Roger Knowles Tribute: David Patriquin on Asymbiotic & Associative N_2 fixation

M403, Robertson Terrace & Ruth & Roger days 1970, 1972-73

CSM Special Symposium, St. John's, N&L, June 2011

M403: 1970, 72-73

Bob Fessenden
(Alberta Sci & Technol)

Jacques Lefebvre
Lee Barro
Jean Brossard

Fred Donawa
(UWI, Trinidad)

Ray Brouzes
(Domtar, Alcan)

Patrick O'Toole
(UC Dublin)

Maurice
Lalonde
(Laval)

David Patriquin
(Dalhousie)

Doug Denike
(Dal...
Paramedic

Ruth & Roger in 2004

Free-living & Associative N_2 fixation Research at Dalhousie 1973-1990

Saltmarsh

Diane Livingstone

Cathy Keddy

Kate Sircom

Spartina alterniflora

Arcobacter nitrofigilis

Rob McClung

Sea Urchins

Vibrio diazotrophicus

Mary Lou Guerinot

*Johanna Dobereiner (Br)

*A.P. Ruschel (Br)

*Colin Hudson (BB)

*Ian Walker (BB)

Cereals, Sugarcane

Nick Hill

A.brasilense X Helicomyces roseus

Don Smith

Peter Dunfield

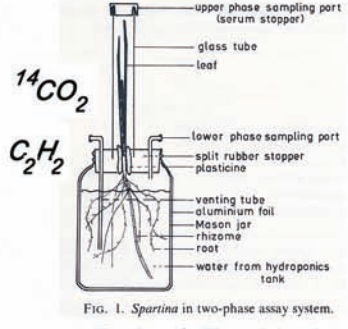


FIG. 1. *Spartina* in two-phase assay system.

Endo- & Exorhizal

Dave Boyle

Devender Jain

N cycling: Kevin Vessey Dave Burton Charlene Van Raalte Leonard Eaton ▪ ▪ ▪

N_2 fixation in sugarcane litter

(Patriquin, 1982; Hill 1988, Hill and Patriquin 1988, 1990a,b, 1991)

FIELD:

- Cane litter N2-ase activity begins, peaks and declines to near zero over 3 months after onset of rainy season
- Cane litter browns concurrent with increase in N2-ase activity
- Cane litter ARA inhibited by N fertilizer, stimulated by Fe (calcareous soils)
- Similar activity in litters of 5 seaside trees, mahogany; NOT by litter from mature tropical forest (Barbados)

sugarcane

mahogany

sea grape

N_2 fixation in sugarcane litter

Lab: Crude Cane Culture (CCC) transferred to wheat straw

- Results in net N gains up to of 6.6 mgN/g straw & enhances breakdown

- Air dried infected straw retains inoculum potential for 5+ years

- Combinations of 8 fungi, 20 strains of diazotrophs and 33 non-diazotrophs could not replicate the activity

- Only microaerophilic or aerobic N fixer isolated from wheat straw systems: *Azospirllum brasilense*

- Cycloheximide reduced N2-ase activity except when malate or xylan (substrates for *A. brasilense*) were added

- Onset of N2-ase activity associated with darkening & proliferation of dermataceous fungus *Helicomyces roseus*; melanin probably acts as N sink

- Knowlsian experiments showed sensitivity to O2 declines with increasing temperature: system may be restricted to tropical litters

straw with indigenous microflora

straw with indigenous microflora + CCC

N_2 fixation in sugarcane litter

Possible significance

Barbados: 10-40 kg N/ha estimated from integrated ARA,
Inhibited by broadcast N, not banded N

Brasil: 16 year experiment comparing N gains
+/- annual burning, +/- vinasse, +/- N fertilizer

de Resende et al. 2006 Plant & Soil 281:339–351

Banding fertilizer on stooles, Barbados

	Burned	Not Burned	+N fert	-Nfert
N removed in crop (16 years)	765	1496	1246	1046
N gain in soil (16 years)	248	562	-68	858

(kg N/ha)

