

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

Susana Garcia-Blanco, Suvid Surendran, Makram
T. Suidan, Daniel B. Oerther

Department of Civil and Environmental Engineering, University of Cincinnati, USA

Albert D. Venosa

National Risk Management Research Laboratory, US EPA, USA

OUTLINE

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

BACKGROUND

1. Bioremediation of oil contaminated sites

- Oil spills
- Biostimulation \Rightarrow optimal nutrient supply?

2. Resource Ratio Theory

3. Development of molecular tools and their application to environmental engineering

- Fingerprinting techniques \Rightarrow 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 \Rightarrow **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**, \Rightarrow 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

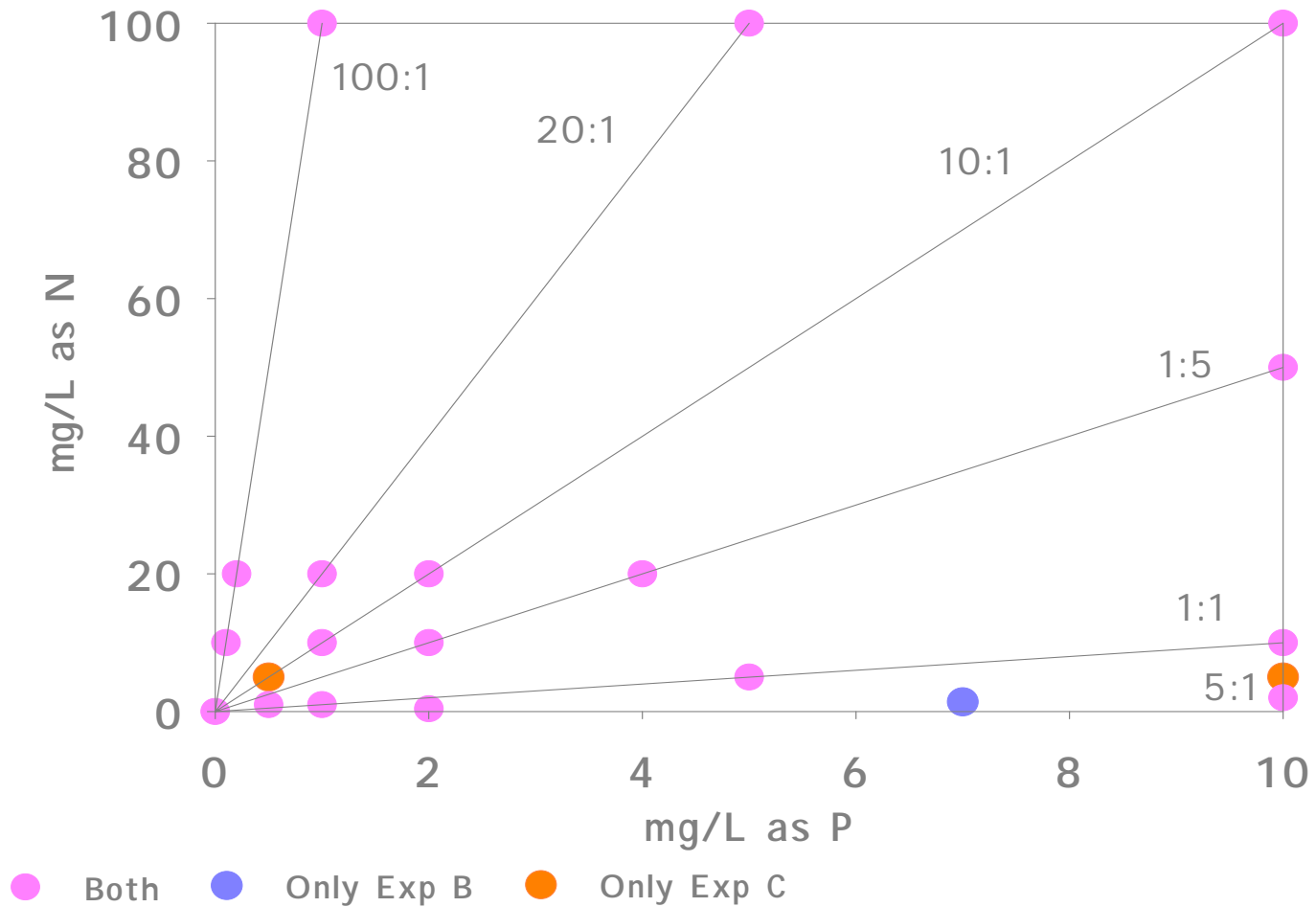
To test the applicability of the resource ratio theory to the biodegradation of oil hydrocarbons

- 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.

