



Shifting cultivation

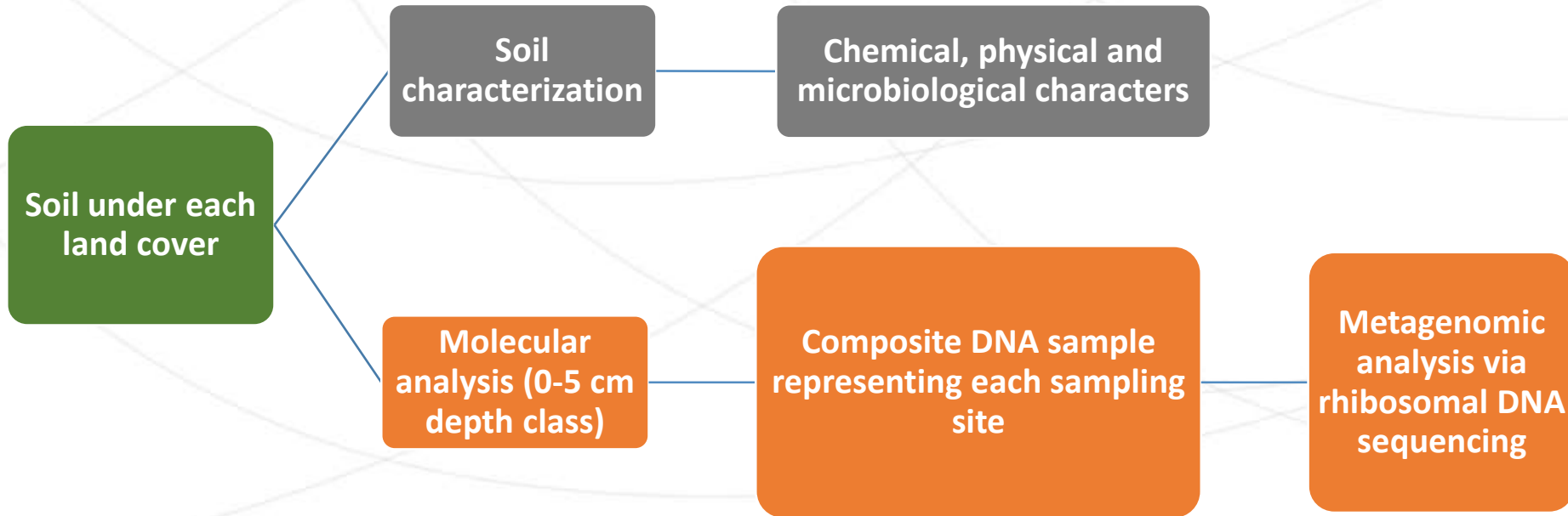




- Five soil samples from 10m X 10m quadrat were collected at each site
- Information on vegetation cover was recorded

