

**Unlocking Microbial  
Communities in *Terra Preta*  
Nucleic acid extraction and purification  
as keys to characterizing biology in black  
carbon soils**

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# Microbiology in TP

- Noted both for their high black carbon (BC) content and fertility.
- Soils high in BC appear to harbor distinct microbial populations (Pietikäinen, Kiikkilä et al. 2000).
- Terra Preta (TP) soils may possess a large and unique microbial community (Thies and Suzuki 2003).
- Preliminary data suggest high microbial populations compared to adjacent (non-TP) soils.

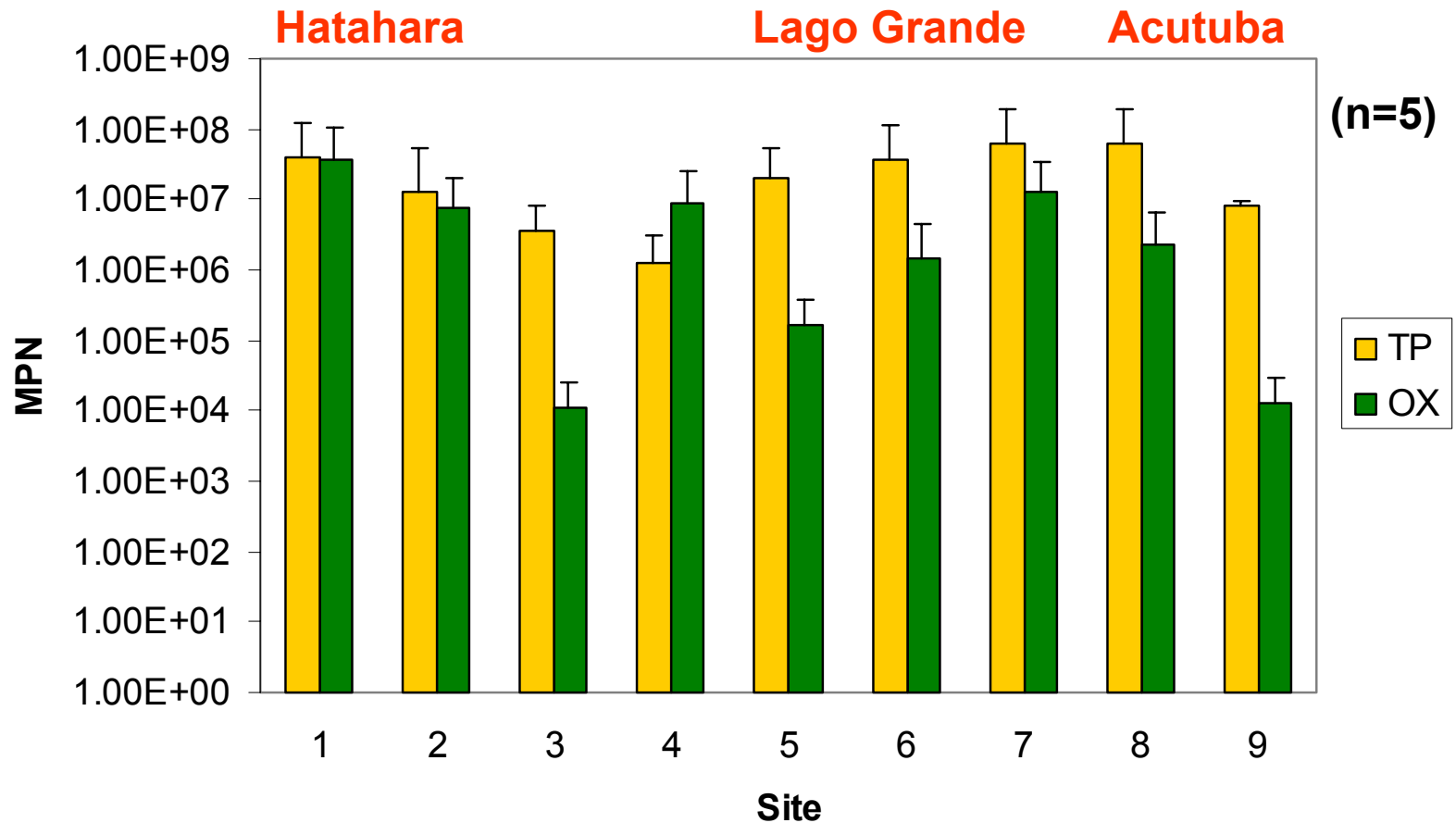
# TP compared to adjacent soils

- Higher C mineralization rates in TP compared to adjacent ferralsols (Glaser et al., 2003)
- Microbial communities clearly differ (Thies and Suzuki, 2003)
- Natural experimental design and unique opportunity for study
- We have shown DNA difficult to extract and purify



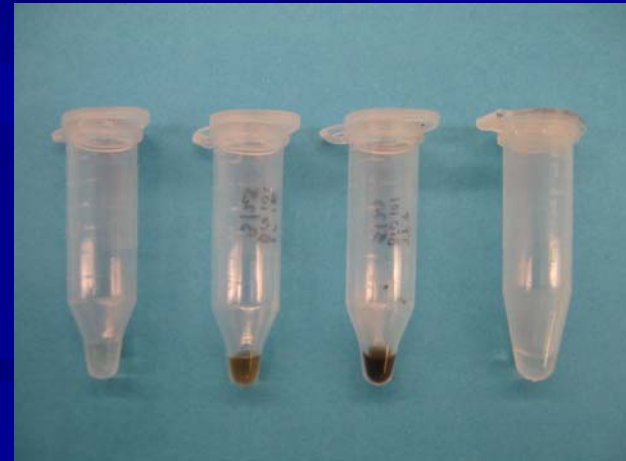
# Abundance of culturable bacteria (Most Probable Number – MPN)

MPN enumeration on liquid R2A Media - TP and Oxisol



# Extracting nucleic acids from soil

- **Kits available for soil**
  1. Cell extraction or Cell lysis
  2. Protein precipitation
  3. Purification
  
- **Problems and Pitfalls**