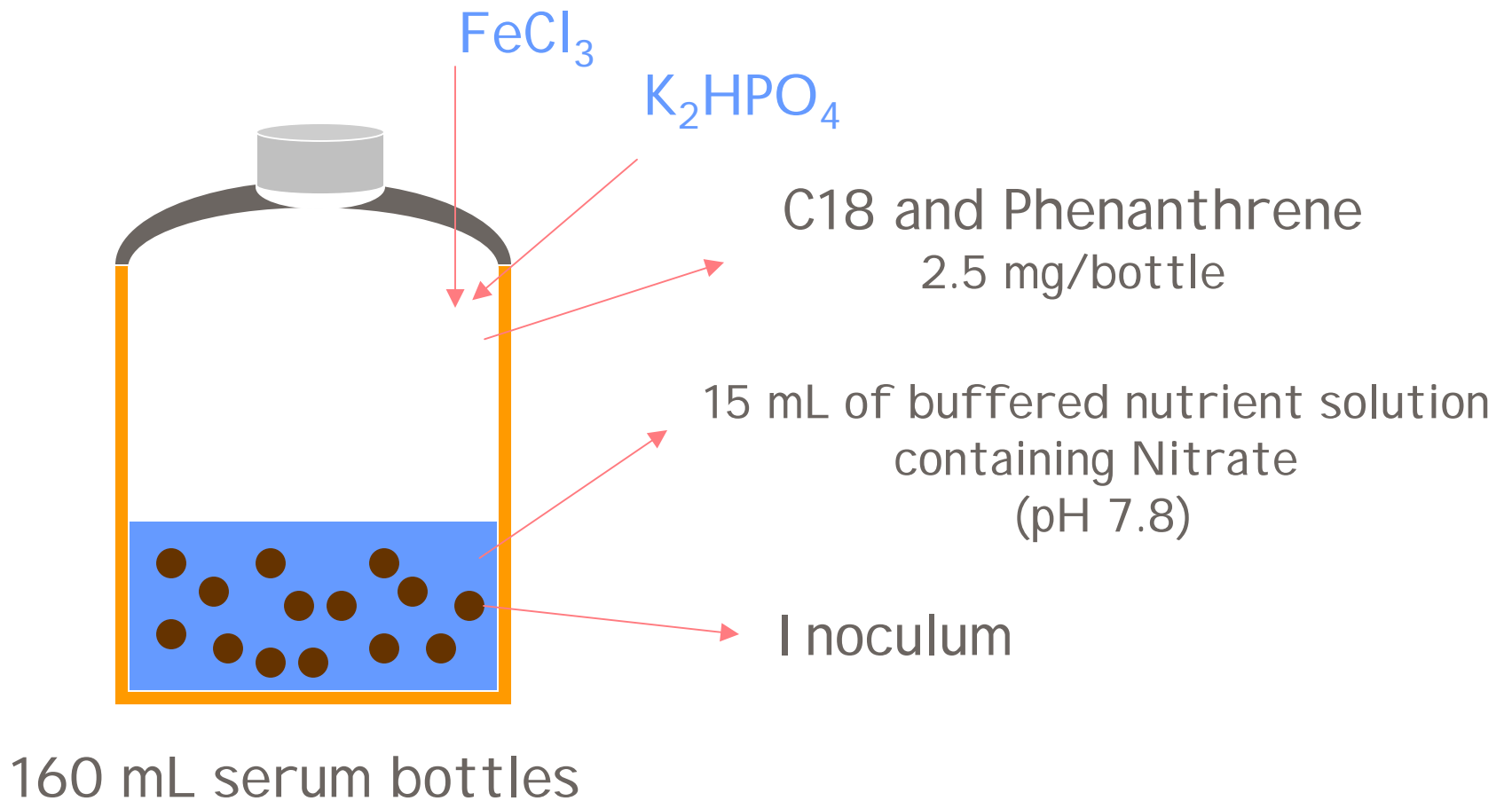
DESIGN PARAMETERS

- 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



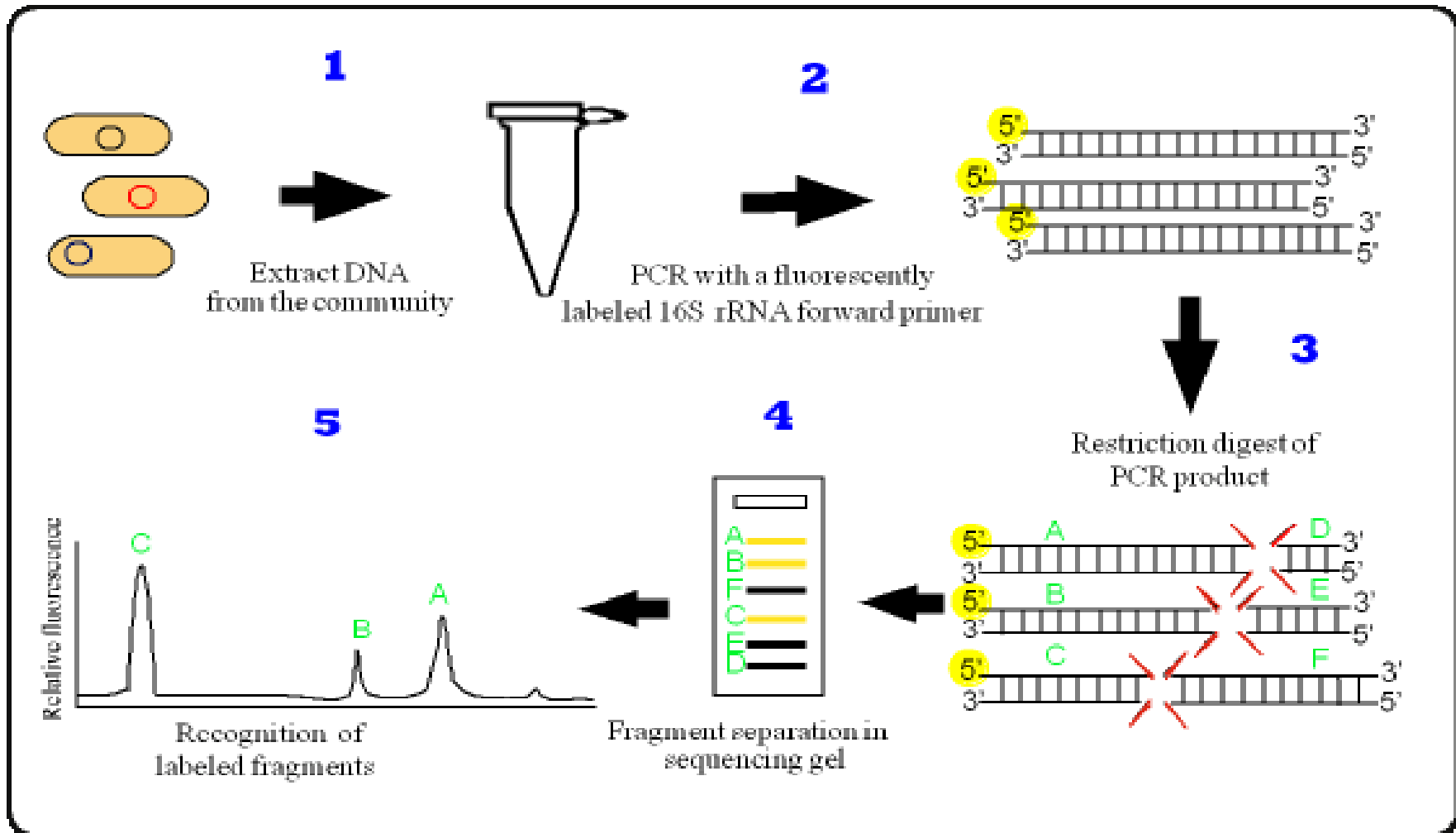
EXPERIMENTAL DESIGN



ANALYTICAL METHODS

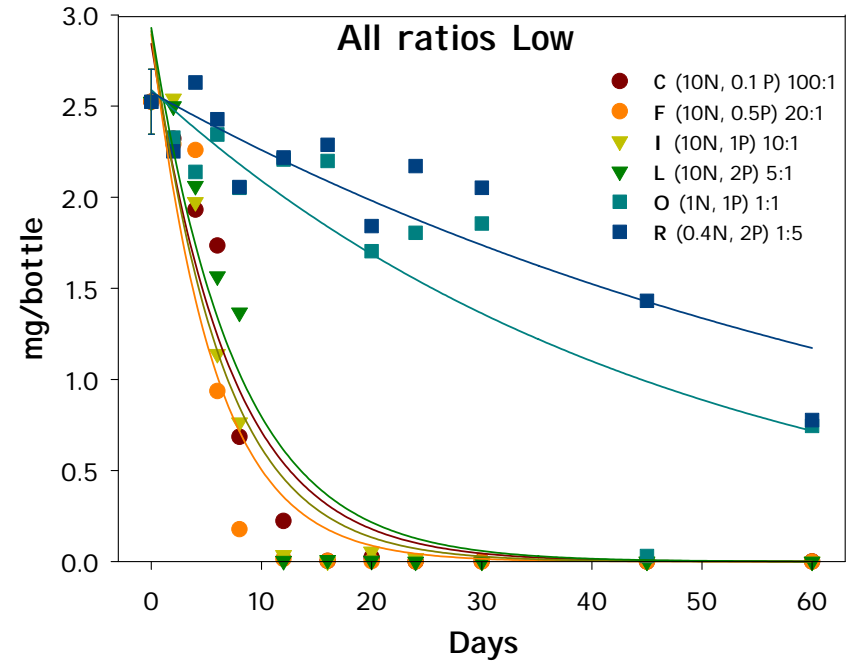
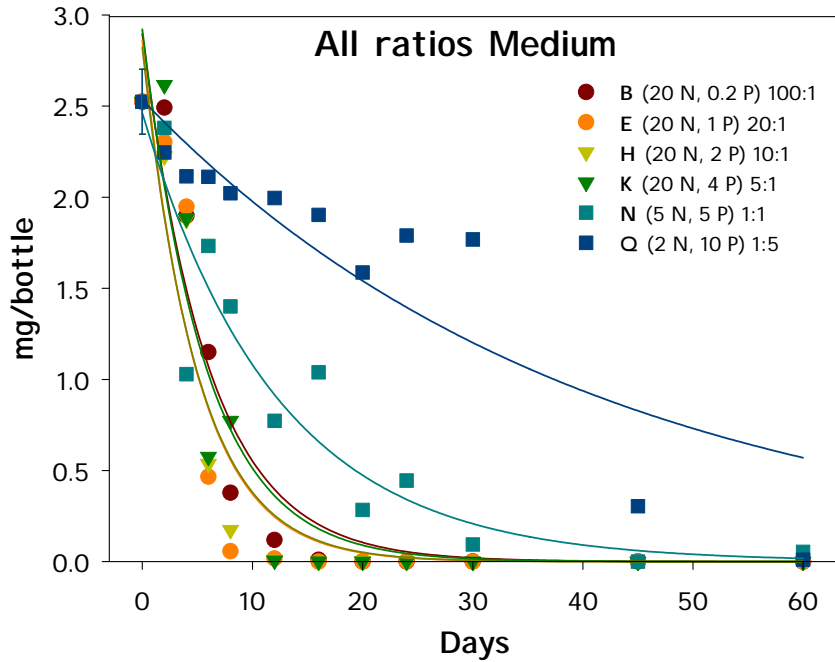
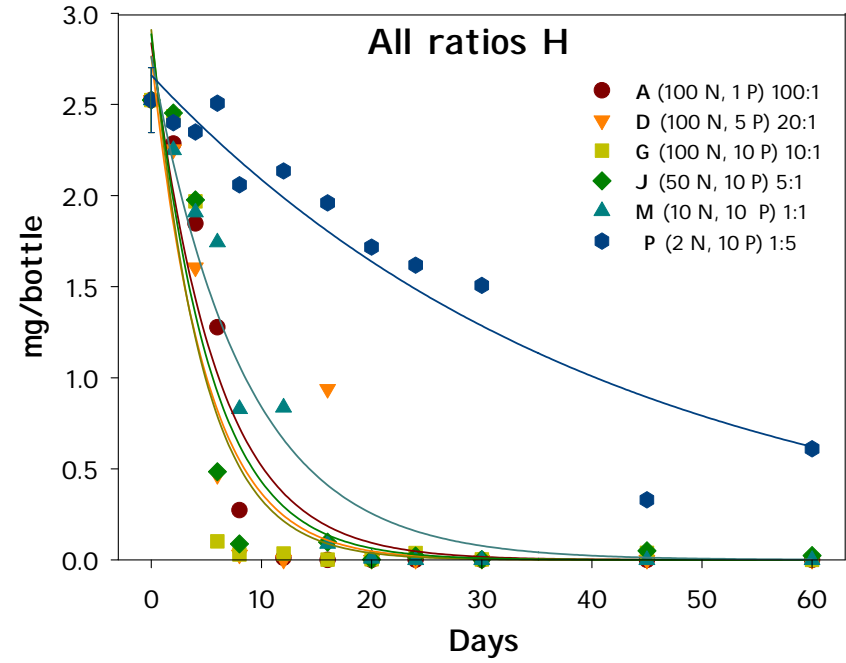
- OIL CHEMISTRY
 - GC/MS analysis of targeted alkanes and PAHs (*Method 8270C*)
- NITRATE AND PHOSPHATE ANALYSIS
 - Ion Exchange Chromatography (*Dionex DX 500 with an IonPacR AS14*)
- MICROBIOLOGY
 - Most Probable Number (MPN) (*Wrenn, B.A. and Venosa, A.D., 1996*)
 - Phospholipids (*Findlay, R. et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