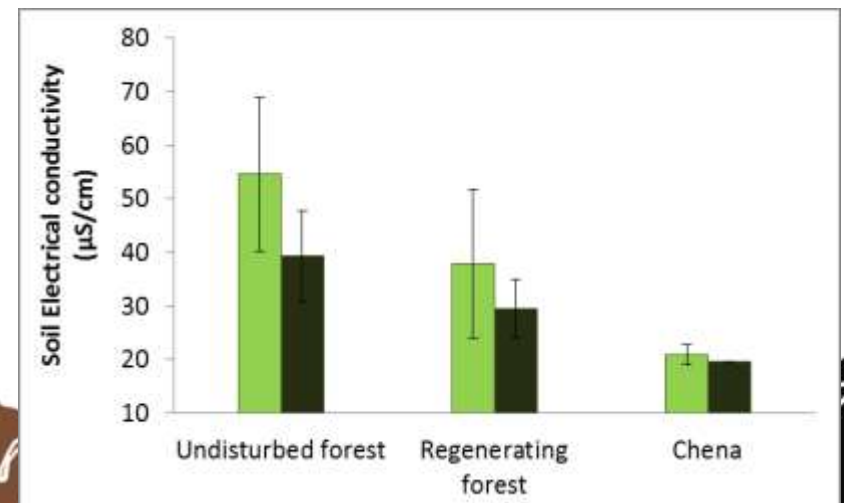
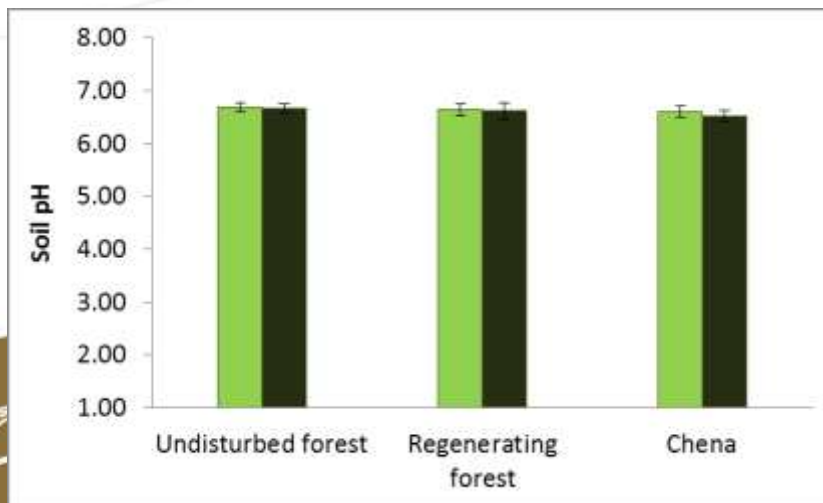
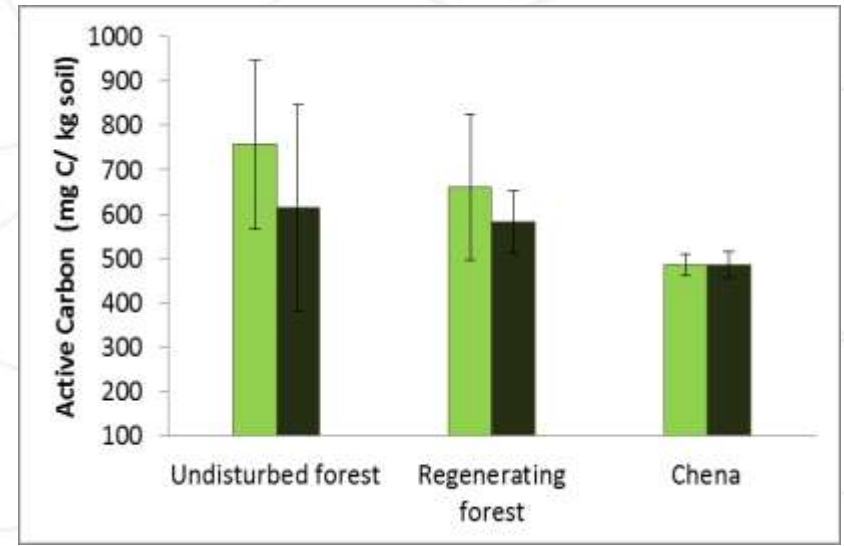
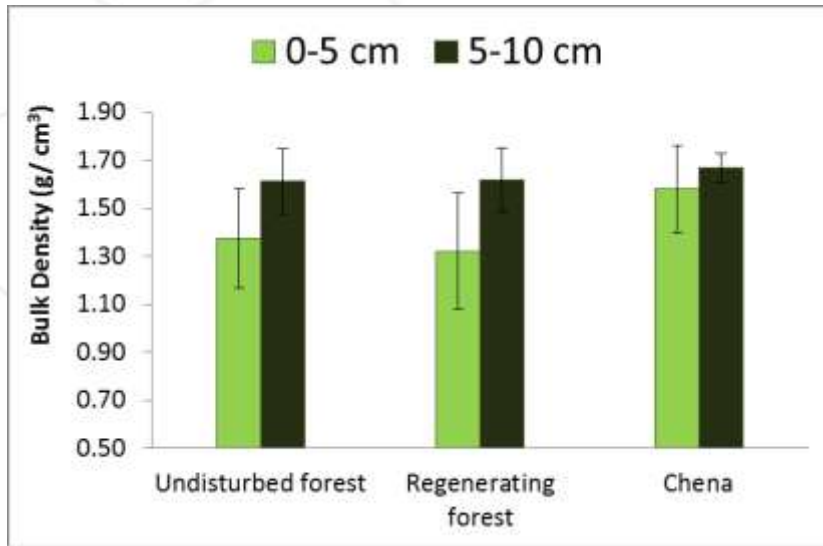