  - Lysis efficiency of cells differs.
  - Humic acids/organic matter may decrease extraction efficiency.
  
- **What is the effect of the chemistry of black carbon on DNA extraction?**



# Methods for improving DNA extraction from soil

## ■ Direct lysis of community DNA

- Bead beating (Miller et al., 1999)
- Heating and freezing (Bruns and Buckley, 2002)

## ■ Purification

- Hexadecyltrimethylammonium (CTAB), polyvinylpolypyrrolidone (PVPP) (Zhou et al., 1996)
- Chemical flocculation  $\text{AlNH}_4(\text{SO}_4)_2$ ,  $\text{MgCl}_2$  (Braid et al., 2003)

# Hypotheses

H<sub>1</sub>: DNA recovery is low due to poor cell lysis

TP soil matrix may protect cells from lysis

H<sub>2</sub>: Cell lysis occurs but DNA binds to the soil

Black carbon in *terra preta* may bind with DNA in solution and reduce yield or purity.

# Tests for this Study

## ■ Improving cell lysis

- Varying bead beating time (30s/150s)

## ■ Reducing DNA binding to the soil matrix.

- Varying DNA extraction kit (MoBio/Bio101)
- Adding chemical flocculants
  - $\text{MgCl}_2$
  - $\text{AlNH}_4(\text{SO}_4)_2$
  - 5X phosphate buffer



# Methods:

## Quantification and PCR

### ■ Quantification

- Stain nucleic acids, image and compare to a DNA standard curve.
- Run on an agarose gel.