T-RFLP



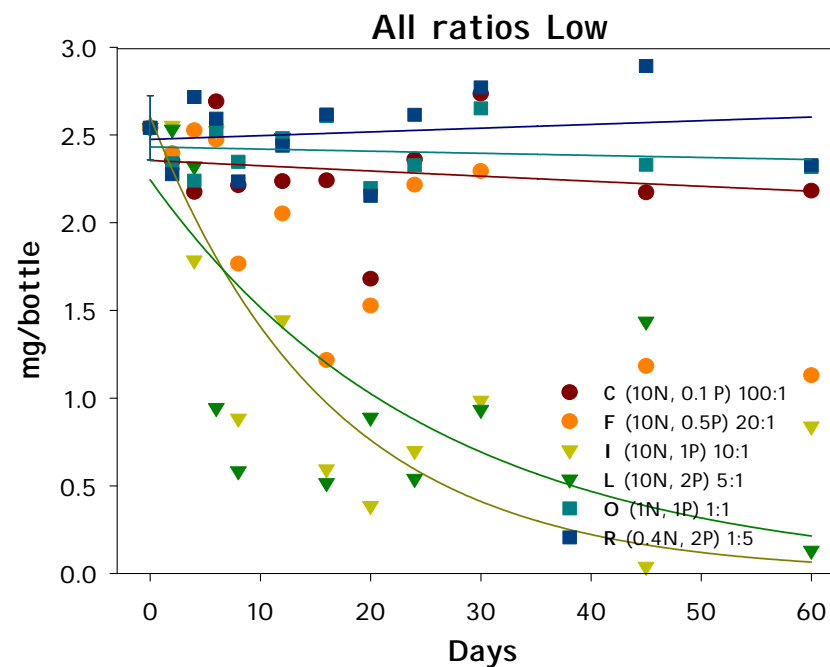
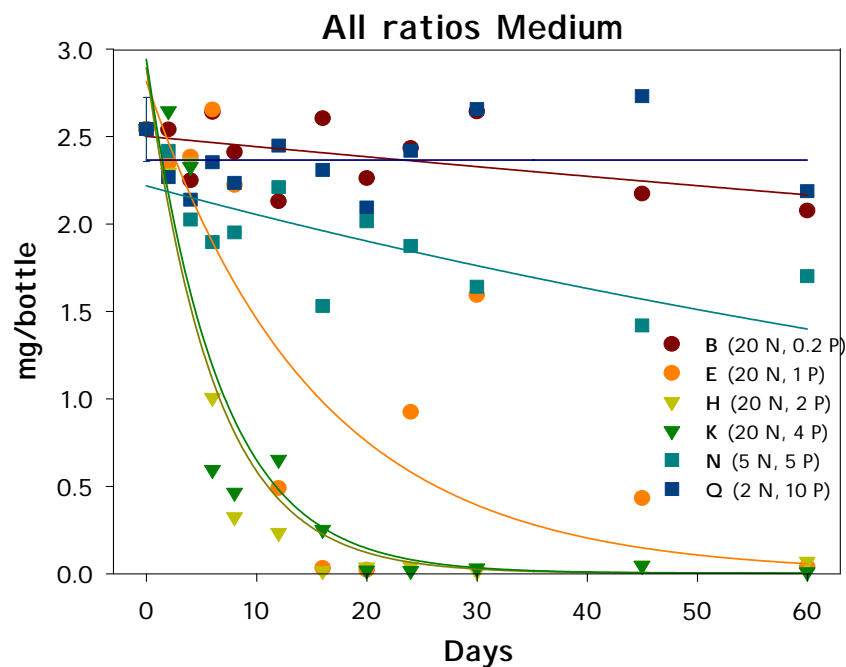
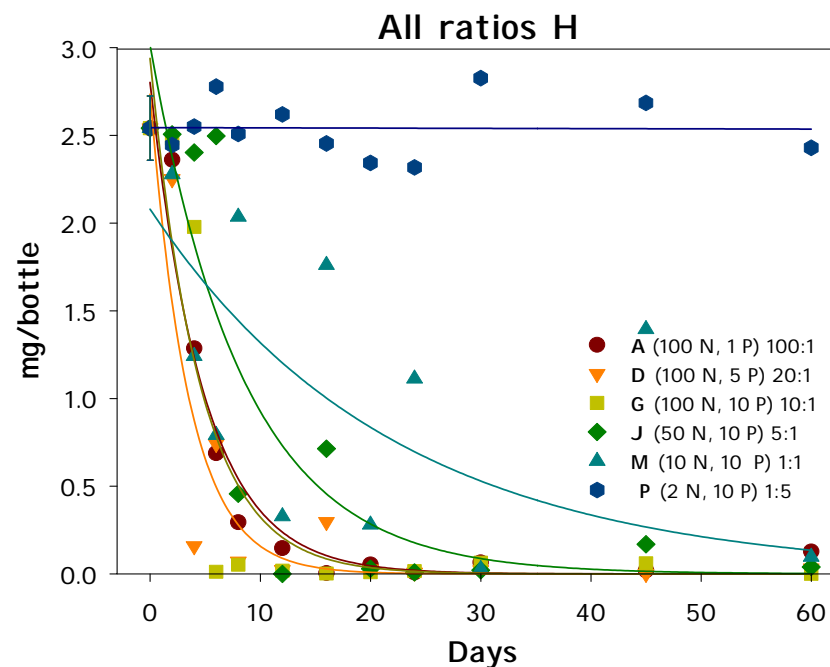
PHENANTHRENE

