EFFECT OF NITRATE AND PHOSPHATE CONCENTRATION ON SIMULTANEOUS PHENANTHRENE AND OCTADECANE BIODEGRADATION

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OUTLINE

- Background
- Objectives
- Experimental Design
- Some experimental results
- Future work

BACKGROUND

1. **Bioremediation of oil contaminated sites**
   - Oil spills
   - Biostimulation ⇒ optimal nutrient supply?
2. **Resource Ratio Theory**
3. **Development of molecular tools and their application to environmental engineering**
   - Fingerprinting techniques ⇒ study of population dynamics (Macnaughton et al., 1999)
   - Detection and identification of bacterial populations in environmental samples

     More than 99% of the organisms present in the environment cannot be cultivated by standard culturing-techniques (Amann *et al.,* 1995)

RESOURCE RATIO THEORY

Theoretical framework in Microbial Ecology to predict the outcome of competitive interactions

RESOURCE: *any substance or factor that can increase microorganism growth rate and, that is consumed by the microorganisms* (Tillman, 1982)

⇓

COMPETITION

Nutrients ⇒ N and P **are resources**

Two hypothesis:

1. Changes in the relative N:P supply ratio will cause significant changes in the microbial community structure, ⇒ rate and extent of biodegradation
2. Changes in absolute nutrient supply levels, at a constant supply ratio, will alter the total hydrocarbon biodegrading biomass, and consequently, the rates of degradation.

OBJECTIVES

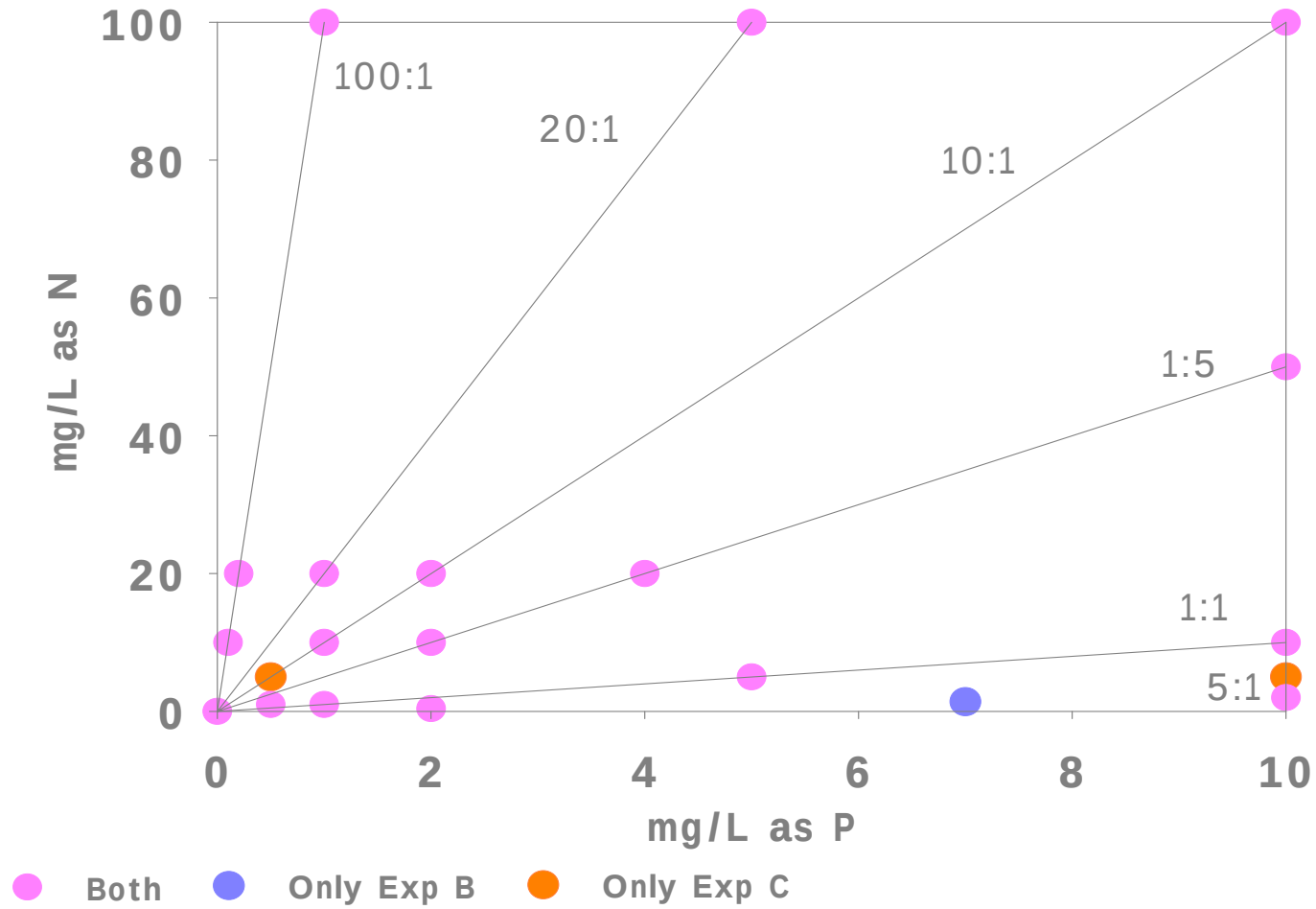
To test the applicability of the resource ratio theory to the biodegradation of oil hydrocarbons

- **To study effect of *initial N:P ratios* and *absolute nutrient supply levels* on:**
  - rate and extent of biodegradation of selected alkanes and PAHs
  - microbiological community structure
- **To investigate *relationships* between rates and extent of degradation and shifts in the active microbial community.**
- **To develop a *bioremediation strategy* within the theoretical framework of the resource-ratio theory.**

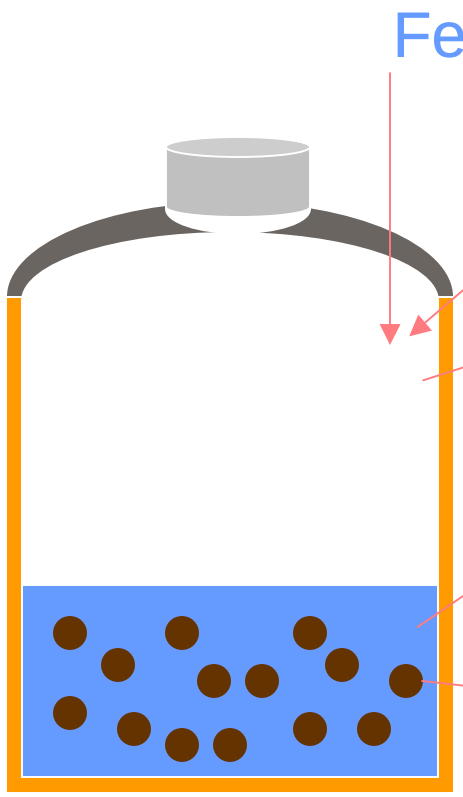
DESIGN PARAMETERS

- **Tested Variables:**
  - *Initial N:P ratio*
  - *Absolute initial N and P supply*
- **N and P sources:** *KNO_3* and *K_2HPO_4*
- **C sources:**
  - C_{18} + Phenanthrene (Experiment B)
  - 14 alkanes + 14 PAH (Experiment C)
- **360 serum bottles**
  - *6 N:P ratios* with *3 concentrations* each
  - An *sterile* control and a *no nutrients* control
  - *12* sampling times over *60* days
    (6 for biological analysis)
- **Mixed culture from EPA's culture collection**

N:P CONCENTRATIONS AND RATIOS



EXPERIMENTAL DESIGN



C18 and Phenanthrene
2.5 mg/bottle

15 mL of buffered nutrient solution containing Nitrate (pH 7.8)