**Measures quantity of raw DNA extract.**

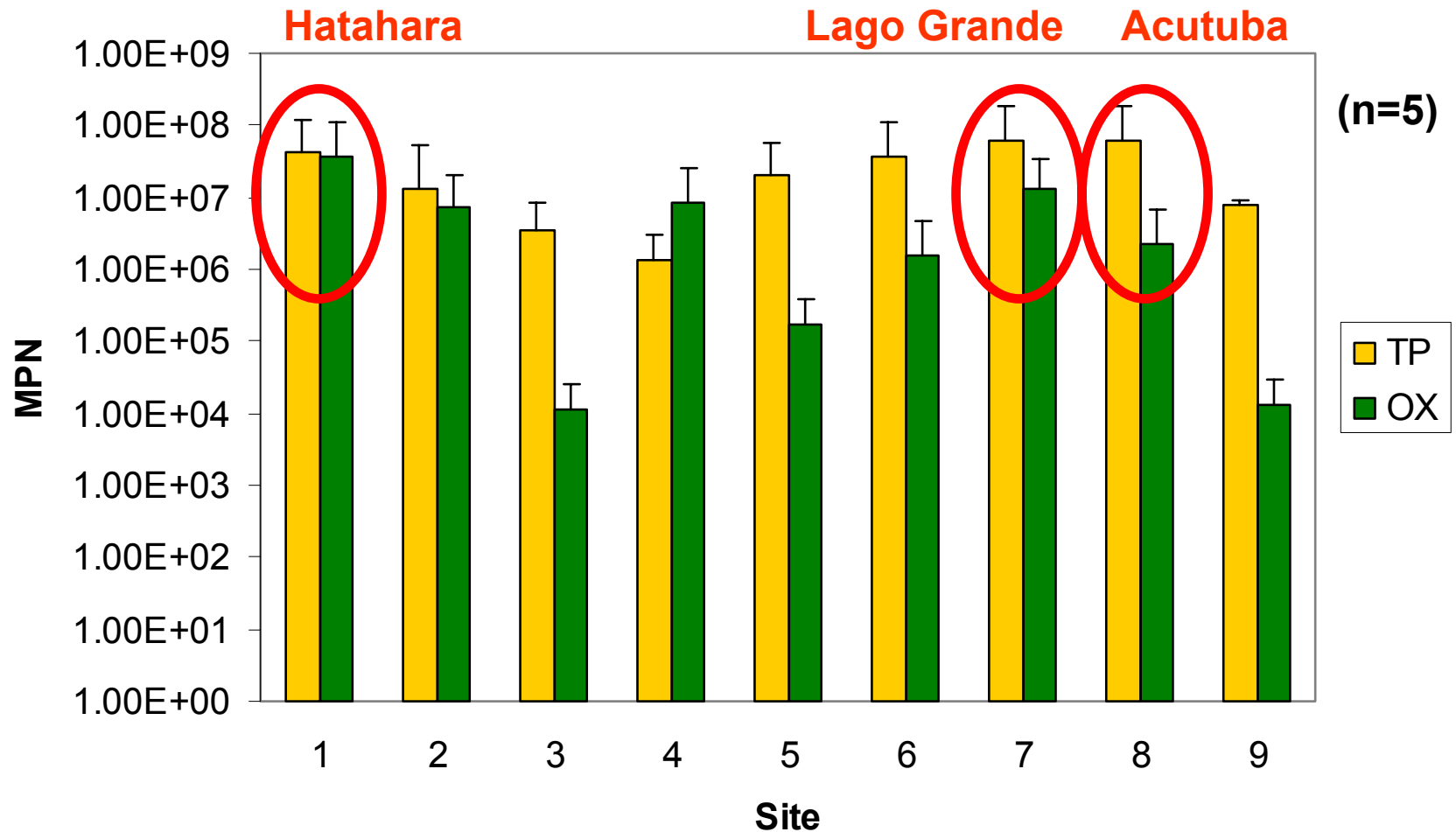
### ■ PCR

- Polymerase chain reaction is used to amplify community DNA fragments.
- Can be used to evaluate suitability of DNA extracts for molecular analysis.