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



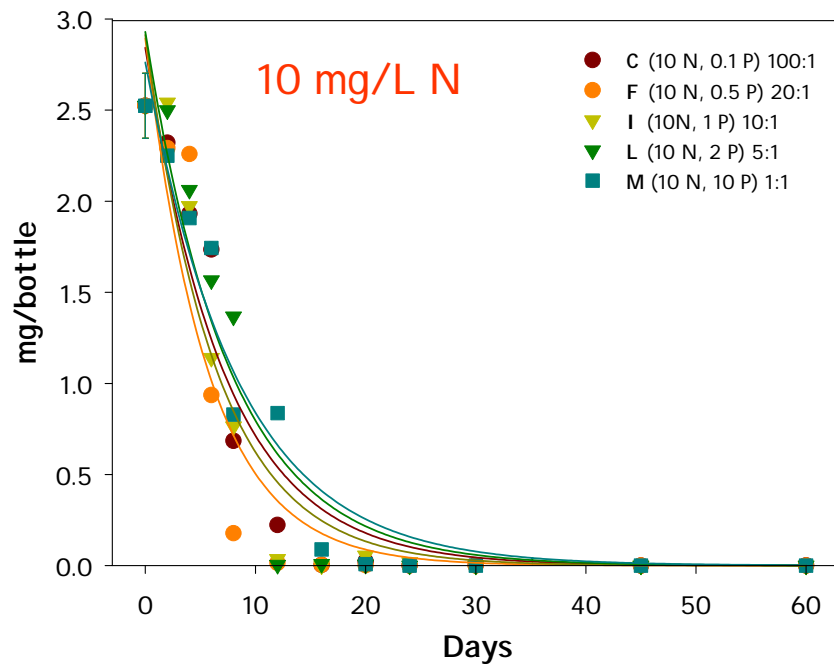
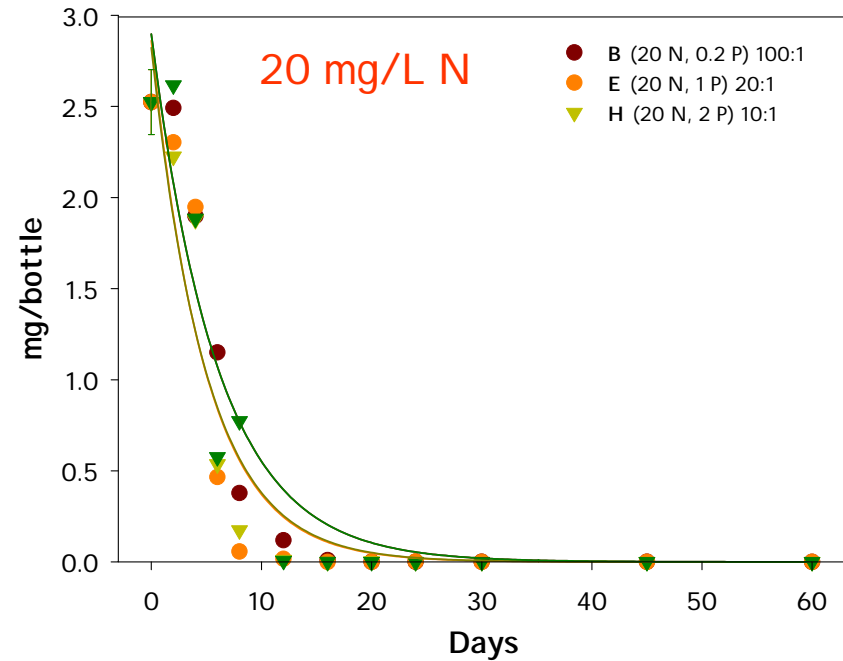
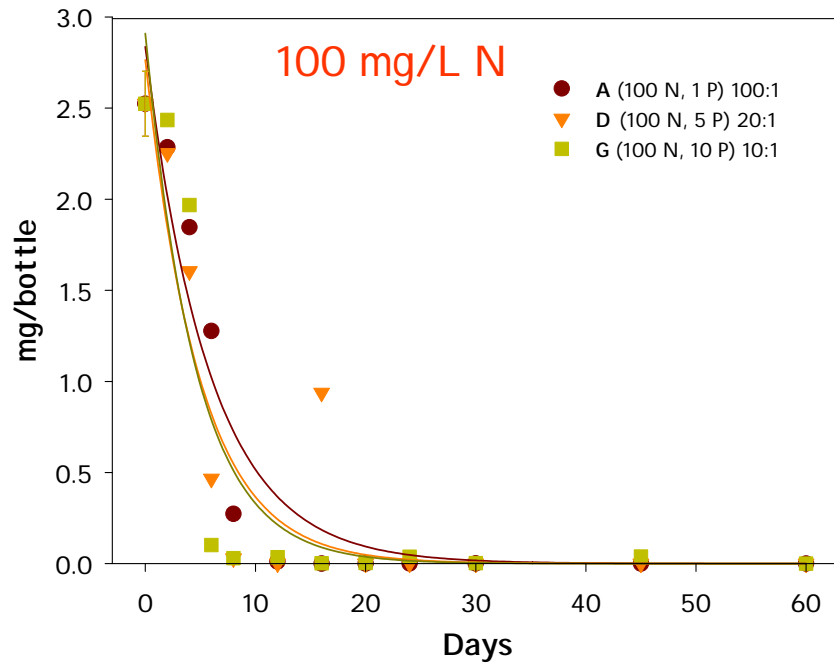
OCTADECANE

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** \Rightarrow rate and extent of biodegradation