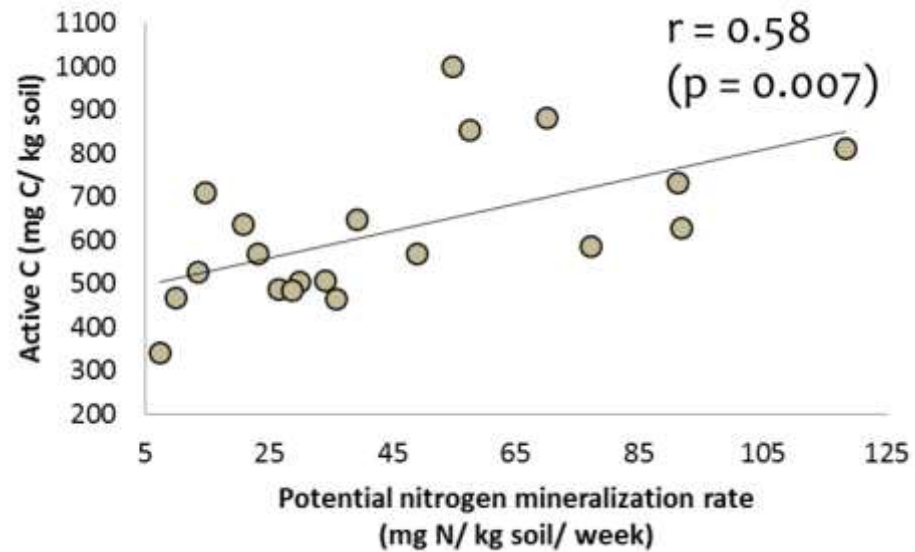
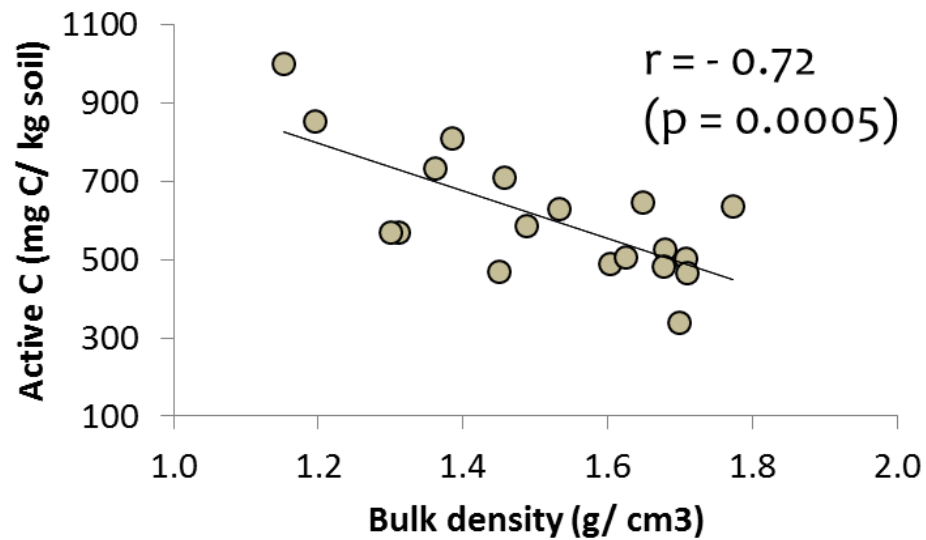
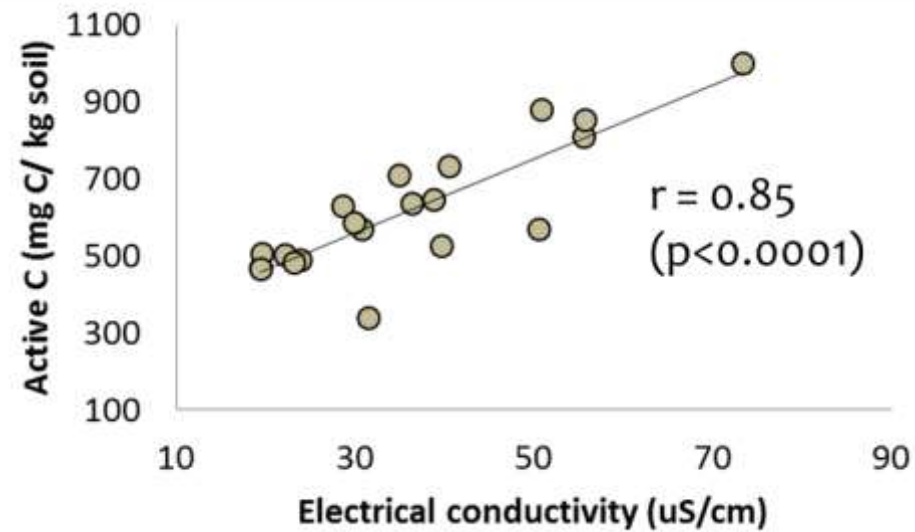
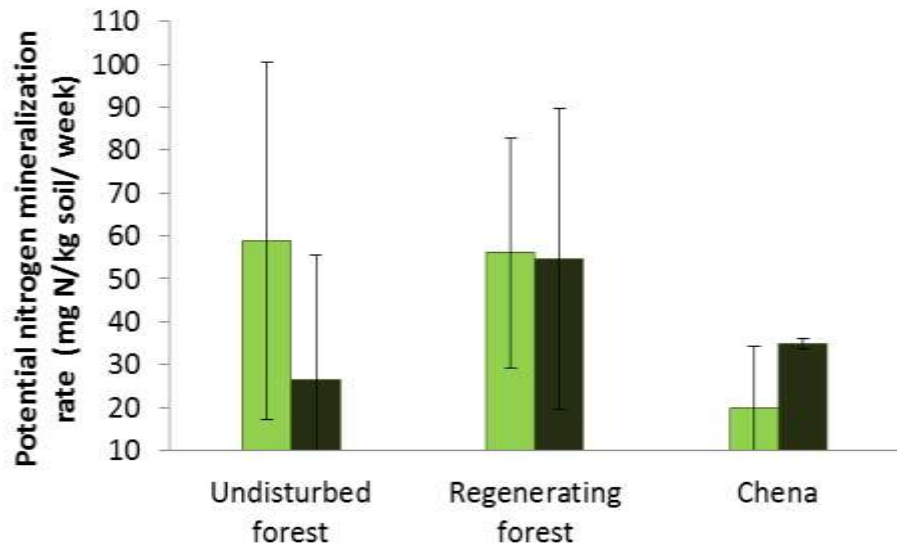
Materials and Methods