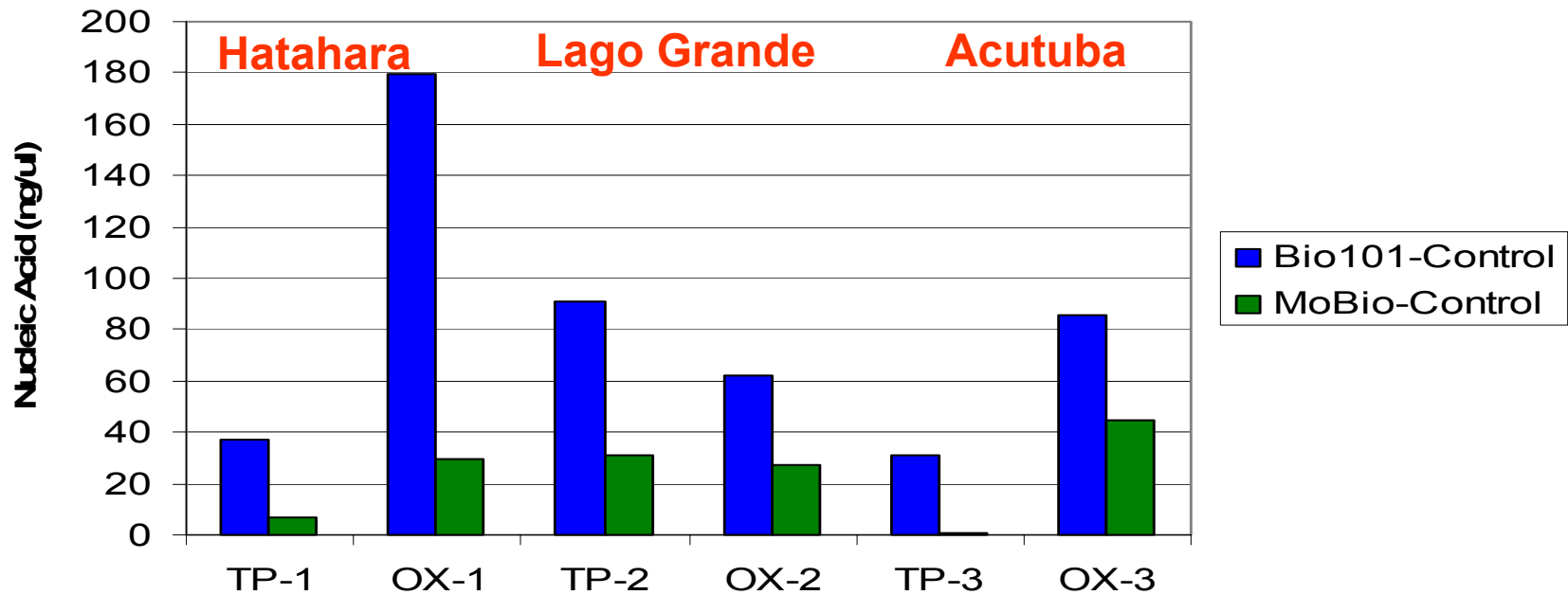
**Measures purity of DNA extract.**

# Microbiological enumeration – sites chosen

MPN enumeration on liquid R2A Media - TP and Oxisol