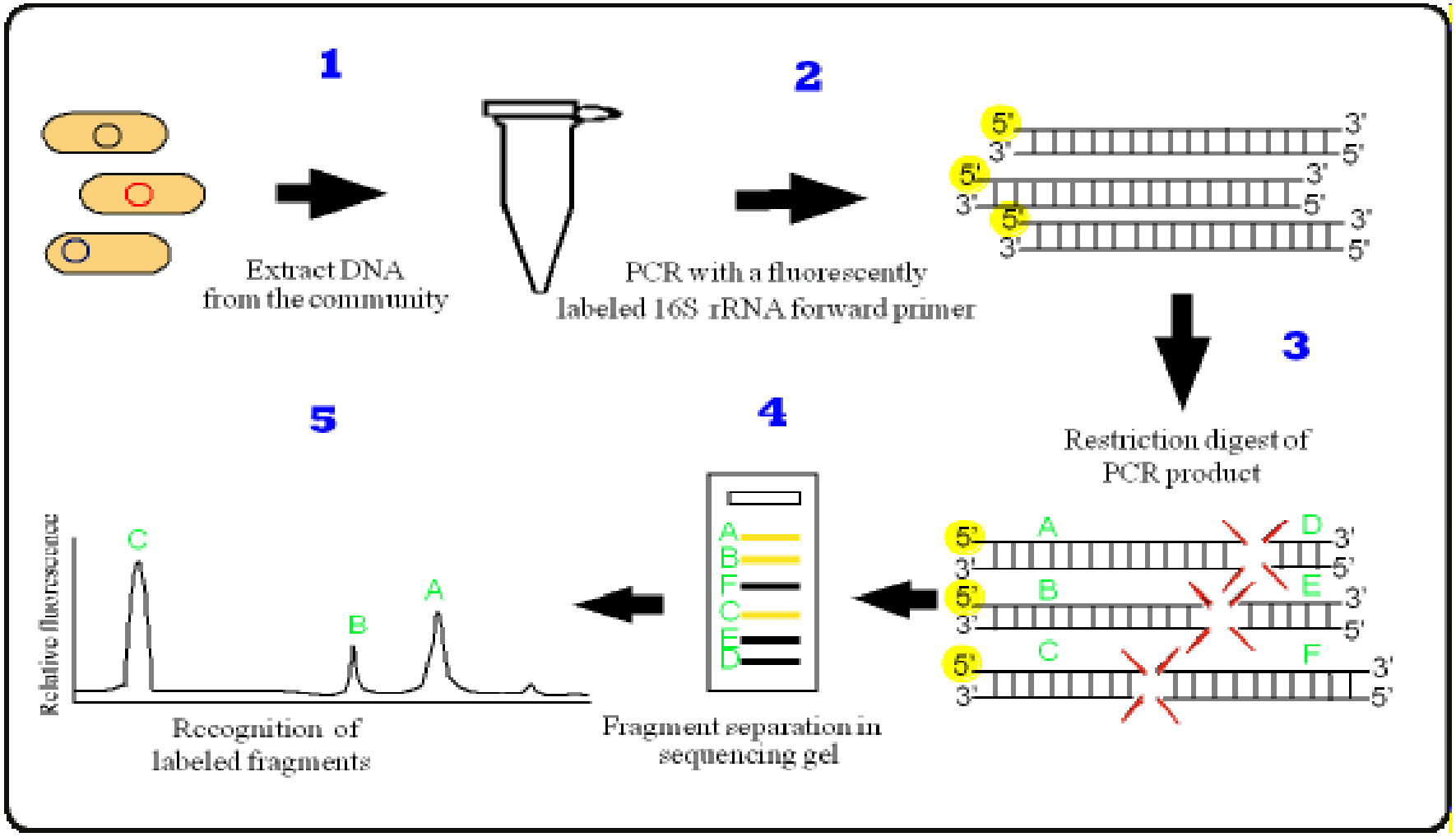
Inoculum

160 mL serum bottles

ANALYTICAL METHODS

- **OIL CHEMISTRY**
  - GC/MS analysis of targeted alkanes and PAHs *(Method 8270C)*
- **NITRATE AND PHOSPHATE ANALYSIS**
  - Ion Exchange Chromatography *(Dionex DX 500 with an IonPacR AS14)*
- **MICROBIOLOGY**
  - Most Probable Number (MPN) *(Wrenn, B.A. and Venosa, A.D., 1996)*
  - Phospholipids *(Findlay, R. et al., 1989)*
- **MOLECULAR METHODS**
  - Terminal Restriction Fragment Length Polymorphism (T-RFLP) *(Liu, W.T. et al., 1997; Marsh, T. L., 1999)*