--> Large N gains when litter is
not burned; N gains supressed by N fertilizer

litter ("trash") conserved Barbados

trash burned Brasil

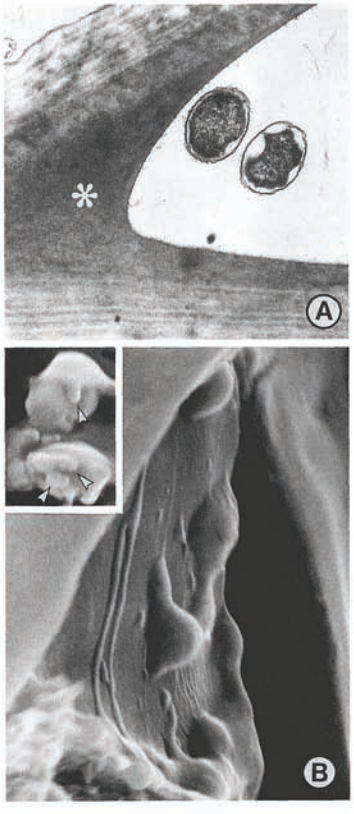


Figure 3. A transverse section through a portion of an intercellular space including two bacteria that have also been sectioned transversely. The asterisk marks the intact middle lamella between the two cells that have partially separated to form the spaces. Transmission electron micrograph; ×22,900. B, A tangential view into an intercellular space showing part of the surface of a cell wall that lines the space. A group of mucoid colonies is attached to this wall. ×10, 500. The inset shows outlines of bacteria (arrows) embedded in such colonies. Scanning electron micrograph. ×7,500.

Dong et al.,Plant Physiol. (1994) 105: 1139-1147

Endophytes *(Dobereiner 1992, Kloepper, 1992)*

- *Reside within healthy plant tissues without eliciting disease*
- *Poor survival in soil*
- *Commensal OR mutualistic OR mildly parasitic*

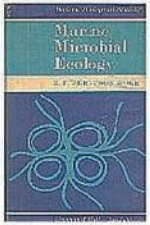
Sugarcane

- *Gluconacetobacter diazotrophicus: max. growth in 10% sucrose →gluconic acid →pH3*
- *also: Herbaspirillum seropedicae, H. rubrisubalbicans, Burkholderia sp.*
- *Mostly inter- and intracellular, vascular tissues, root cortex, stems, leaves*
- *N isotope studies: up to 69% of plant N from N fixation. but highly variable x cultivar, site, country.*
- *Global: 40 kg N/ha Brazil; 20 kg N/ha elsewhere (Herridge et al 2008)*

Kallar Grass

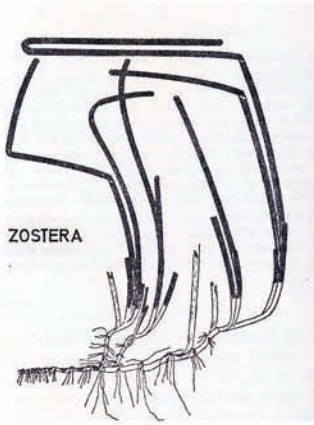
- *Salt tolerant grass, Pakistan*
- *Azoarcus spp. infect roots, xtlem vessels, fungal resting stages; **possibly endemic***
- *Azoarcus sp. strain BH72 expresses structural genes that encode nitrogenase in association with the host plant and supplies N to plant*

Origin of N & P for growth of turtle grass (*Thalassia testudinum*)



E.J. Ferguson Wood (1965)

- seagrasses stimulate SO_4 reduction (SR)
- H_2S releases P from FeP; P assumed to be limiting



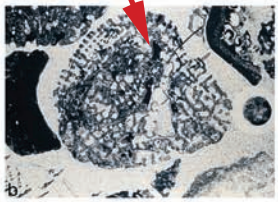
DP (1972) evidence that

- N rather than P limiting
- N taken up by roots & rhizomes from sediments (vs leaves)
- reducing (sulfidic) conditions required for high growth rate
- source of new N?? - anaerobic N_2 fixation??