# Results: Initial DNA extraction using two commercial kits

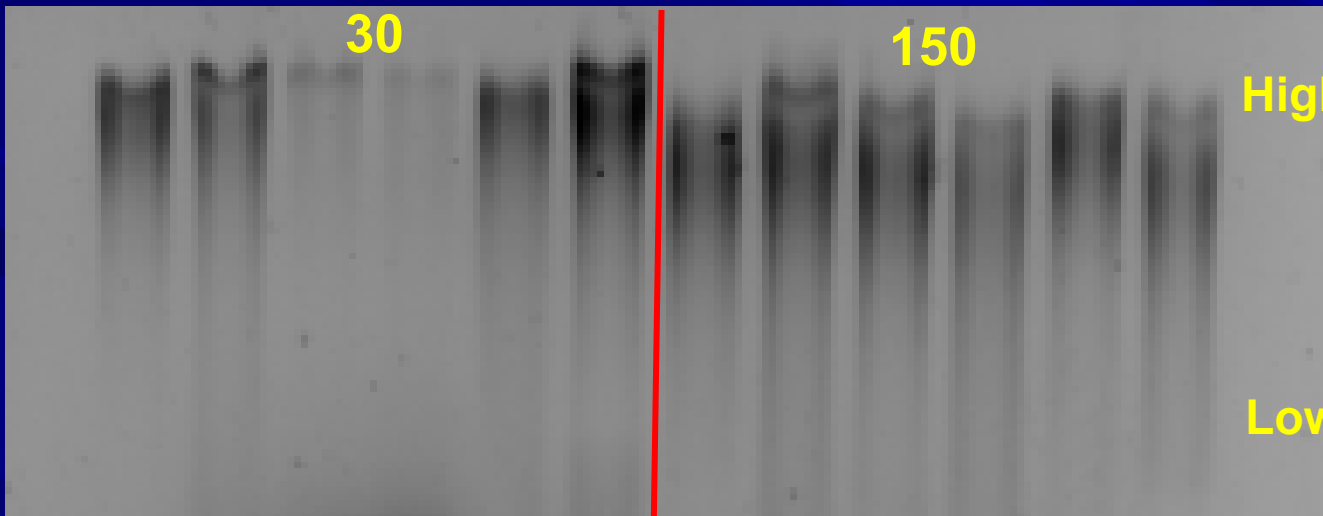
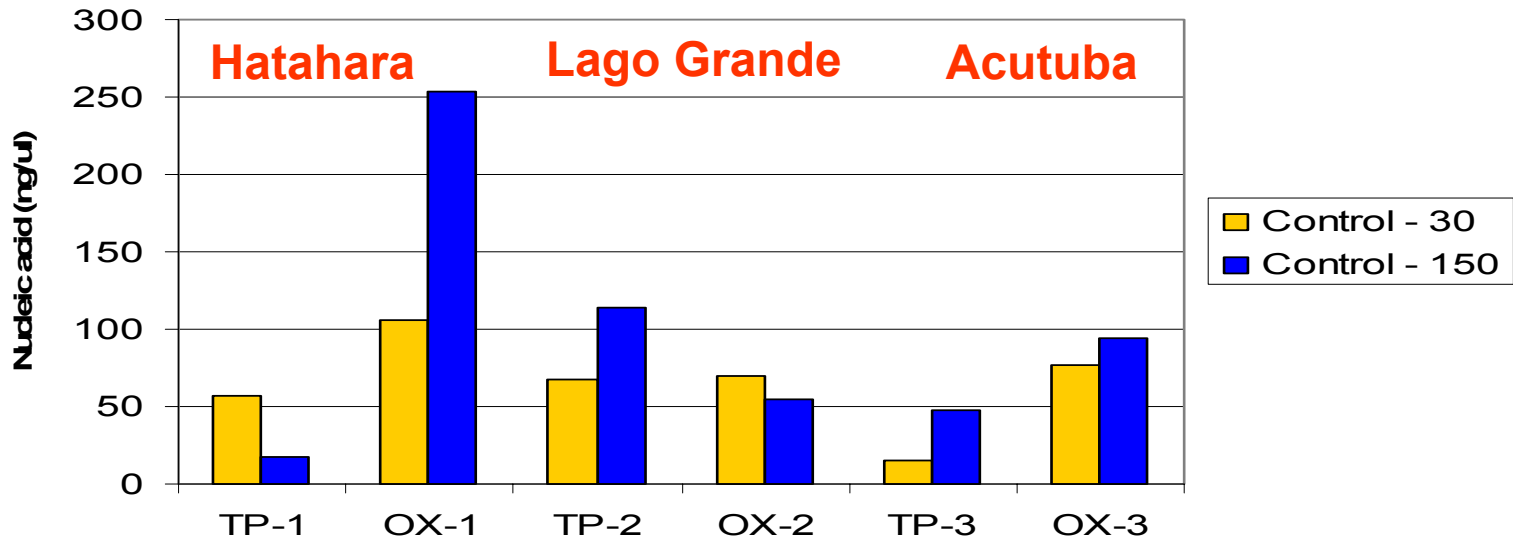