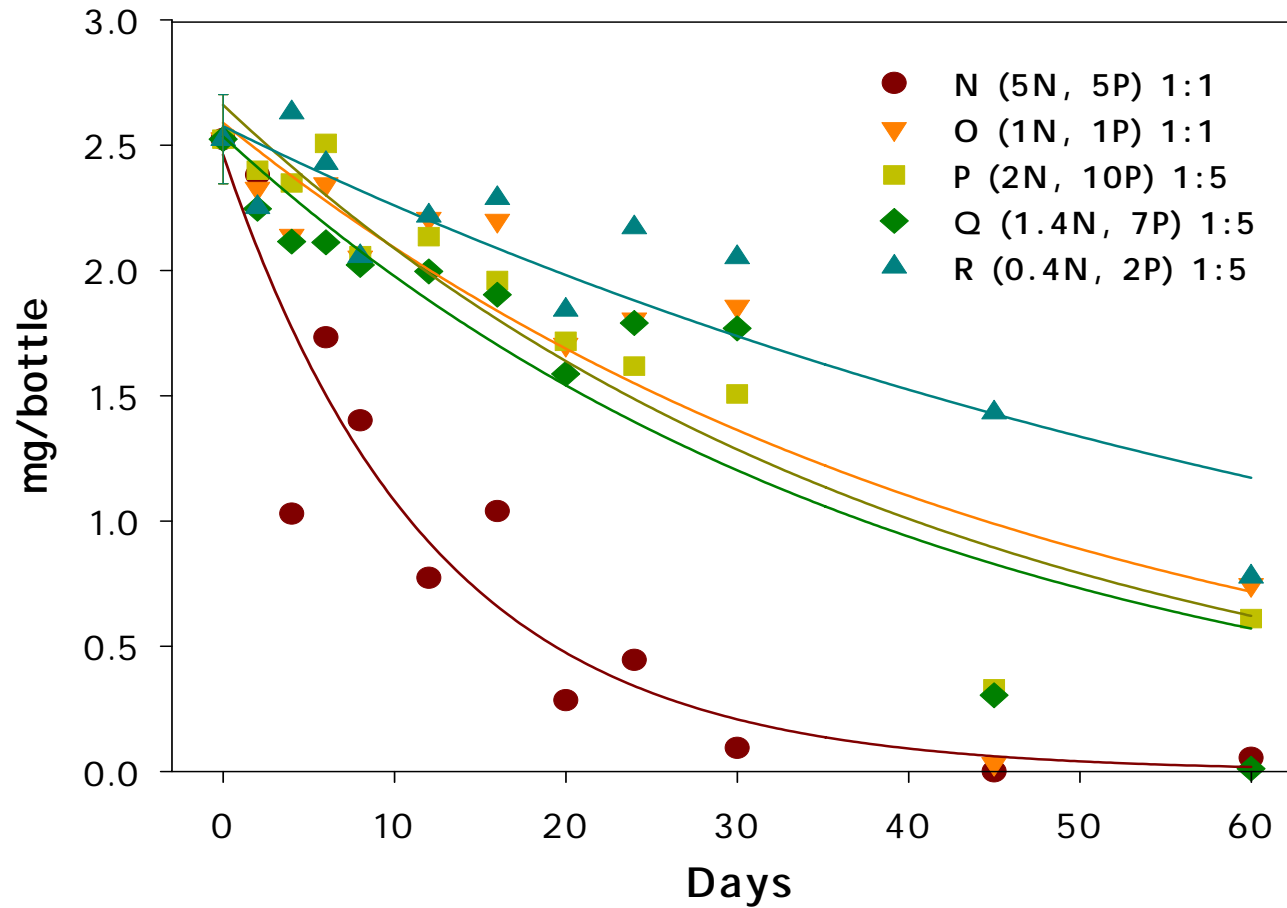


PHENANTHRENE

Constant N /Variable P

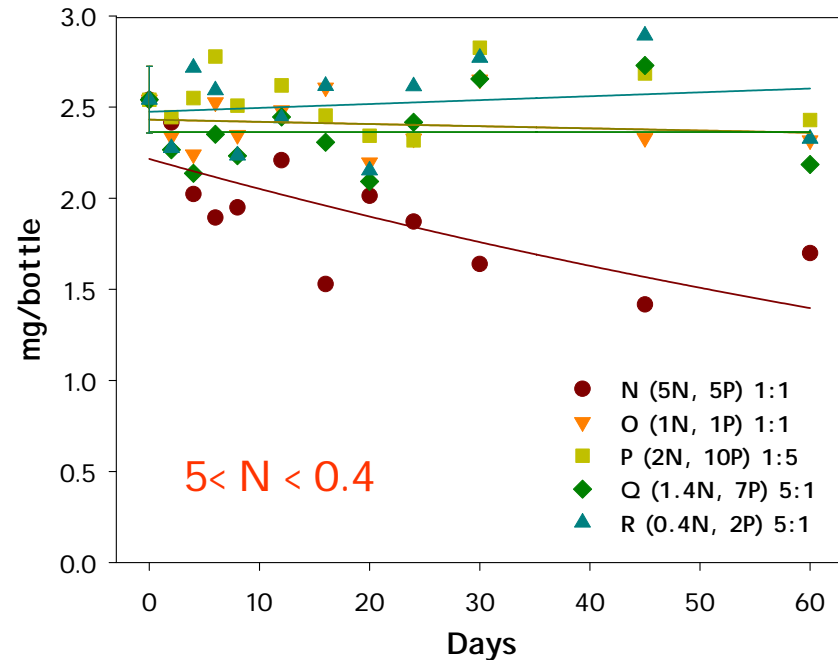
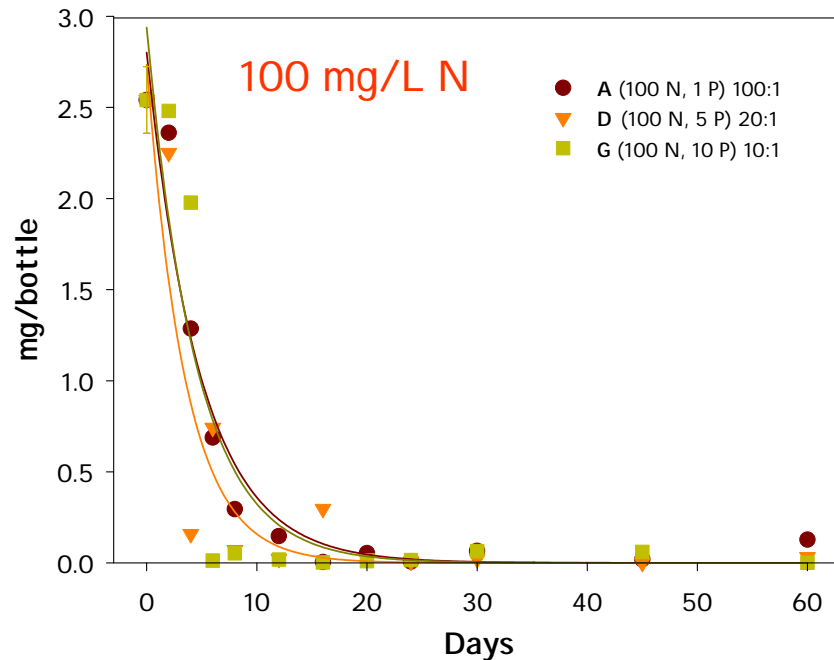
For 100, 20 or 10 mg/L of N,
P does not have a significant effect
 on phenanthrene biodegradation

PHENANTHRENE



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

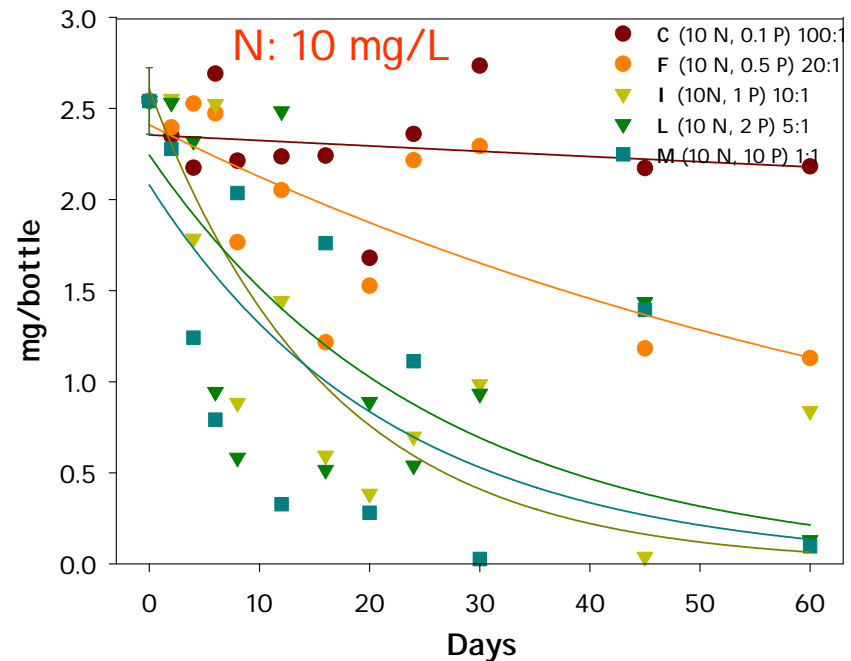
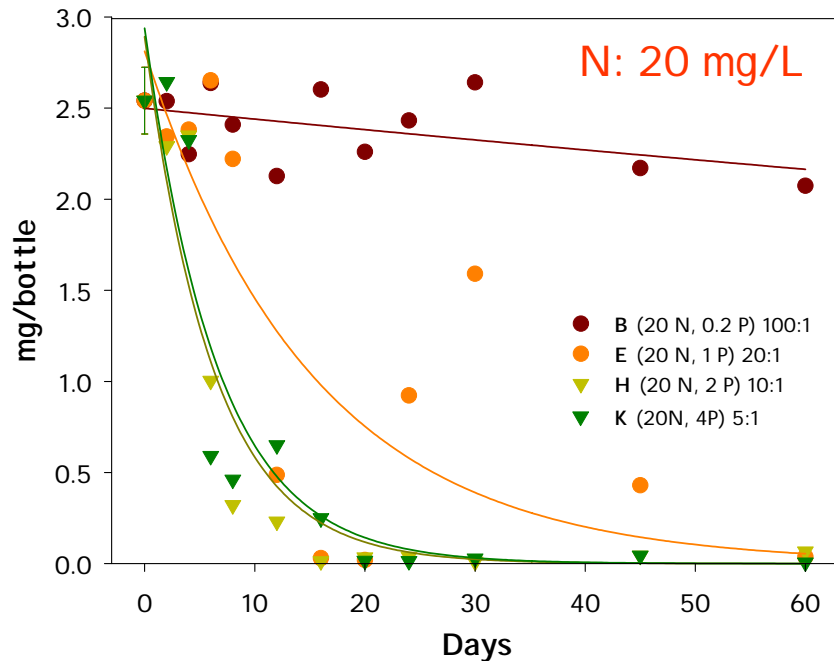
OCTADECANE