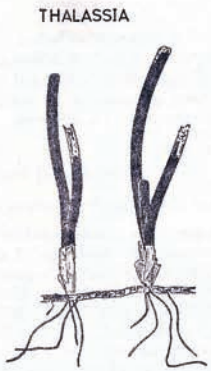
w. Roger Knowles (1973)

- high ARA; $^{15}N_2$ fixation by rhizosphere sediments, washed rhizomes & roots of 4 seagrass spp. (tropical>temperate)
- diverse N_2 fixers enriched in rhizosphere 50-300 fold over non-rhizosphere

FeS



- N_2-ase activity in skeletal carbonate sand grains occurred in anaerobic microsites (1975)



N_2 fixation x Seagrasses (F. Hydrocharitaceae) & Saltmarsh Spartina spp (F. Poaceae) - by year 2000:

- **< 5-12 % (temperate spp.) to >30-50% of annual N requirements**
- **Multitude of diazotroph species**
  - -including *Vibrio parahaemolyticus* in *S. alterniflora*
- **Key role of sulphate-reducing bacteria (SRB)**
  - -25 to 95% of N2 fixation inhibited by inhibitors of SR
  - -SR closely linked to seagrass photosynthesis
  - -enrichment of SRB rhizoplane & root interior
  - -acetogen clusters on root surface
- **Excess N2 fixation by SRB:**
  10% (sediment), 134% (root), 155% (rhizome) of N requirements for SRB from N2 fixation (9%, 140%, 556% in Spartina stricta)
- **O_2 versus H_2S**
  - -rapid oxidation of H2S in roots
  - -SRB tolerant of O_2 flushes
  - -roots can shift to anaerobic metabolism
  - -possible inhibition of SR at root tips (meristematic regions)
- **Global (Ocean) scale**:
  Seagrasses & Saltmarshes 7.8 tg/a
  = approx, 10% of oceanic N2 fixation (30-130 Tg/a) ... hotspots?

N_2 fixation x Seagrasses (F. Hydrocharitaceae) & Saltmarsh Spartina spp (F. Poaceae)

Crump & Koch 2008

Examined phylogenetic diversities of leaf- and root-attached bacteria on four aquatic/marine angiosperms

"Aquatic angiosperms host specialized communities including several broadly distributed and potentially mutualistic bacterial populations"

Amongst capabilities/activities:

sulfide oxidation, methane oxidation iron reduction, sulfate reduction, nitrogen fixation

Marine N_2 fixation post-2000: How little we know!

New N budgets for the oceans:

(e.g. Brandes et al. 2007)

- New components (e.g., ANAMMOX)
- More, improved measurements: much higher N losses (sedimentary losses late 80's: 85 Tg; now 200-300 Tg
- Where is the missing N?

From N_2 fixation or is the cycle not balanced?

Some nos.

- N removal: 300-400 Tg (versus approx. 100 earlier estimates)
- Fixed N sources: 125 (atmosphere, rivers)
- Deficit of 150 - 250 tg by marine N2 fixation?

Pre-2000 estimates of marine N2 fixation: 30-130 tg, most <50

Terrestrial N2 fix: biological 110 Tg (approx 50 in agriculture)
Industrial N2 fix/fuel combustion: 160 Tg

Uncertainty = major limitation to modeling x climatic change, ocean acidification, impacts of anthropgenic N fixation etc ...i.e. to understanding/predicting transition Holocene to Anthropocene

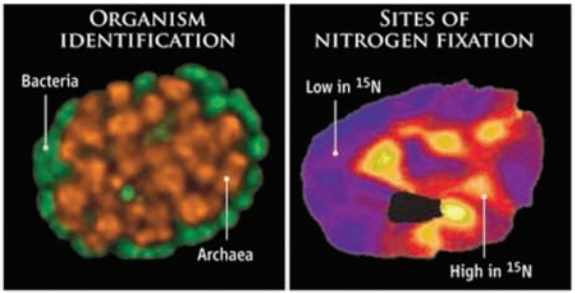
Where is the missing N?

To Answer: Improved measurements & efforts to link biochemical processes at cellular scale with global biogeochemical cycles

Methodologies:

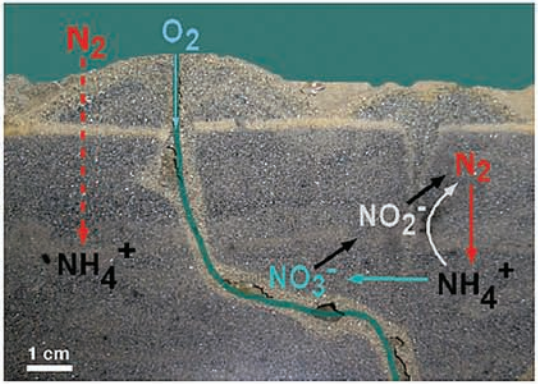
- *Geochemical Tracers*
- *Satellite x Trichodesmium*
- *Micro labeling (MIMS/nanoSIMS)*
- *Metagenomics…*

A few highlights (benthic systems)



R W Fulweiler Science 2009;326:377-378

reporting on Dekas et al. in Science (2009) Deep-Sea Archaea Fix and Share Nitrogen in Methane-Consuming Microbial Consortia



Burrowing shrimp introduce oxygen deep into their burrows, oxidizing the surrounding sediment, where N_2-fixation leads to the production of new bioavailable nitrogen.