MoBio

Bio101

# Results: Bead beating and DNA yield

Nucleic Acid Yield with 30 and 150 Seconds Bead Beating for Cell Lysis

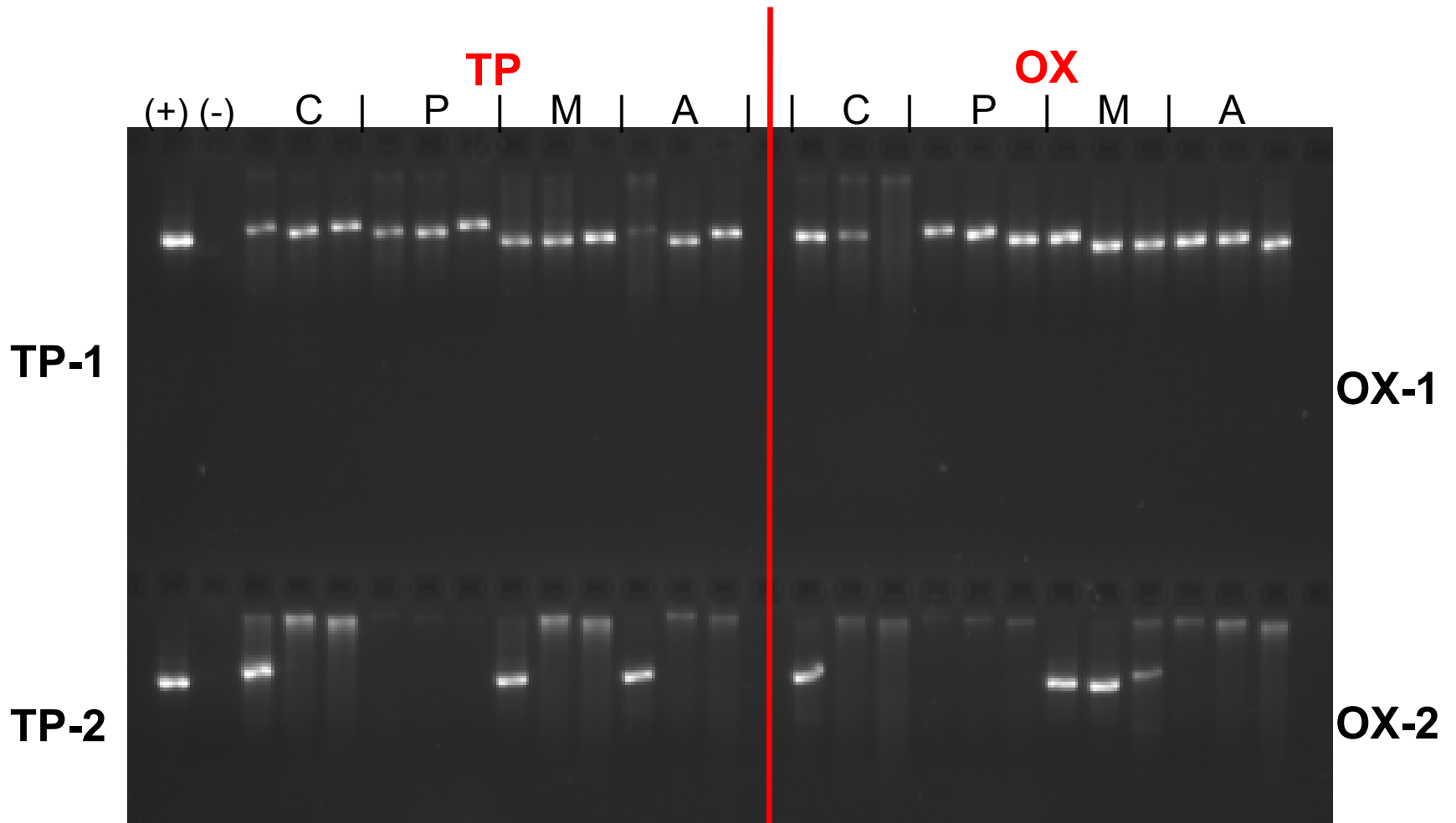


High Molecular Weight



Low Molecular Weight

# Results: PCR with different chemical treatments

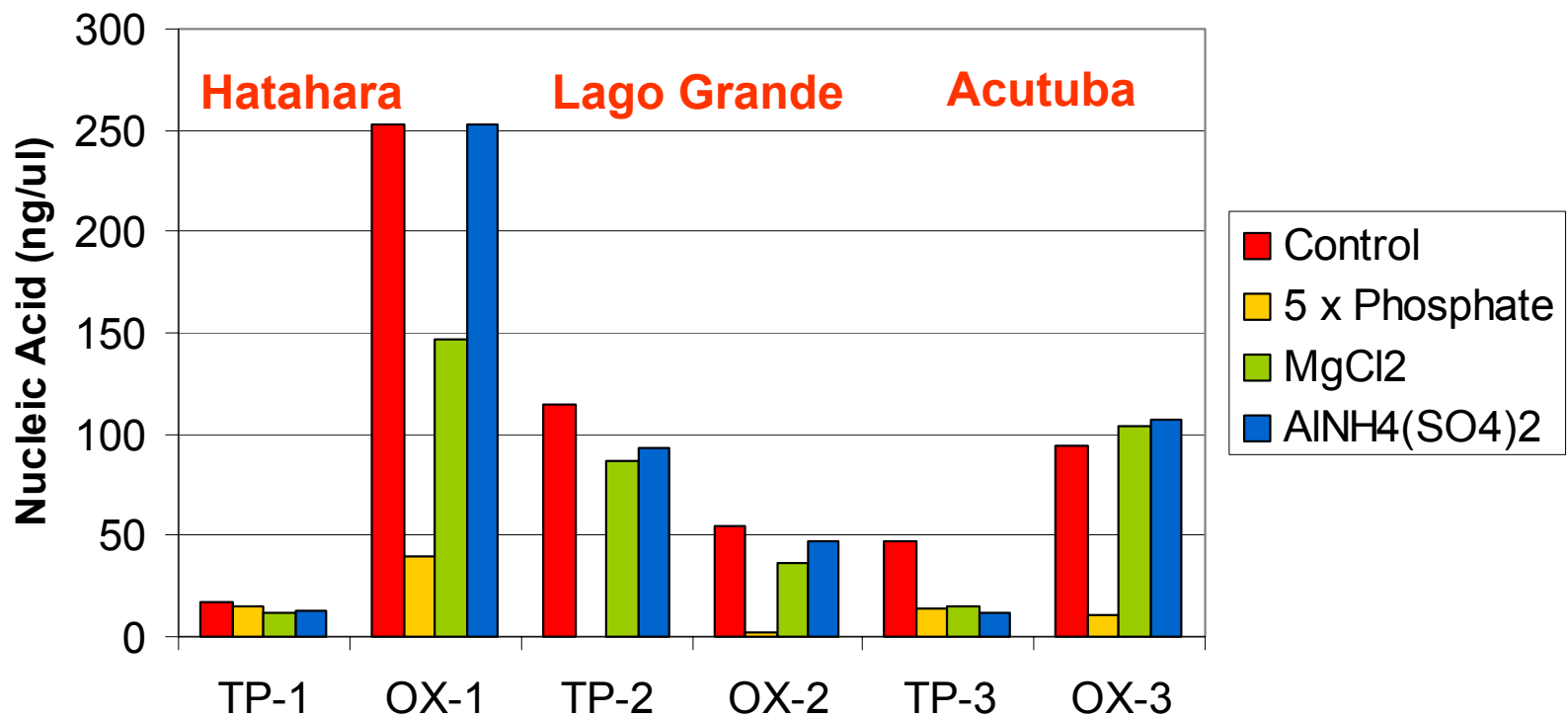


C = Control (no addition to commercial kit)  
M =  $MgCl_2$

P = 5 x Phosphate buffer  
A =  $AlNH_4(SO_4)_2$