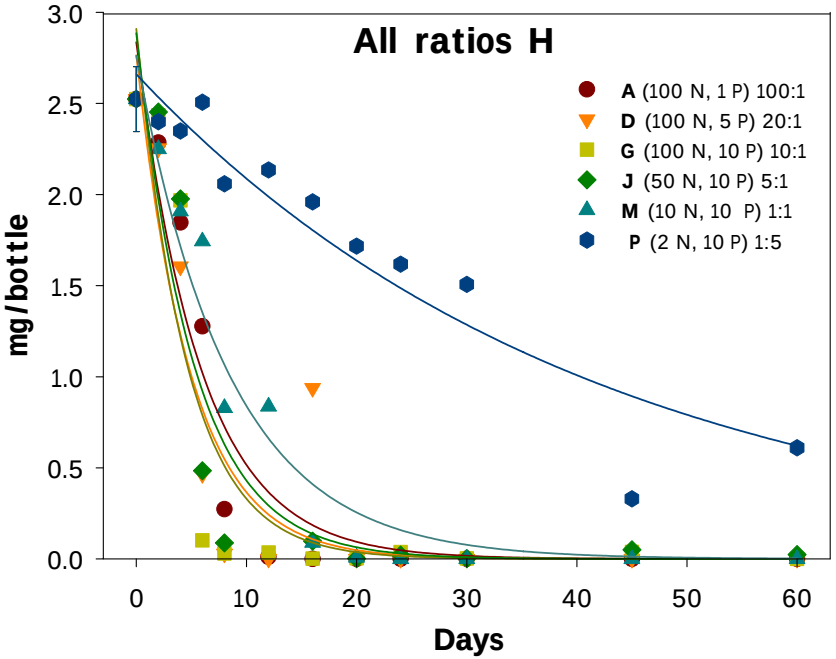
T-RFLP

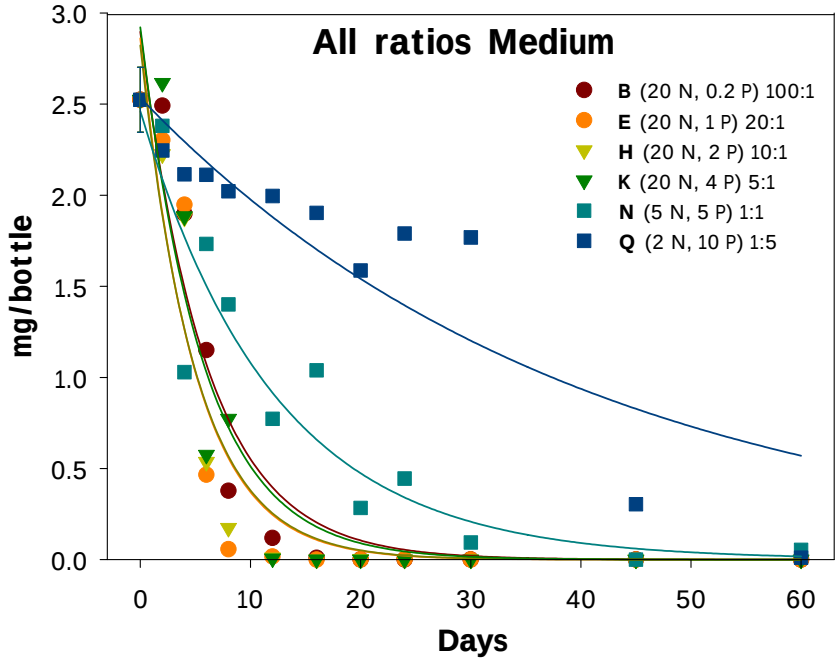


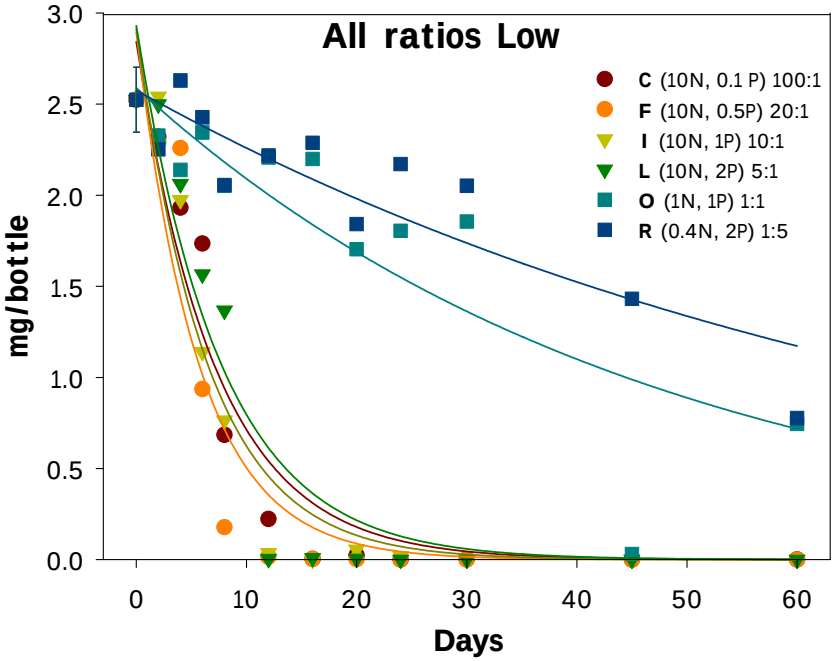
V. Grüntzig, B. Stres, H. L. Ayala del Río, and J. M. Tiedje,2002 (http://rdp.cme.msu.edu/html/t-rflp_jul02.html)

PHENANTHRENE

There is an effect of N and P on the biodegradation rate of phenanthrene

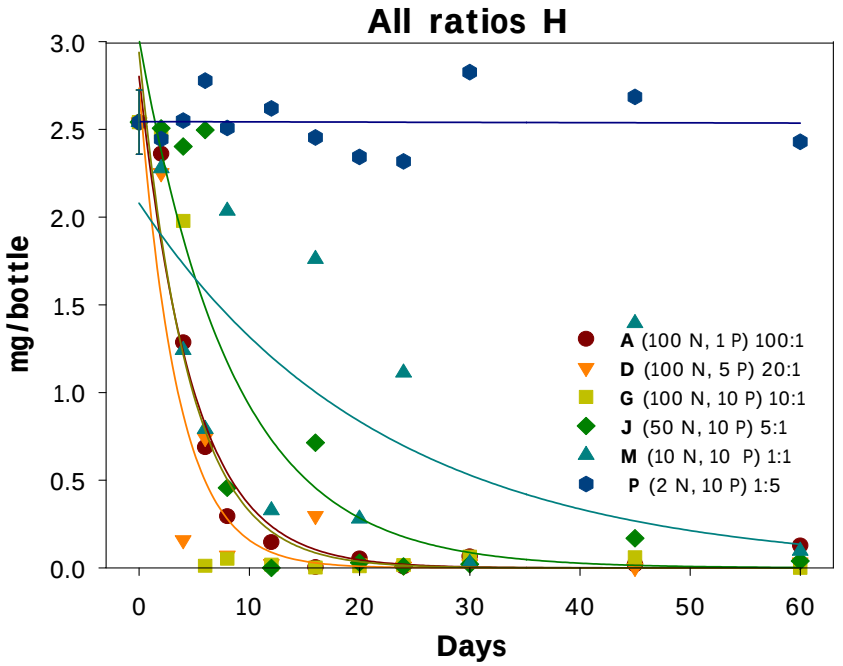


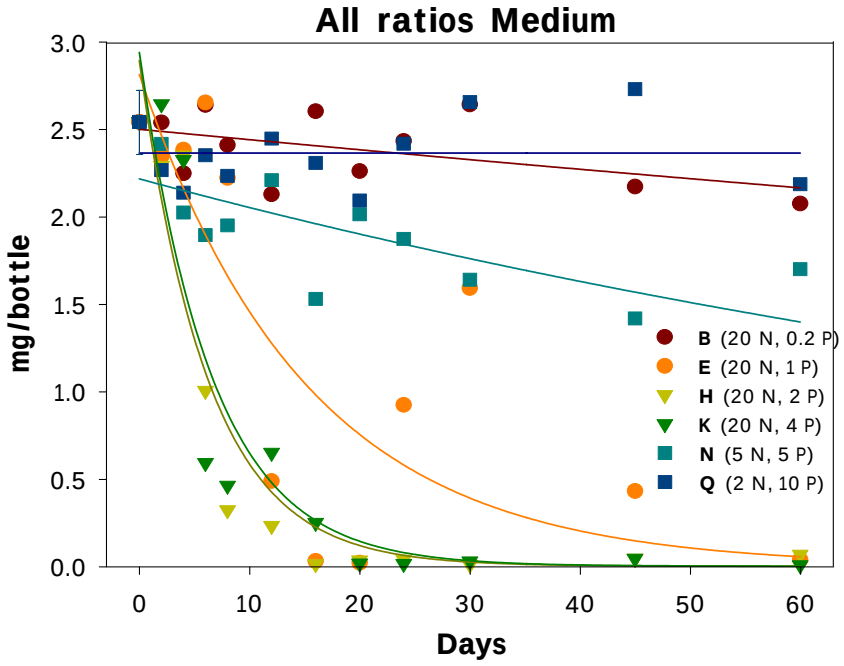


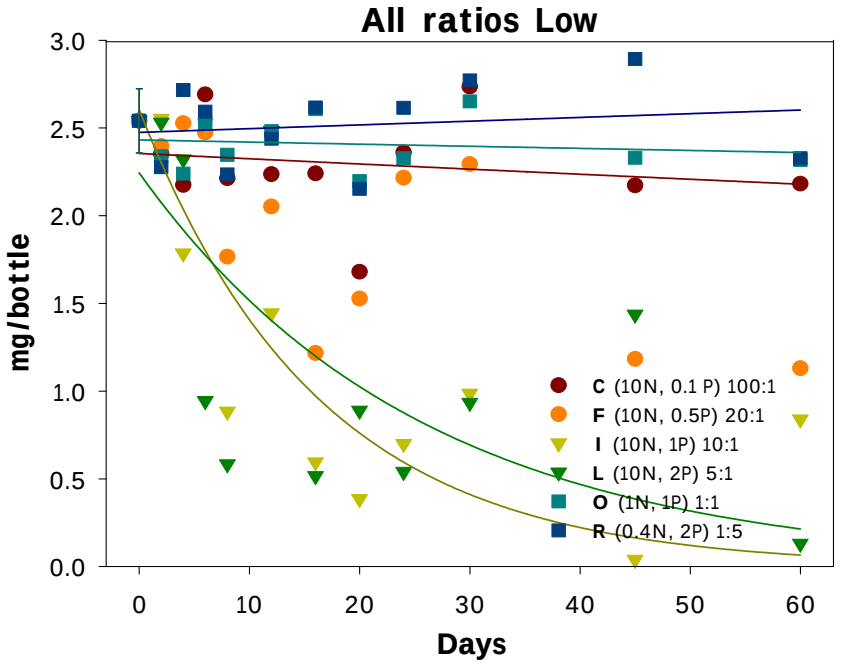


OCTADECANE

There is more pronounced effect of N and P on the biodegradation rate of octadecane