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 C₁₈ 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 C₁₈ degradation
at intermediate N concentrations

Further study: T-RFLP \Rightarrow community structure

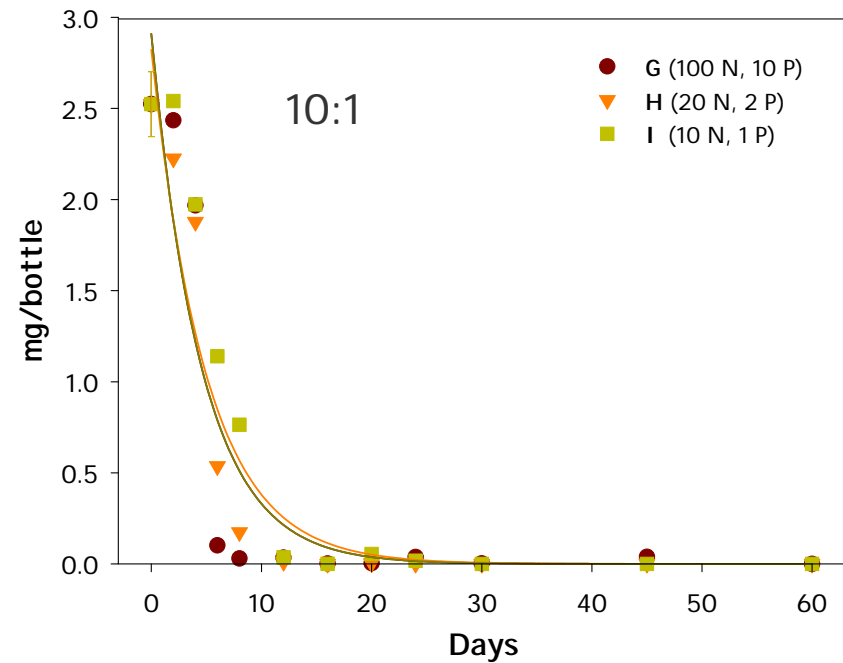
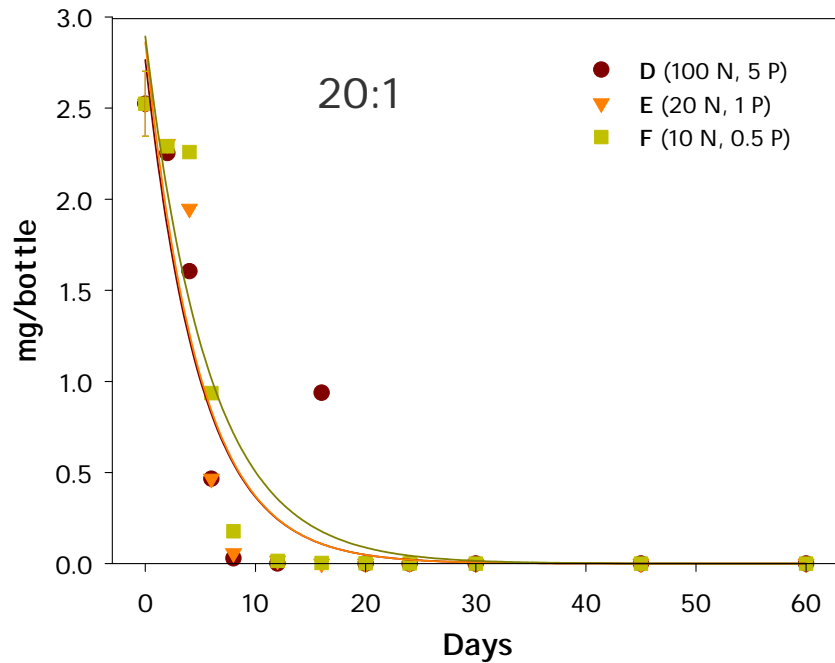
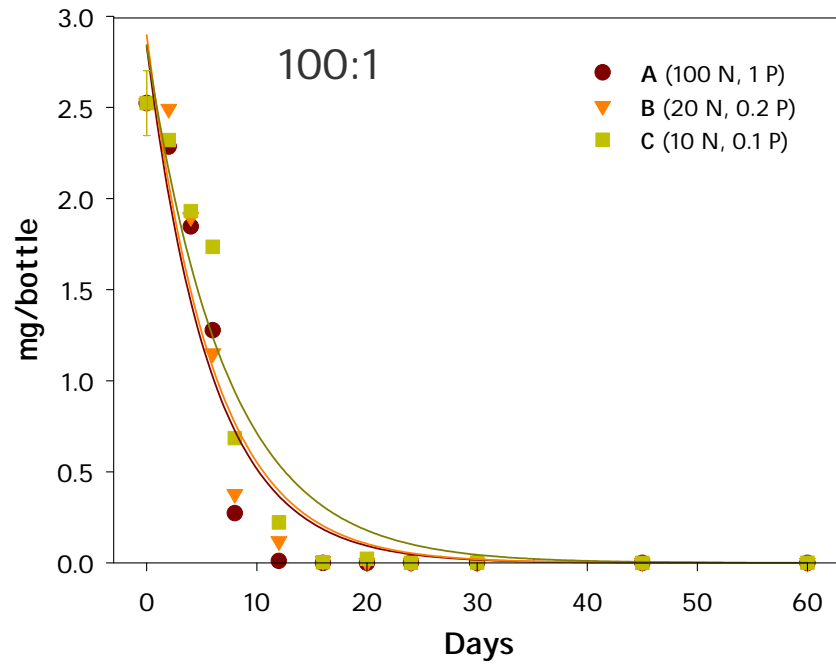
- Degradation rate coefficients were in most cases higher for phenanthrene than for C₁₈.