DNA extraction → MoBioPowerSoil DNA extraction kit
Next Generation Sequencing (NGS) using Illumina-MiSeq platform
→ V3-V4 regions of the 16S rRNA gene using bacteria specific primers
Sequences files were analyzed by using DADA2 pipeline and MG-RAST server
Taxonomic assessment was done based on SILVA_SSU sequences data base
Shannon and Simpson indices and alpha diversity were estimated

Soil characteristics



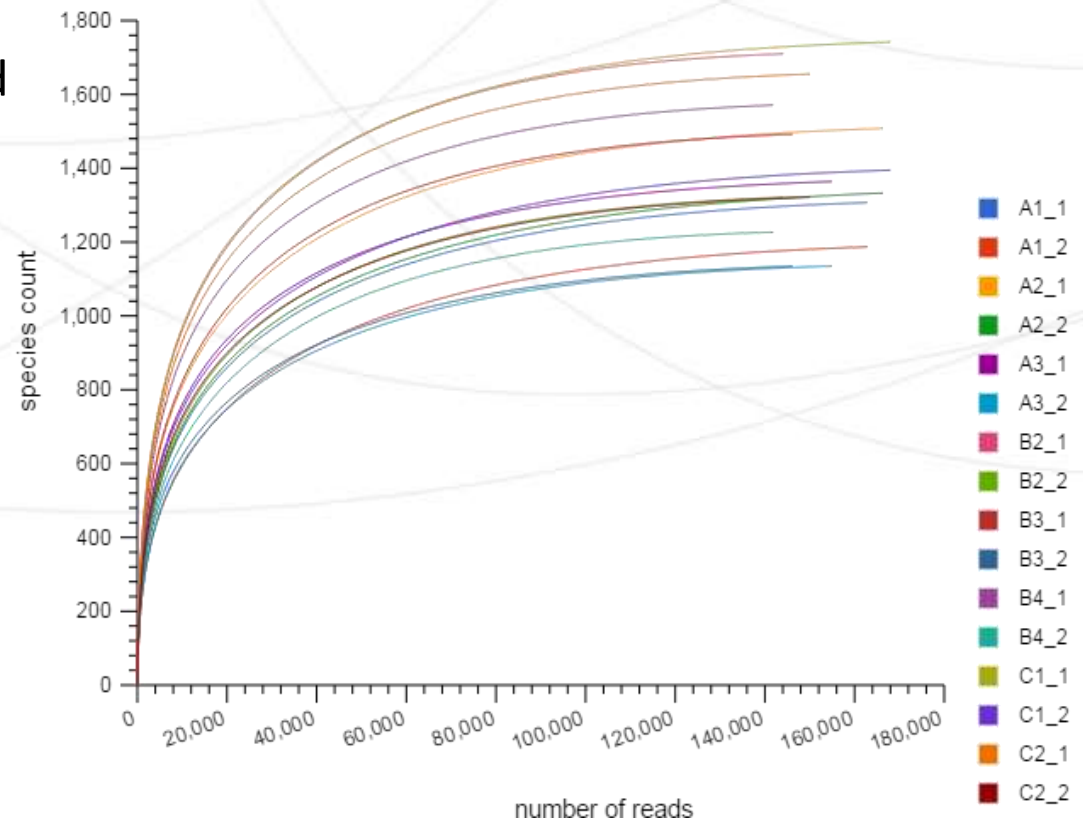


Results: Bacterial diversity

Land cover	Sampling Site	Sequences in	Sequences out	Sequence recovery after initial quality control (%)
Relatively undisturbed forest	A1	162,858	147,919	90.82
	A2	166,358	149,453	89.83
	A3	154,939	149,074	96.21
Regenerating forest	B2	144,265	108,402	75.14
	B3	146,228	113,756	77.79
	B4	141,984	104,408	73.53
Shifting cultivation	C1	168,156	130,030	77.32
	C2	150,071	112,741	75.12