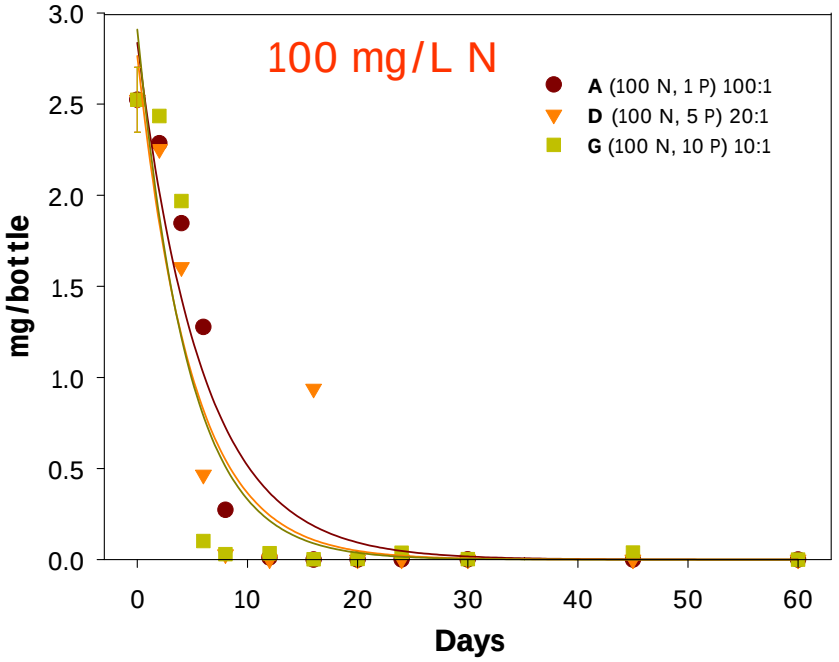


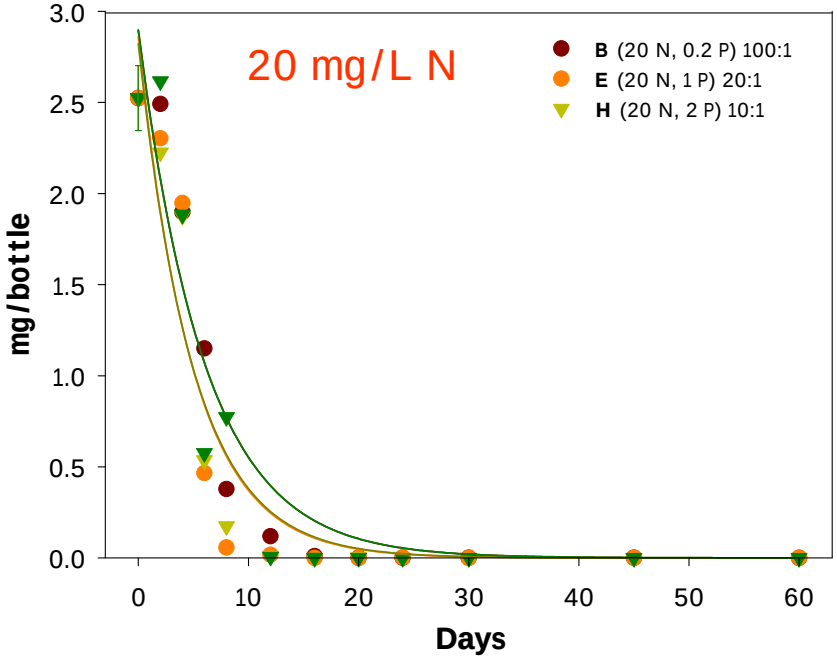


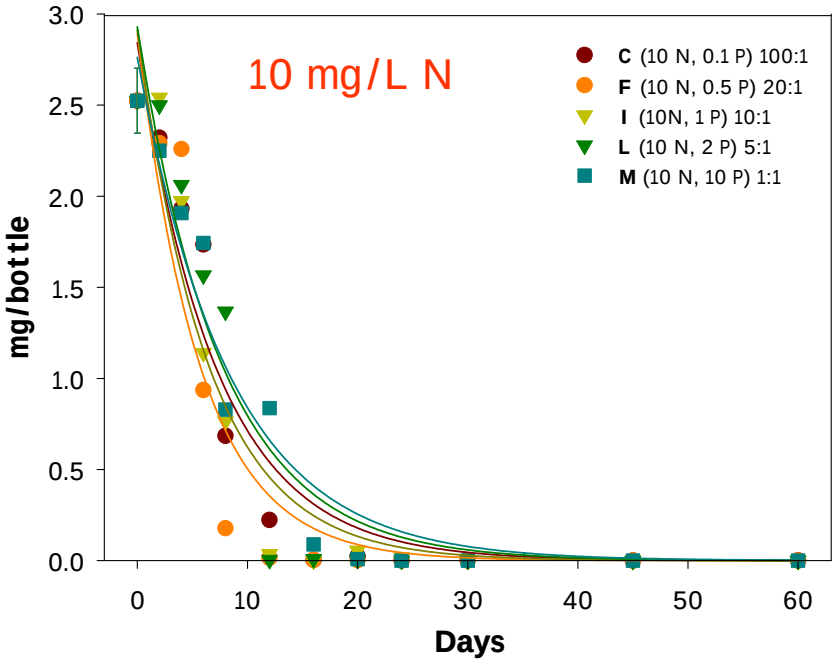


HYPOTHESIS # 1

Changes in the relative N:P supply ratio will cause significant changes in the microbial community structure ⇒ rate and extent of biodegradation