# Results: Effect of chemical treatments on community DNA yield

DNA Yield Using Chemical Treatments - TP and Adjacent Soils

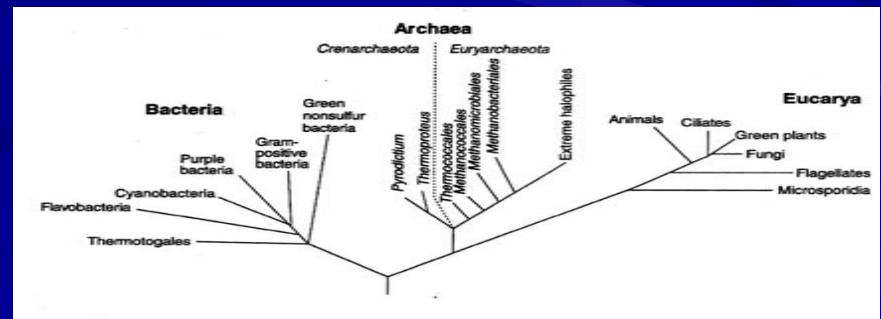
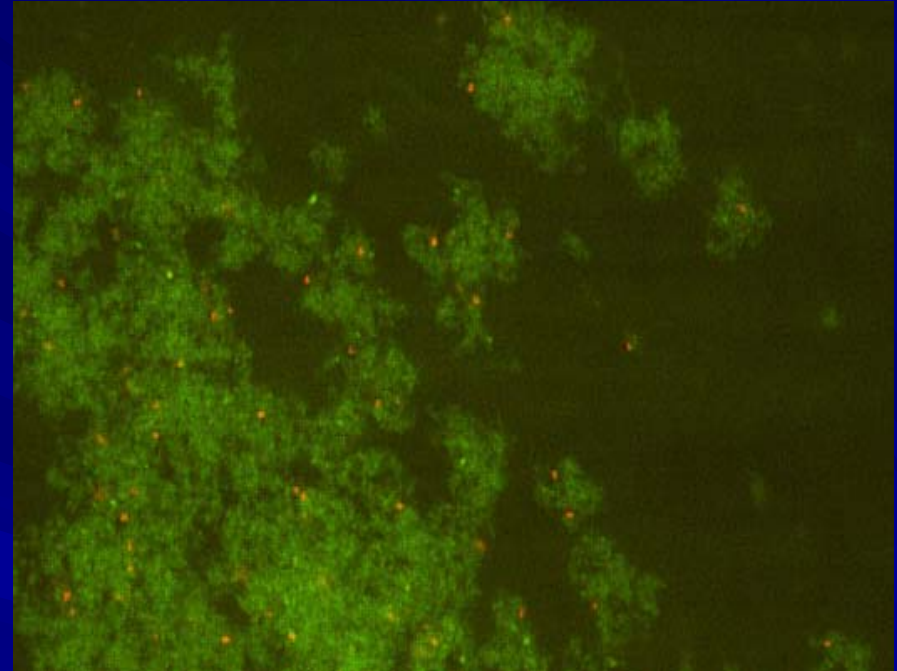


# Conclusions

- Some extraction kits are better than others for particular soils.
- In some cases, increased bead beating time does increase DNA yield, but it can increase DNA shearing, which may impact downstream molecular analyses.
- Some additions and modifications of kits may improve PCR.  $\text{MgCl}_2$  is the more promising among them, but not best for all soils.

# Further work and optimization

- Use microscopy to assess efficiency of cell lysis.
- Determine best purifiers for each sample to improve downstream molecular analyses
- Characterize microbial community in *terra preta* and adjacent soils using molecular methods such as T-RFLP and DGGE.





Still more  
digging to get  
to the bottom  
of *terra preta*!

Thank You

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