Results: Bacterial diversity

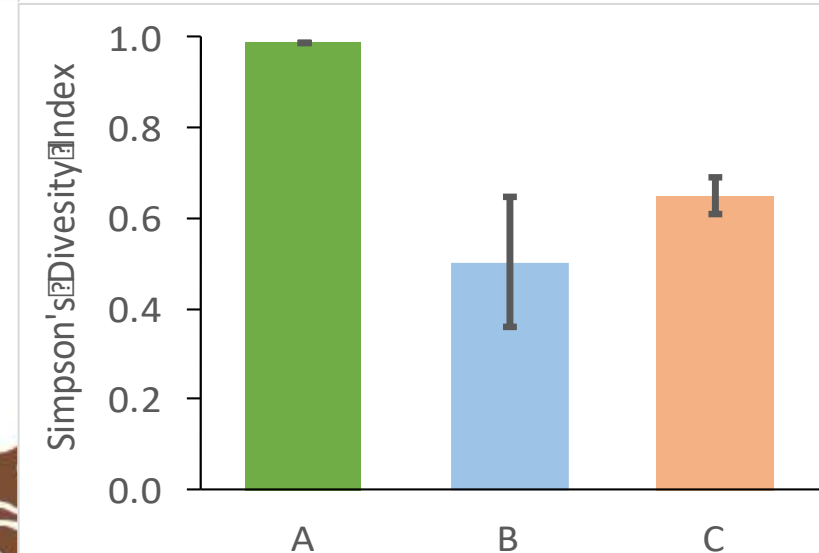
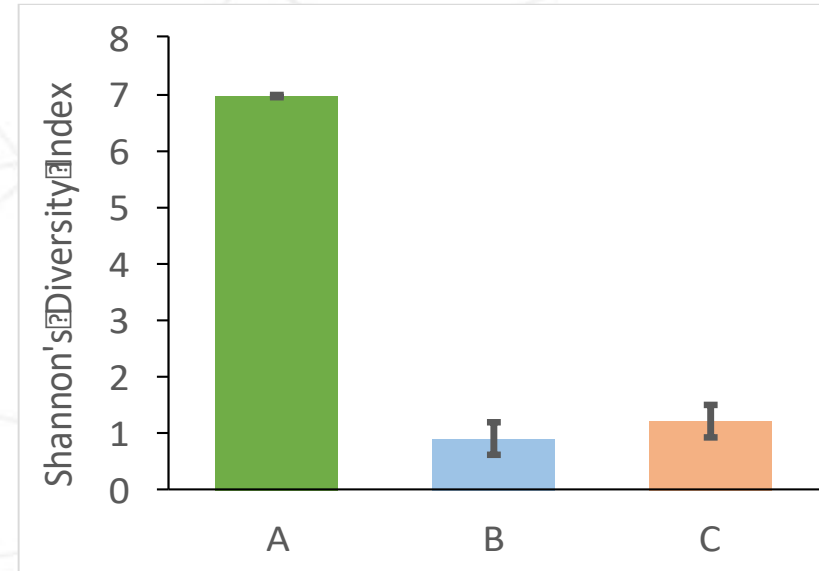
- Rarefaction curves started plateauing after 2×10^4 reads
- Thus, species coverage obtained from each sequence runs were optimum and species richness for each site was exhaustingly sampled



Results: Bacterial diversity

Both Shannon Index and Simpson Index indicates the presence of **high bacterial diversity** and **less species dominance** in relatively undisturbed forest soils compared to soil under other two land covers