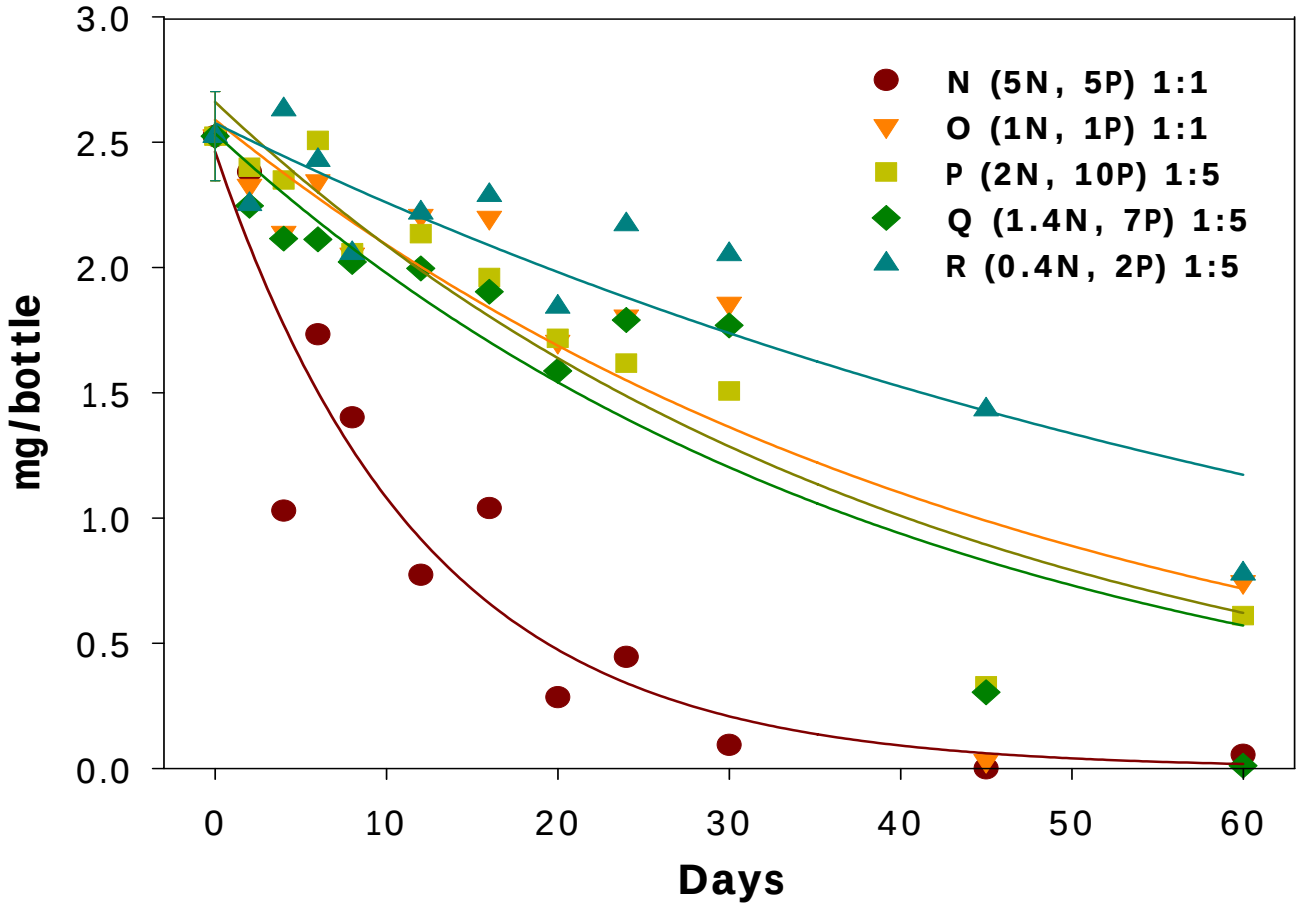


PHENANTHRENE

Constant N /Variable P

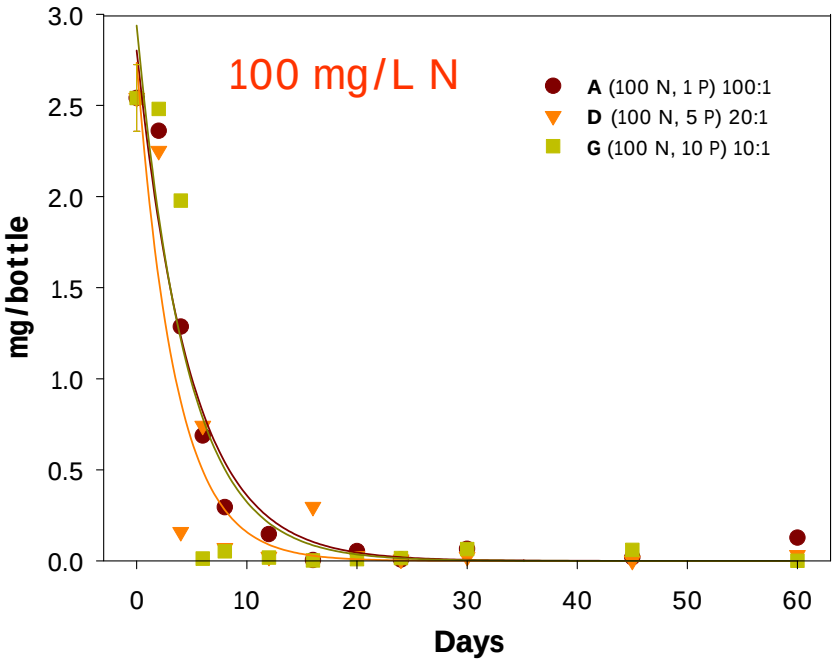
For 100, 20 or 10 mg/L of N,
P does not have a significant effect
on phenanthrene biodegradation

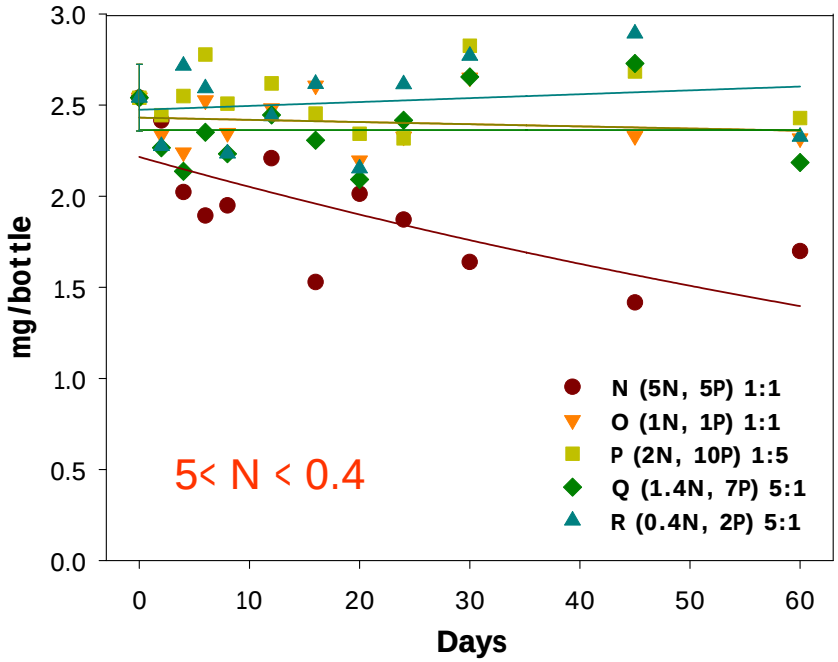
PHENANTHRENE



At lower values of N, phenanthrene degradation slows down even if the concentration of P is high

OCTADECANE

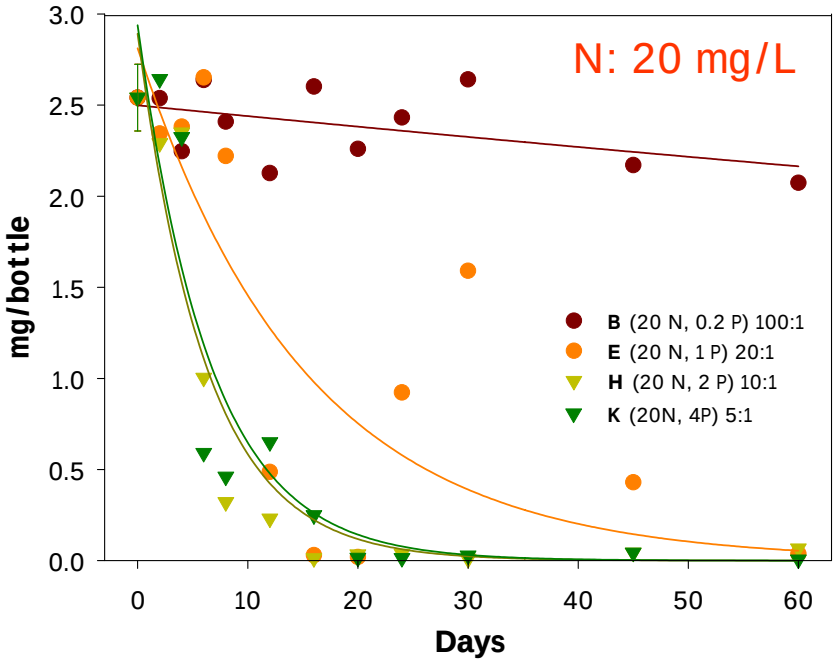


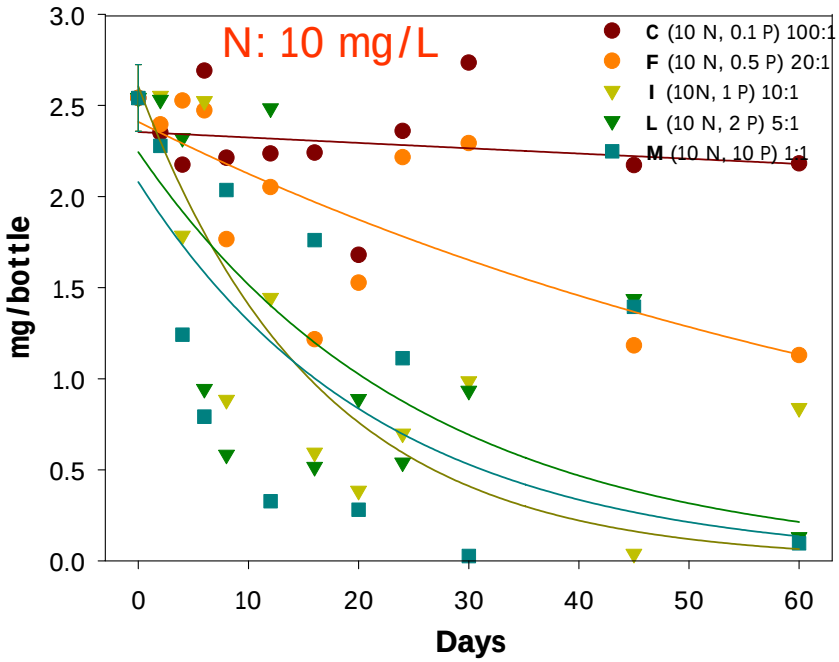


Constant N/ Variable P

- When N is highest (100 mg/L) or lowest (0.4 to 1 mg/L), no effect of P
- Octadecane is not degraded for N <2 mg/L

