The effect of nitrogen enrichment on $C_1$-cycling microbes and methane flux in salt marsh sediments

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CH$_4$ over 20 times more potent greenhouse gas than CO$_2$
60% of emissions are anthropogenic

~3% from salt marshes
Global Change

- N limits in most environments
- Salt marshes vulnerable
- Conservation & functioning

Source: Millennium Ecosystem Assessment
- All domains
- Greenhouse gases
- Bioremediation
- Mutualisms
- N shifts interactions
Methanogens

Strictly anaerobic archaea

Make methane (CH$_4$) from:

- Organic acids
- Alcohols
- Methylamines

CO$_2$ + H$_2$
Methanotrophs

Oxidize methane (aerobic & anaerobic)

Methanotroph

obligate & facultative
Methylothrophs

Oxidize methanol, methylamines, other $C_1$ (aerobic & anaerobic)

CH$_3$OH

Methylothroph

obligate & facultative
Main Questions

1. What nutrients limit $C_1$-cyclers in salt marshes (C, N)?

2. Are plants indirectly involved through root exudates?

3. How does N perturbation affect CH$_4$ flux?
Experimental System

Salt Marshes

Morro Bay
Carpinteria
Tijuana
Experimental Design

N fertilization gradient (g N/m²/year)

Control (background)  +10  +20  +40  +80  +160  +320

3 marshes × 7 treatments × 5 replicates

- Slow-release urea
- 9 buried tubes per plot
- Refill every 10 weeks

105 plots
Ecosystem Measurements

**Sediment**
- pH
- Gravimetric H₂O content
- Temperature
- NH₄ (resin bag)
- NO₃ (resin bag)
- NH₄ (dry sediment)
- NO₃ (dry sediment)
- C:N
- Net nitrification
- Net ammonification
- Net N mineralization

**Plants**
- Plant biomass
- Foliar C:N
- Root ingrowth

**Gases**
- CH₄ flux
- [CH₄] initial
- CO₂ flux
- [CO₂] initial
- C mineralization
Plant Response to N
Linear Biomass & Leaf N Increases
NH$_4^+$ Availability Increase in Sediment

N-NH$_4^+$ availability (µg N-NH$_4^+$ m$^{-2}$)

N addition (g m$^{-2}$ yr$^{-1}$)

$R^2=0.94$

$R^2=0.97$

MBE
CSM
TRE
N Addition Increases CH$_4$ Emissions

1.23 μg CH$_4$/g N/day

linear regression $R^2=0.21$, $P=0.03$
CH$_4$ Flux is Changing with N. Why would it change?

• Methanotrophs inhibited?
• Methanogens stimulated?

![Graph showing the relationship between treatment (g N/m$^2$/yr) and mean CH$_4$ (CH$_4$ ppm/min). The graph includes error bars and a red line indicating linear regression with $R^2=0.643$, $P=0.03$.](image)
Nutrient Addition Microcosms

To tease apart responses to C and N

Methanogen anaerobic
C = acetate
N = NH$_4$Cl

Methanotroph aerobic
C = CH$_4$
N = NH$_4$Cl
Methanotrophs are C-limited but Not N-limited

C addition ≥831% over controls

C = P<0.0001
N = NS
CxN = NS
2-way ANOVA
Methanogens are N & C-limited

C+N 44% over controls

C= P=0.013
N= P=0.021
C×N=NS
2-way ANOVA
Conclusions

1. What nutrients limit $C_1$-cyclers in salt marshes?
   a. methanogens N and C-limited
   b. methanotrophs C-limited

2. Are plants indirectly involved through root exudates?
   a. appears so - by stimulating methanogens

3. How does N addition affect $\text{CH}_4$ flux?
   a. linear, predictable increase in $\text{CH}_4$ emissions
Thanks!

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Suddenly, Bob realizes that he’s "part of the problem".