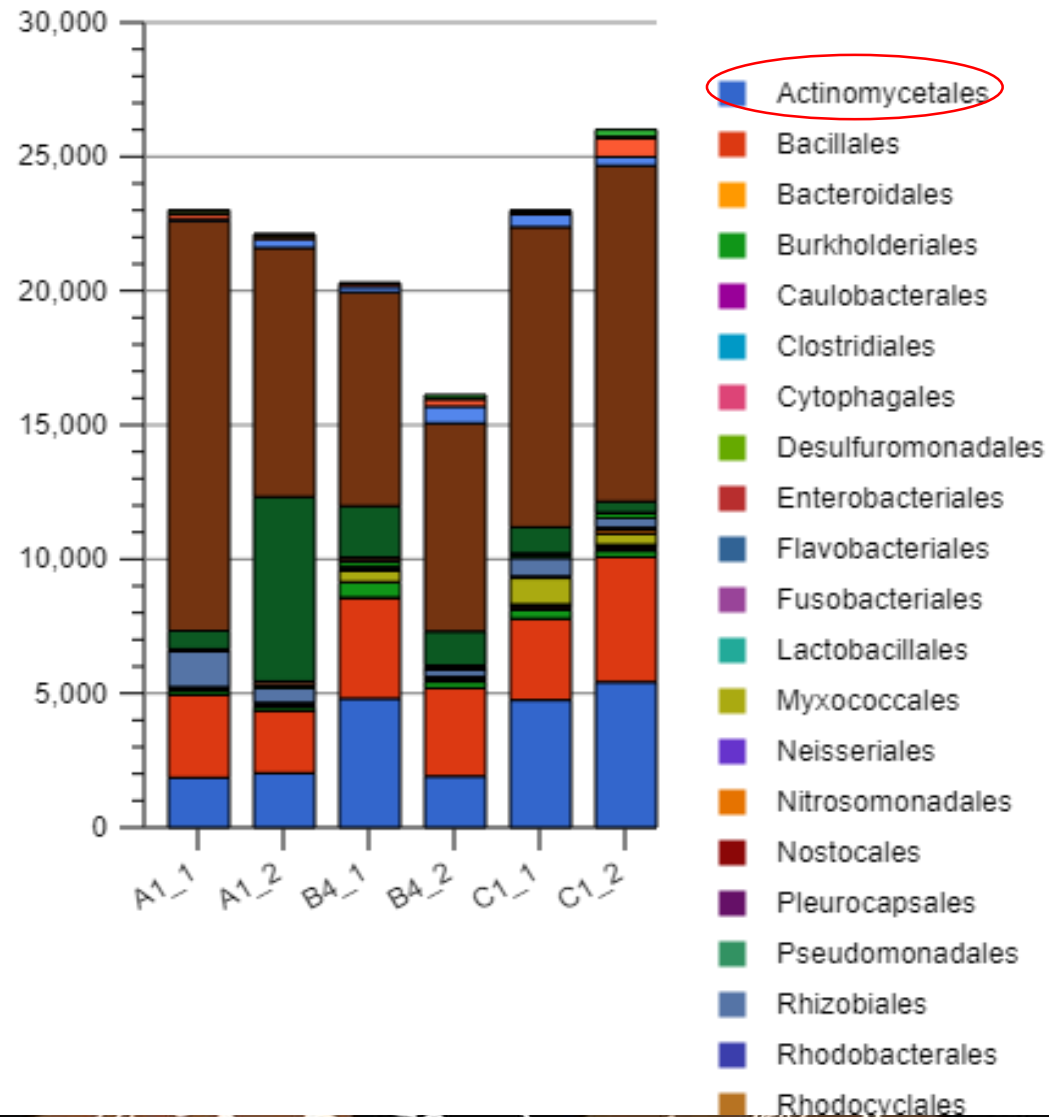
Location	Representative Site
Relatively undisturbed forest (A)	A1
Regenerating forest (B)	B4
Shifting cultivation (C)	C1



Results: Bacterial diversity

Prevalence of bacteria orders in representative samples as analyzed using both forward (1) and reverses (2) reads

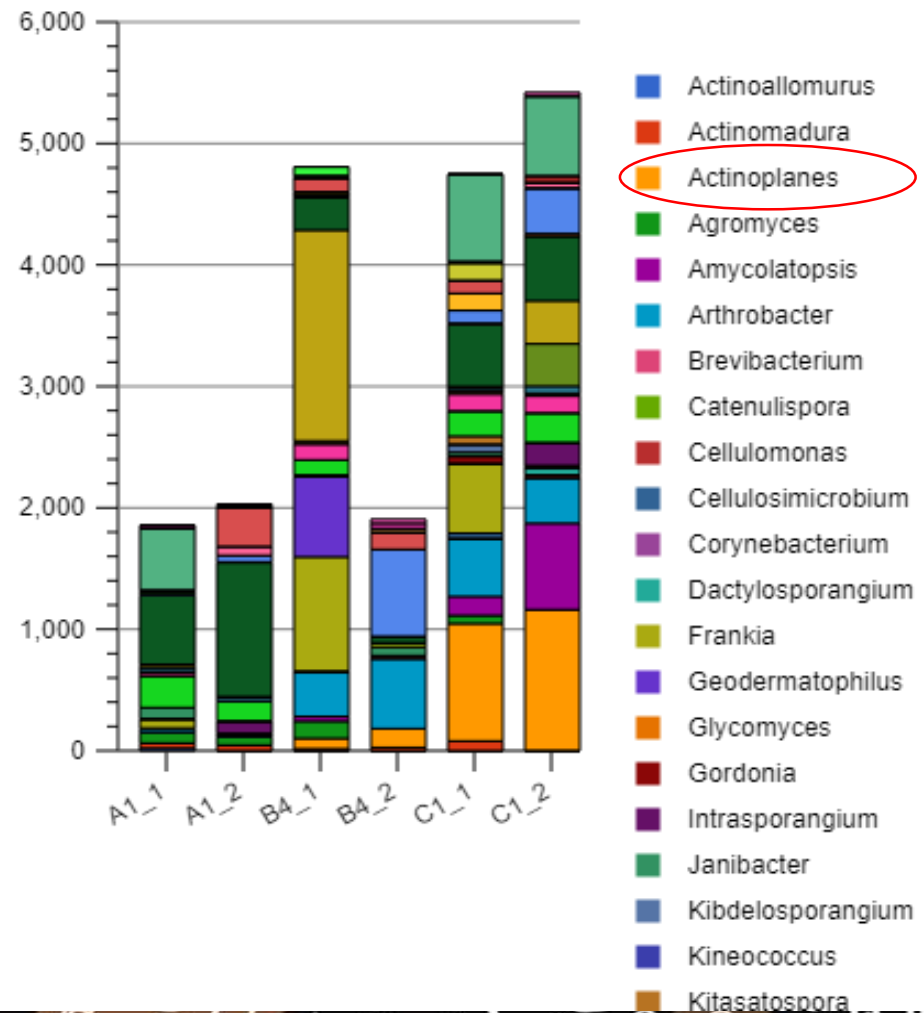
- Actinomycetales, Bacillales, Clostridiales, and Burkholderiales were commonly found in all samples
- The abundance of members of Actinomycetales were higher in disturbed soils