OCTADECANE





Constant N/ Variable P

At intermediate concentrations of N,

- Octadecane degradation INCREASES with decreasing ratio (or with increasing P concentration)
- No C_{18} degradation when P < 0.5 mg/L

HYPOTHESIS # 1

Changes in the relative N:P supply ratio for a constant concentration of N…

… **did not affect phenanthrene degradation**

that seems independent on P concentration

… **had some effect on C_{18} degradation**

at intermediate N concentrations

Further study: T-RFLP ⇒ community structure

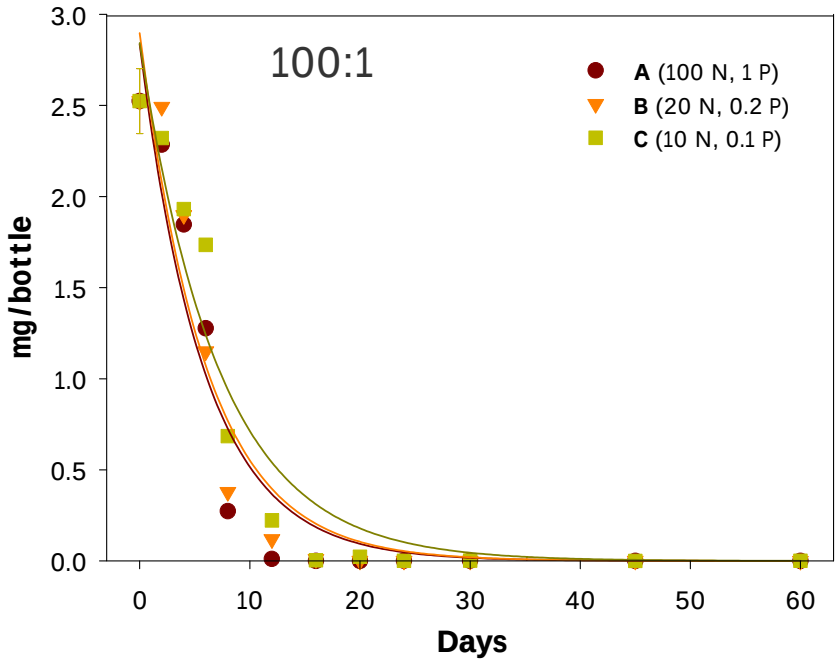
- **Degradation rate coefficients were in most cases higher for phenanthrene than for C_{18}.**

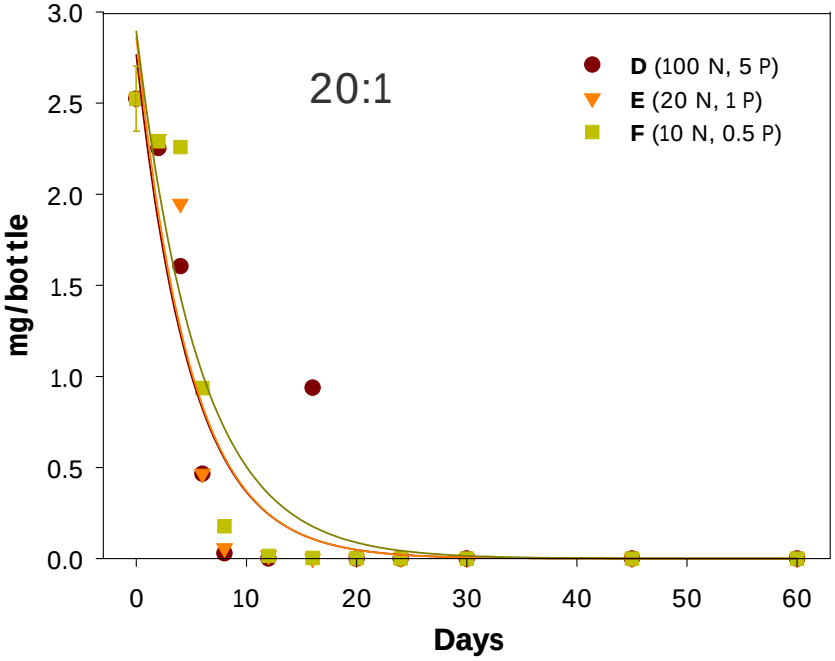
HYPOTHESIS # 2

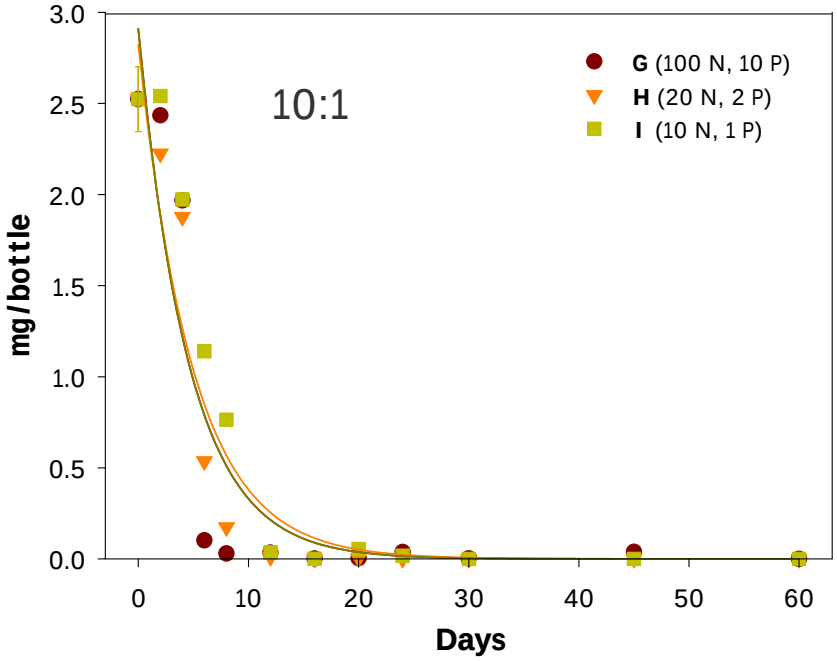
Changes in **absolute nutrient supply** levels, at a constant supply ratio, will alter the total hydrocarbon biodegrading biomass, and consequently, the **rates of degradation**