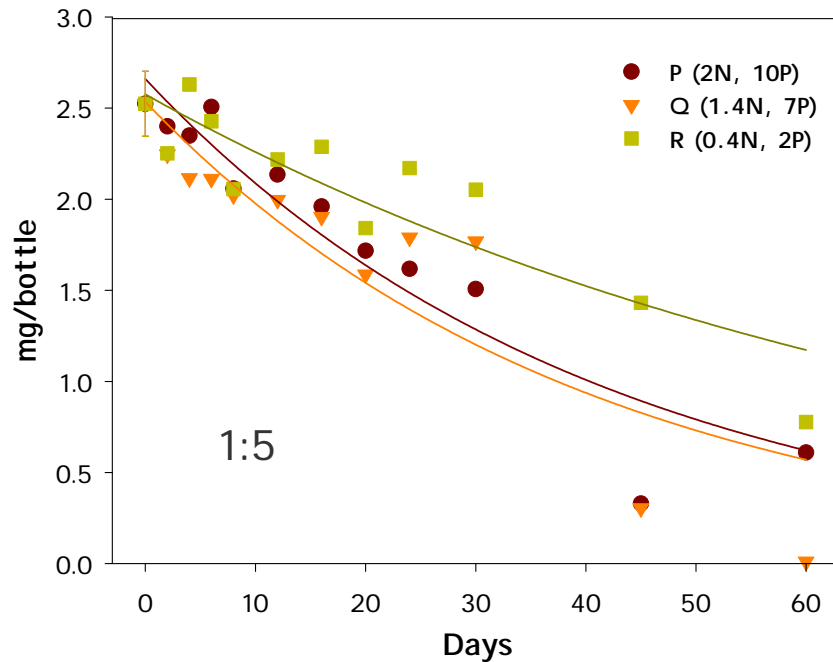
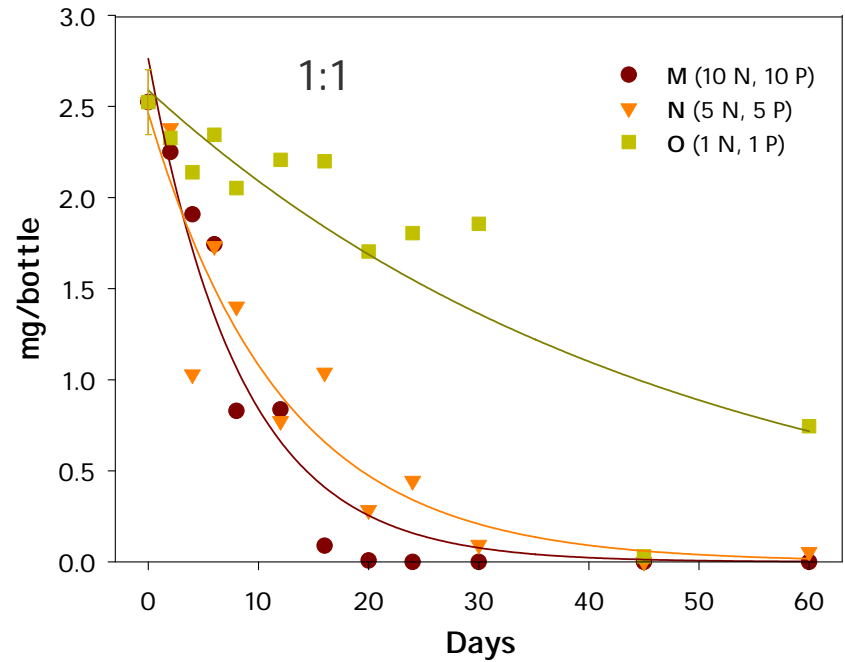
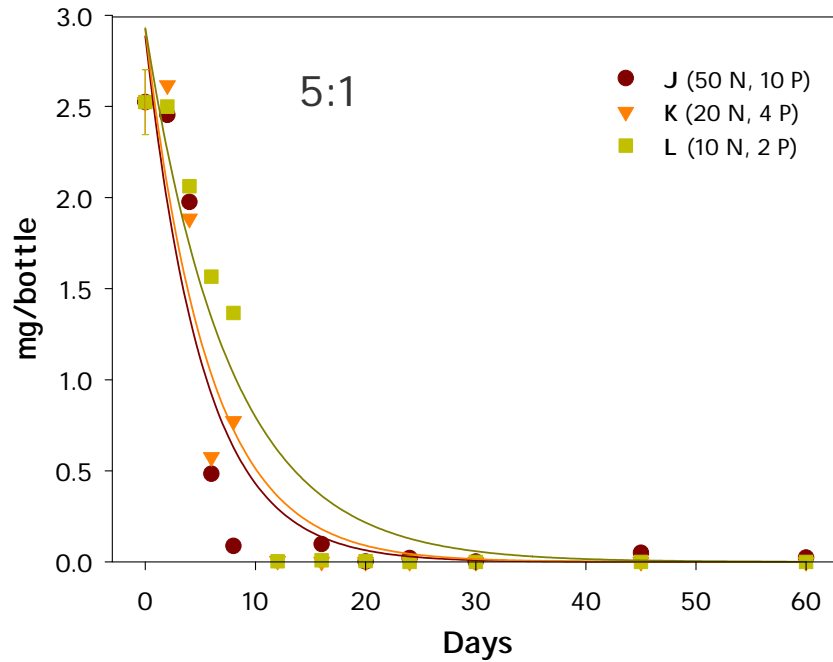
HYPOTHESIS # 2

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





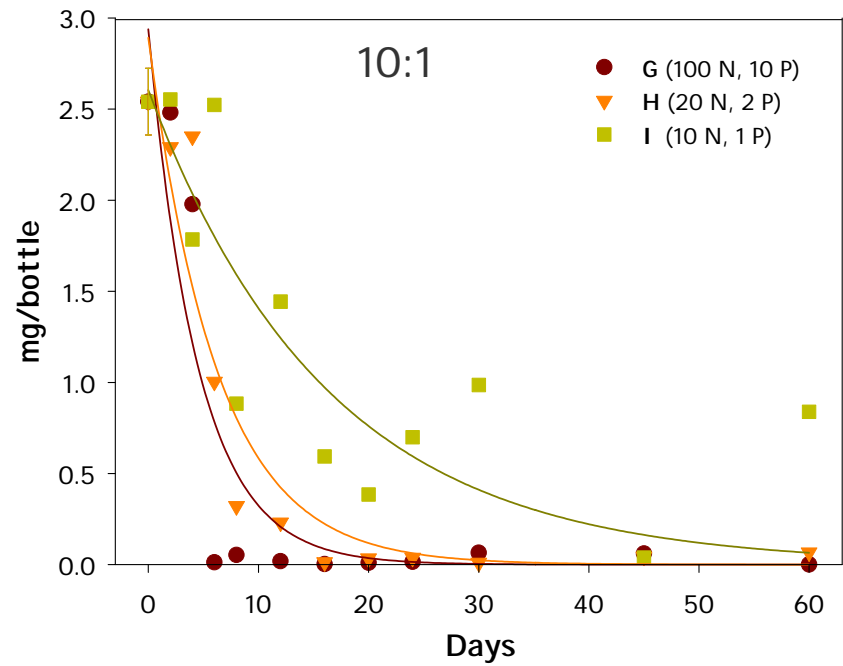
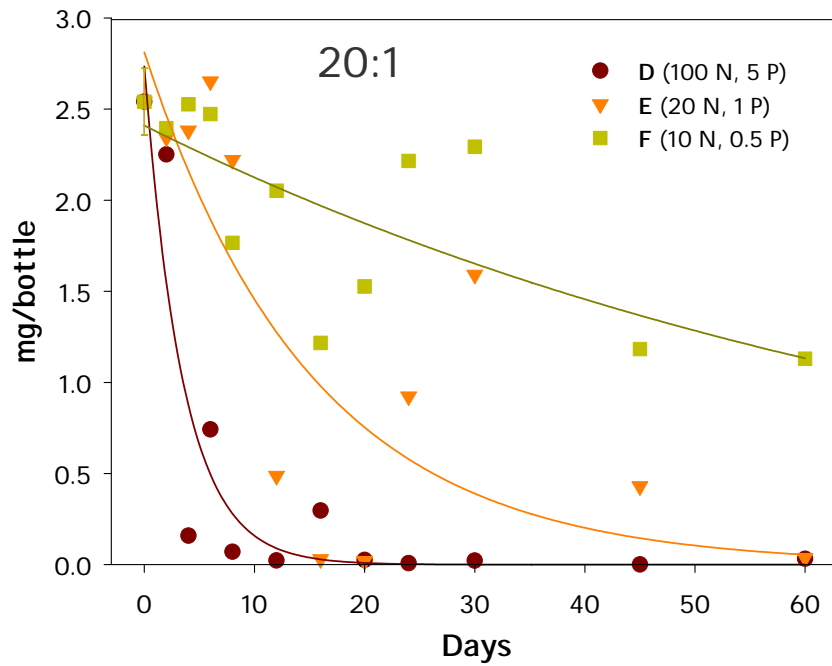
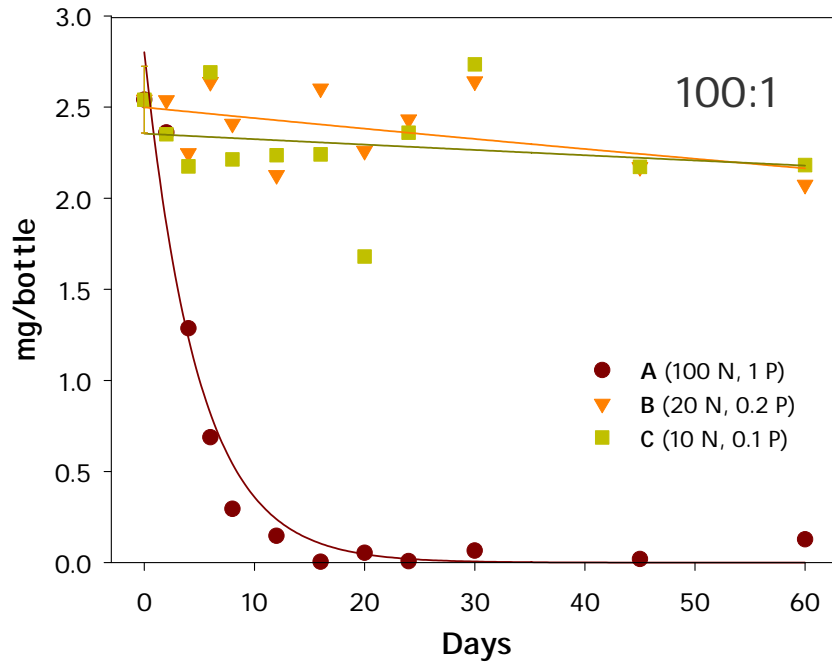
As predicted, for a N:P ratio,