Results: Bacterial diversity

E.g. Dominant genera in order *Actinomycetales* in representative samples

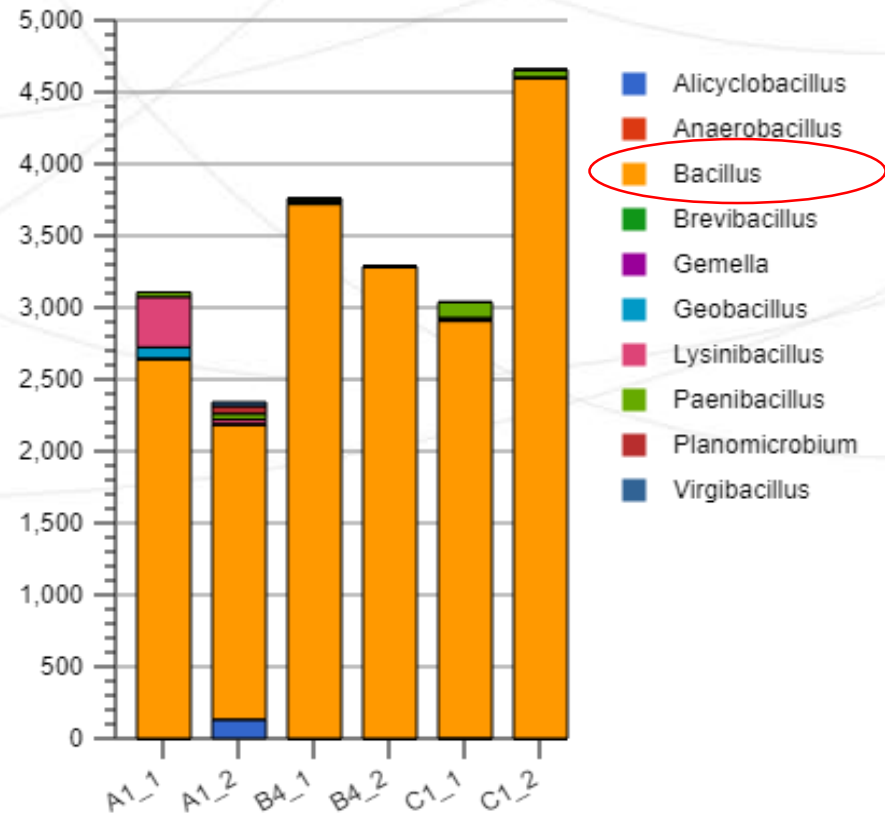
Genus *Actinoplanes* were more prominent in soil under shifting cultivation than in soil under forest cover