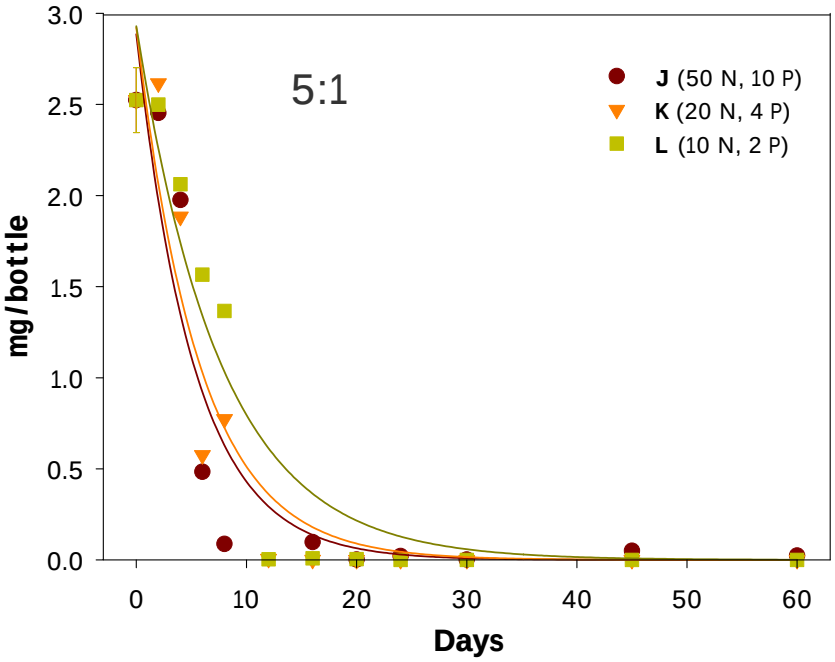
PHENANTHRENE

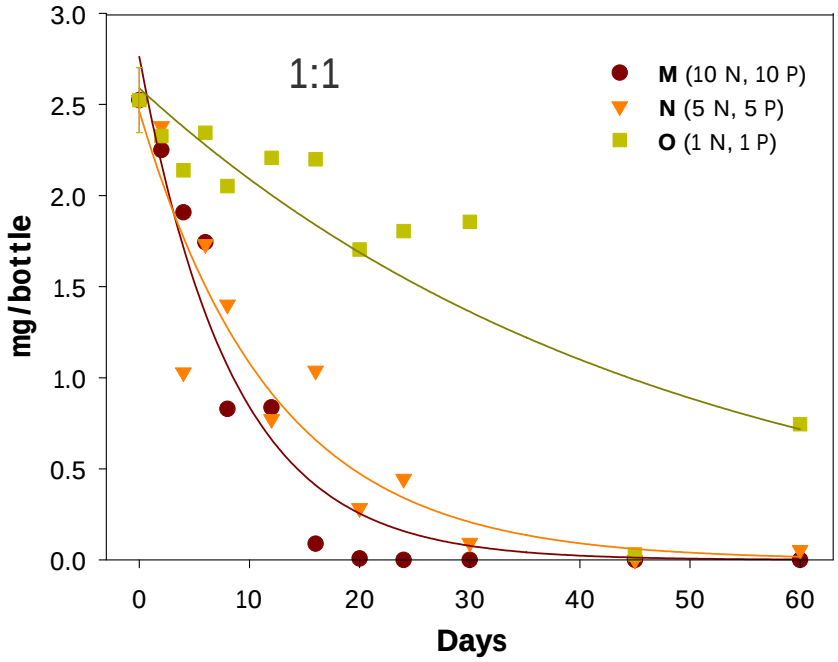
Effect of absolute nutrient supply for the same N:P ratios

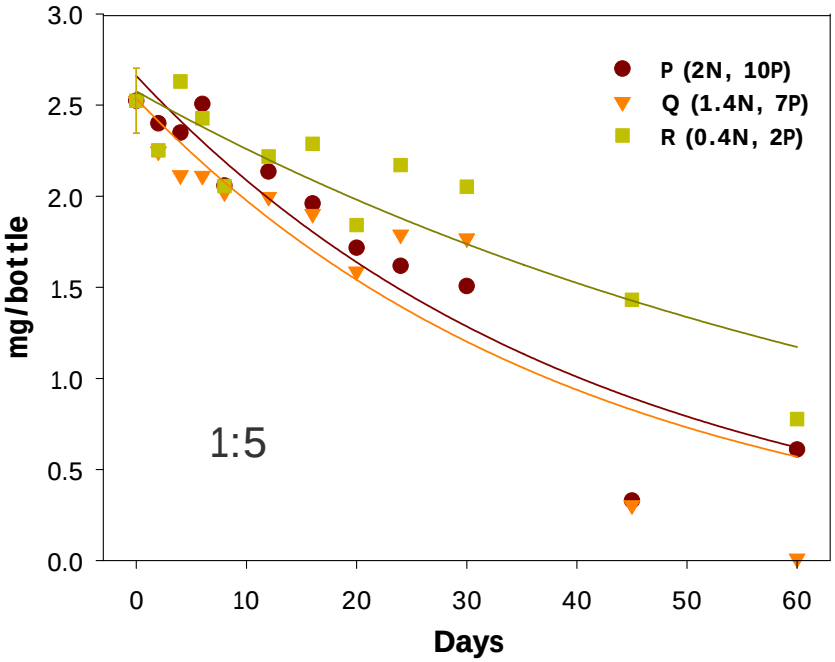






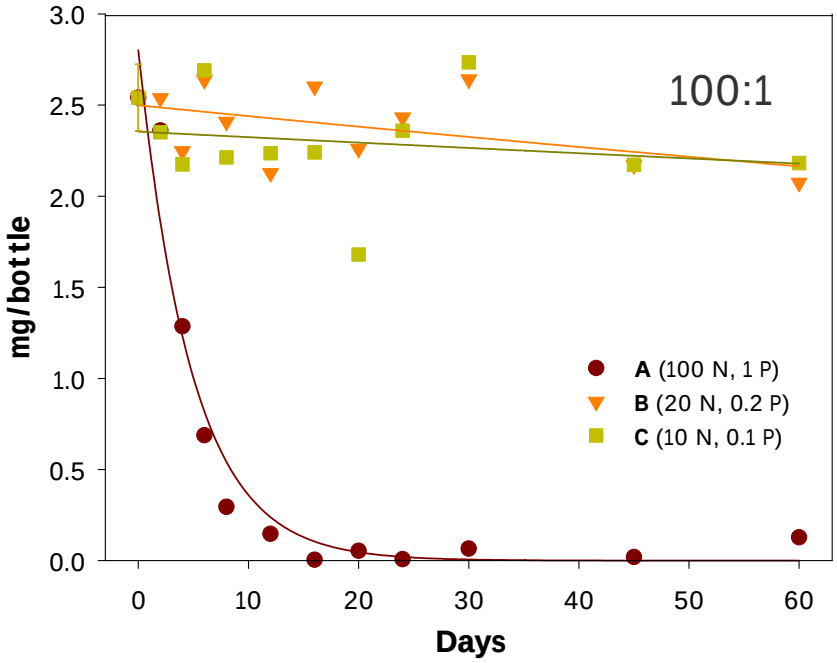






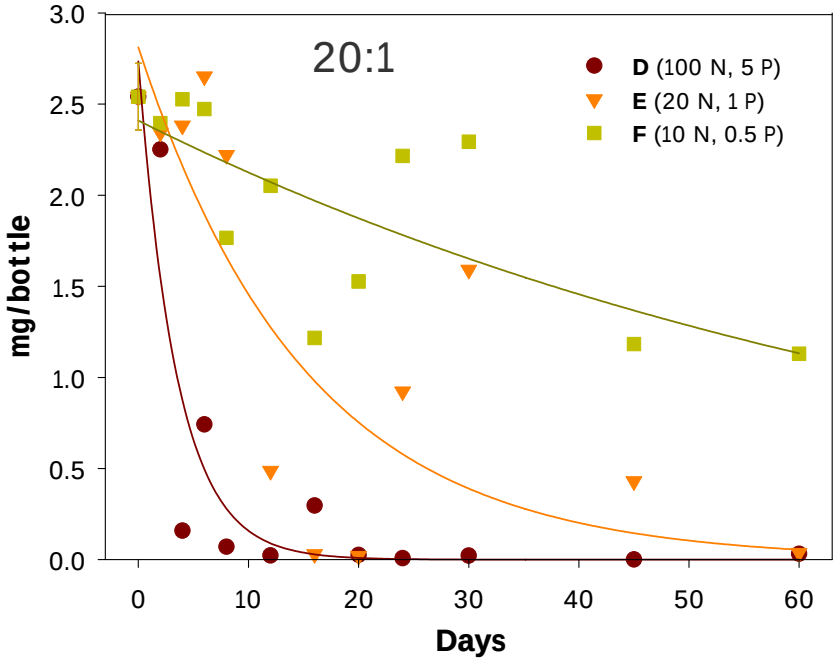
As predicted, for a N:P ratio, degradation rate INCREASES with absolute concentrations

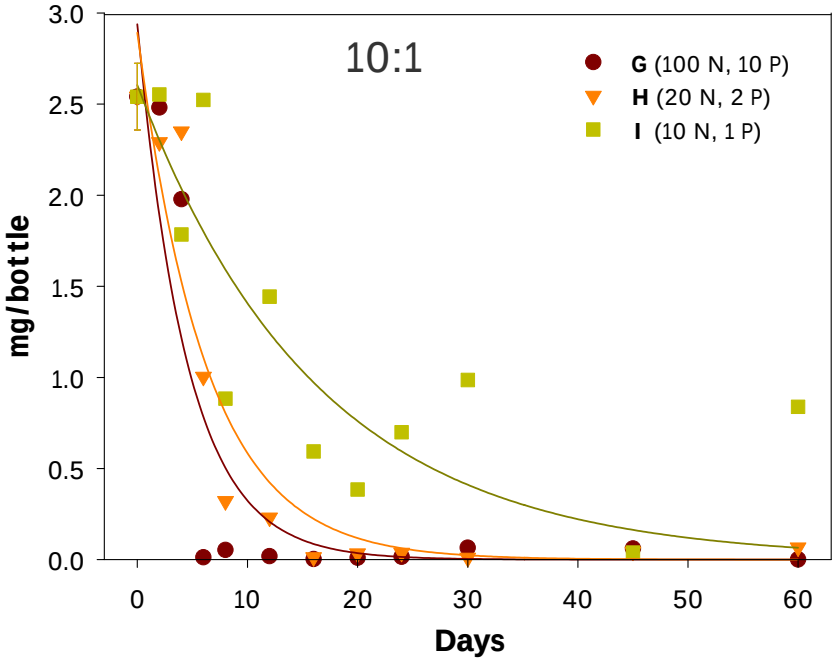
Differences are not significant for high ratios