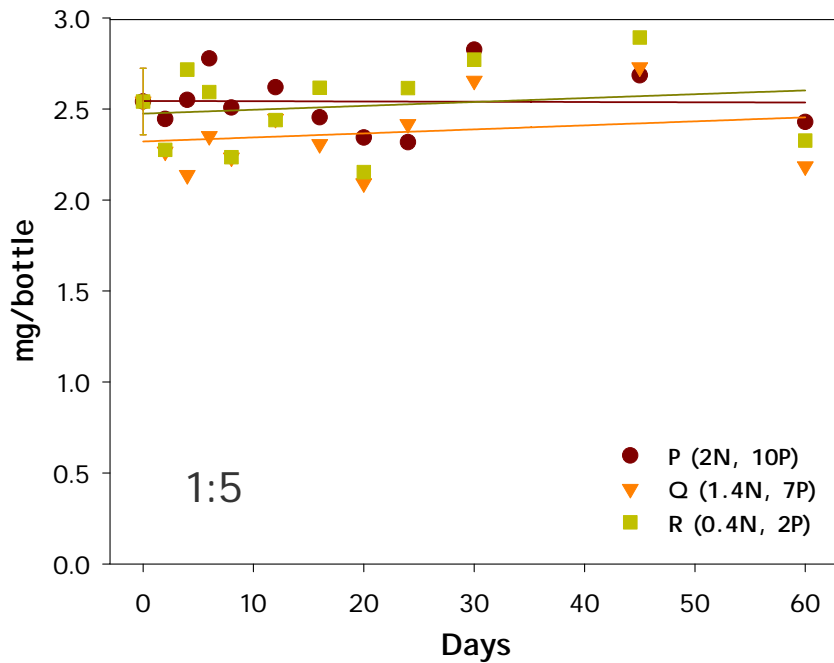
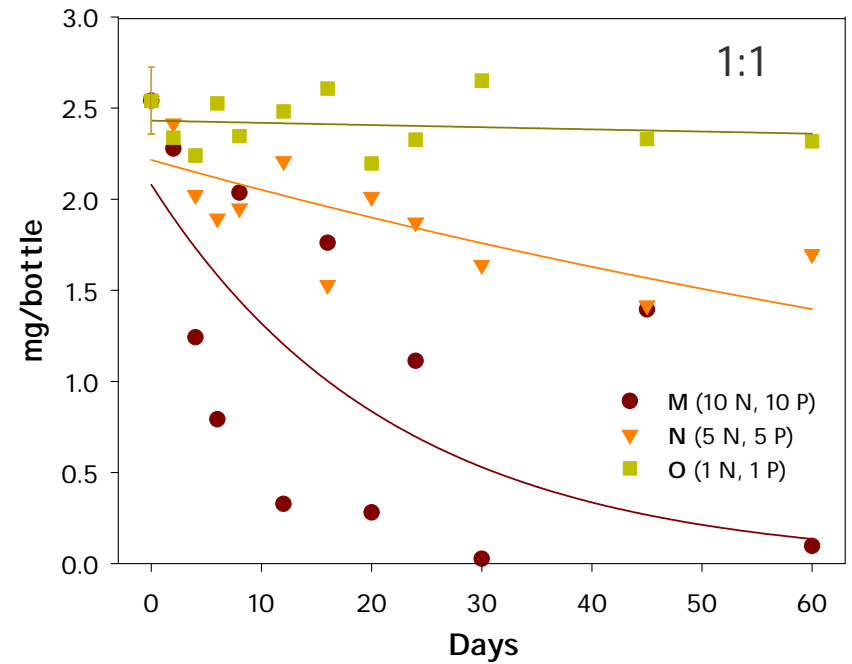
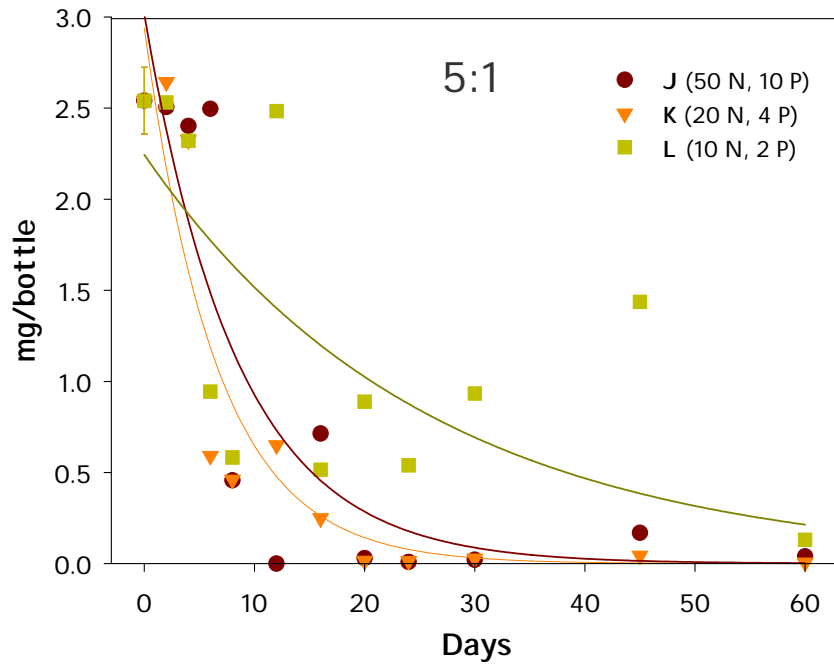
INCREASES
with absolute concentrations

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 C₁₈
- 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 C₁₈ 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 ⇒ 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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