Results: Bacterial diversity

E.g. Dominant genera in order *Bacillales* in representative samples

Bacillus was the most dominant genera in this order in all samples

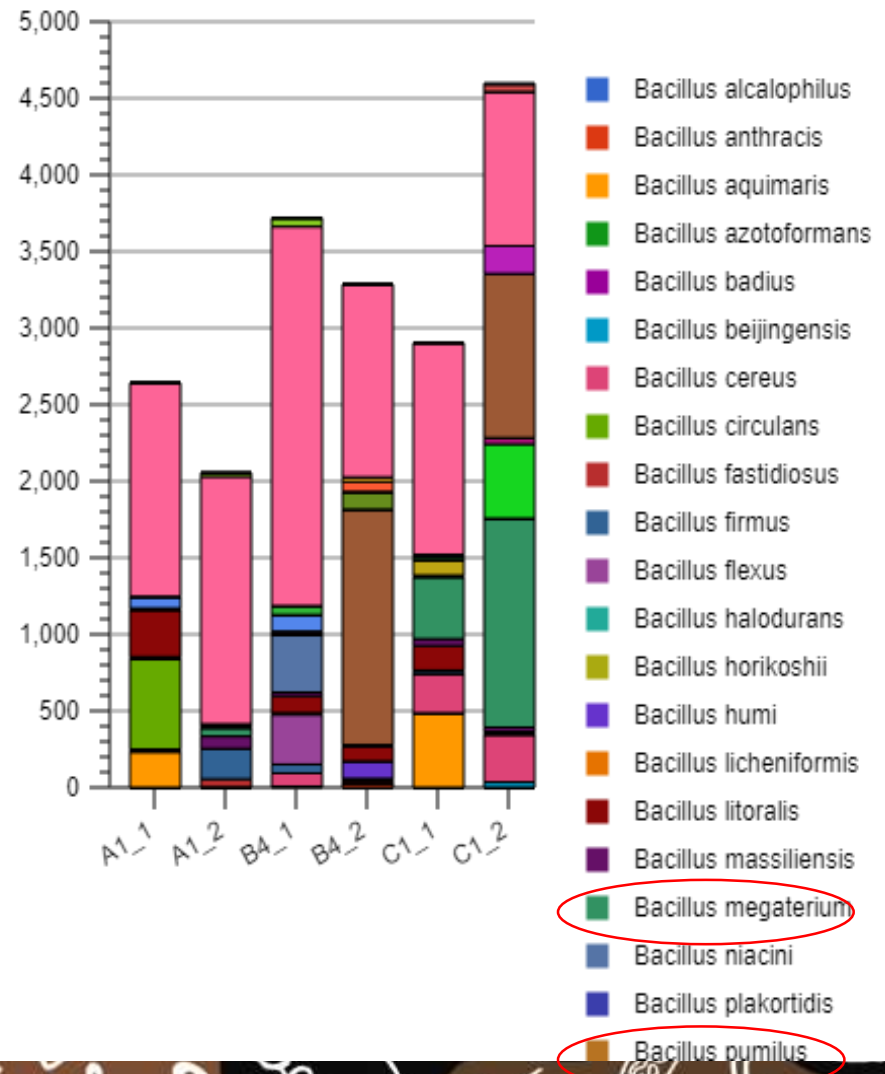


Results: Bacterial diversity

E.g. Dominant species in genus *Bacillus* in representative samples

Bacillus circulans was the most abundant species of this genera in all samples

Bacillus megaterium and *Bacillus pumilus* were found only in soil samples from area under shifting cultivation



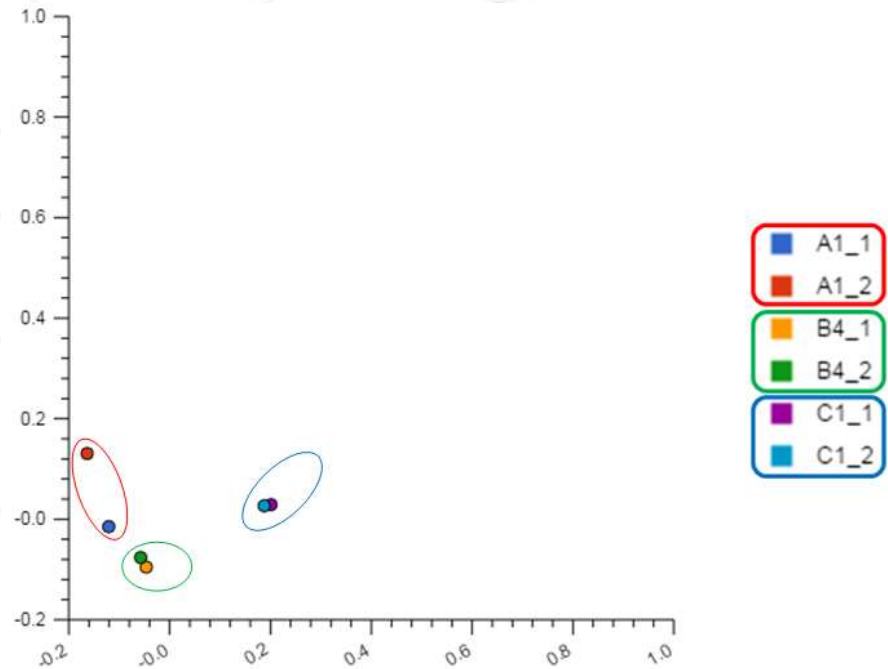
Results: Bacterial diversity

Forward and reverse sequences of sample

- B4 and C1 → more similar
- A1 → slightly different

This beta (α) diversity analysis indicated that as a whole sample A1 is more close to sample B4 from distances and correlation than to sample C1

Thus, sample came from site in active shifting cultivation was significantly different from other sampled sites



Conclusion

Of the studied three land-cover types forest soil with minimum anthropological soil disturbances harbored the highest bacterial diversity at genus level and less domination of single genus over the others in soil bacterial communities

Disturbing the forest ecosystem in Hurulu dry mixed evergreen forest in Sri Lanka for cultivation reduced soil bacterial diversity



Acknowledgement

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**Thank you for
your attention**