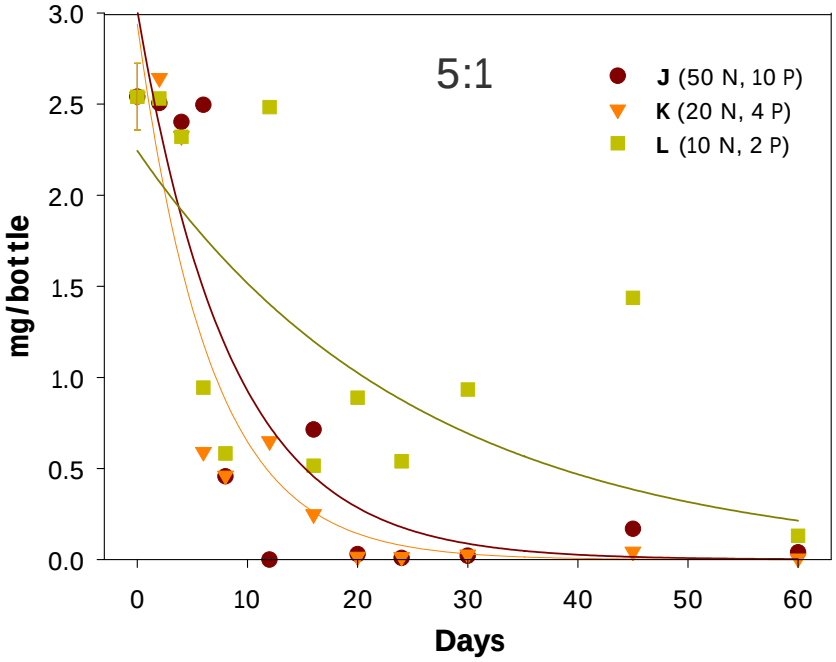


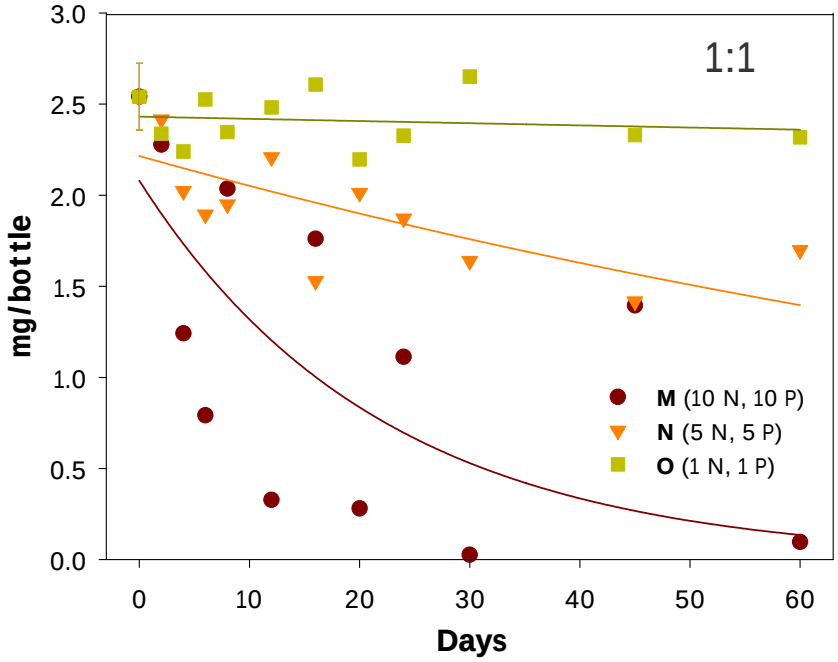
OCTADECANE

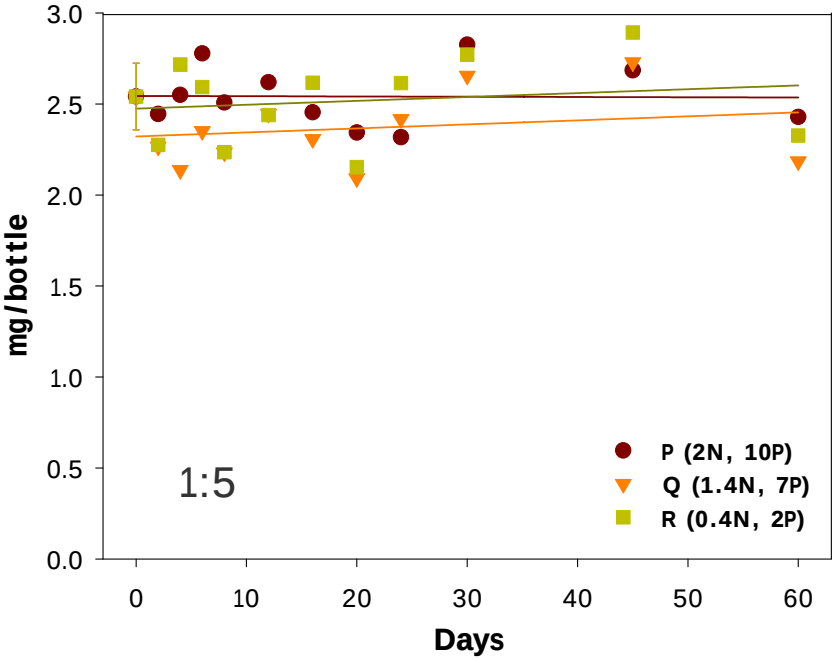
Same N:P ratios
different absolute amounts











As predicted,
for a same N:P ratio,
degradation rate
INCREASES
with absolute concentrations

Differences are more dramatic
than
for phenanthrene

HYPOTHESIS # 2

Changes in absolute nutrient supply levels,

at a constant supply ratio…

… affected hydrocarbon biodegrading biomass

not strong correlation **(data not shown)**

… influenced degradation rate

- No significant for high ratios and phenanthrene
- More dramatic for C_{18}

- **No octadecane degradation for N<2 mg/L (C:N 150:1) and P<0.5 mg/L (C:P 600:1)**

CONCLUSIONS

1. **Faster degradation rate for phenanthrene than C_{18} (Jackson and Pardue,1997;1999)**
   - Cultures richer in PAH degraders
   - Phenanthrene solubility in water is higher than for C_{18}
2. **Best treatment for simultaneous degradation (more than 95% gone after 6 days)**
   - **100 mg/L N, 10 mg/L P C:N:P 30:10:1**
3. **Same degradation extend could be achieved after**
   - 16 days: 100 mg/L N, 1 mg/L P **300:100:1**
   - 20 days: 20 mg/L N, 2 of mg/L P **150:100:1**
   - even 60 days: 10 mg/L N, 10 mg/L P **30:1:1**

CONCLUSIONS

4. Background P could be enough to support phenanthrene degradation, if N is supplied to **C:N 30:1**
5. For C_{18} degradation N and P must be at least in the ratios **C:N 150:1** and **C:P 600:1**
6. By varying N and P concentrations we can select for simultaneous degradation or for degradation of only phenanthrene first
7. Data suggest different microbial community structures $\Rightarrow$ Molecular tools

   relationship: **rate** - **microbial community structure**

PRESENT AND FUTURE WORK

1. Study of effect of N and P on microbial community structure ⇒ T-RFLP
2. Second parametric study with a more complex carbon source:

   14 n-alkanes + 14 PAHs

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- Dr. Makram T. Suidan
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- Suvid Surendran