Image: W. Ziebis and V. J. Bertics

Bertics et al., 2010 Mar Ecol Prog Ser 409: 1-16

Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction

More detailed marine N cycle

Brandes et al.

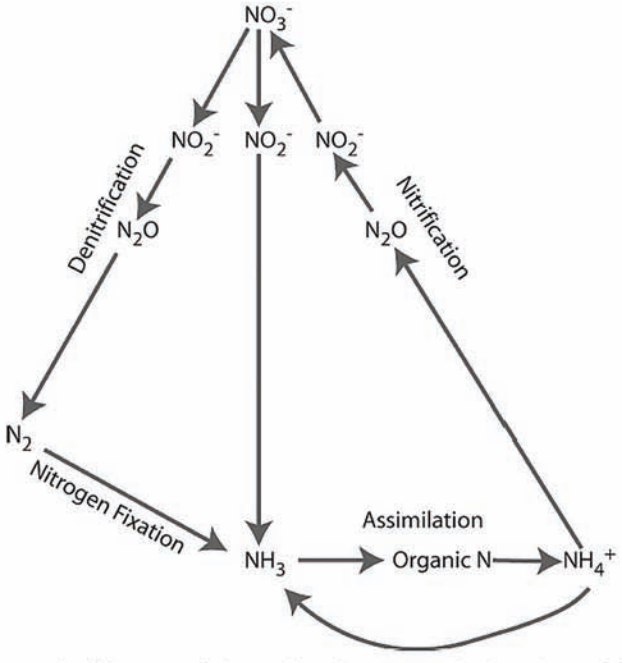


Figure 1. Diagram of the marine nitrogen cycle, based on ref 35. Arrows represent the direction of named reactions.

1979

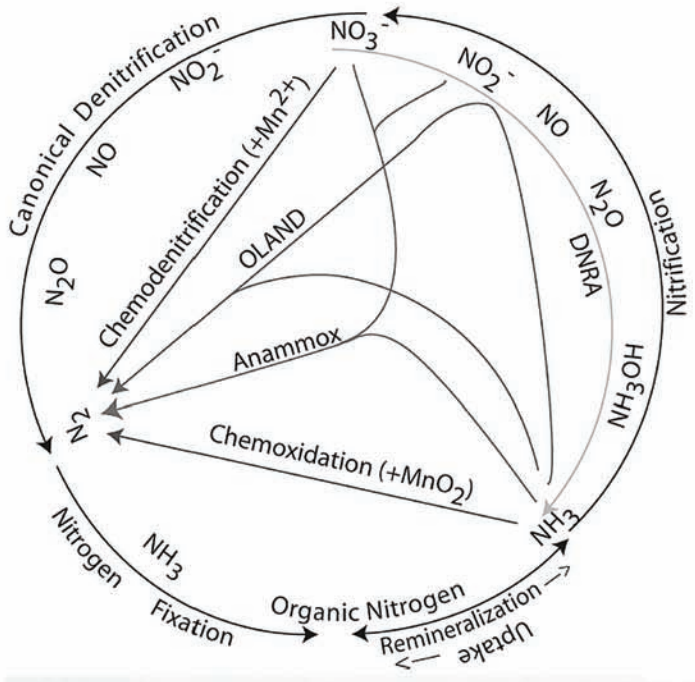


Figure 2. Diagram of the nitrogen cycle as it is understood today. Processes given on the outside of the circle of nitrogen compounds are redrawn from Figure 1. Processes on the inside of the circle are those discovered or identified in the last 15 years. Arrows represent the direction of reactions. Chemonitrification and chemodenitrification reactions are listed with their respective manganese species used as a redox pair. The reduction of NO_3^- to NH_3 during assimilation by photosynthetic organisms is not drawn for clarity.

2007

Corals, Sponges: Spatial-Temporal Coupling of N_2 fixation, nitrification, denitrification

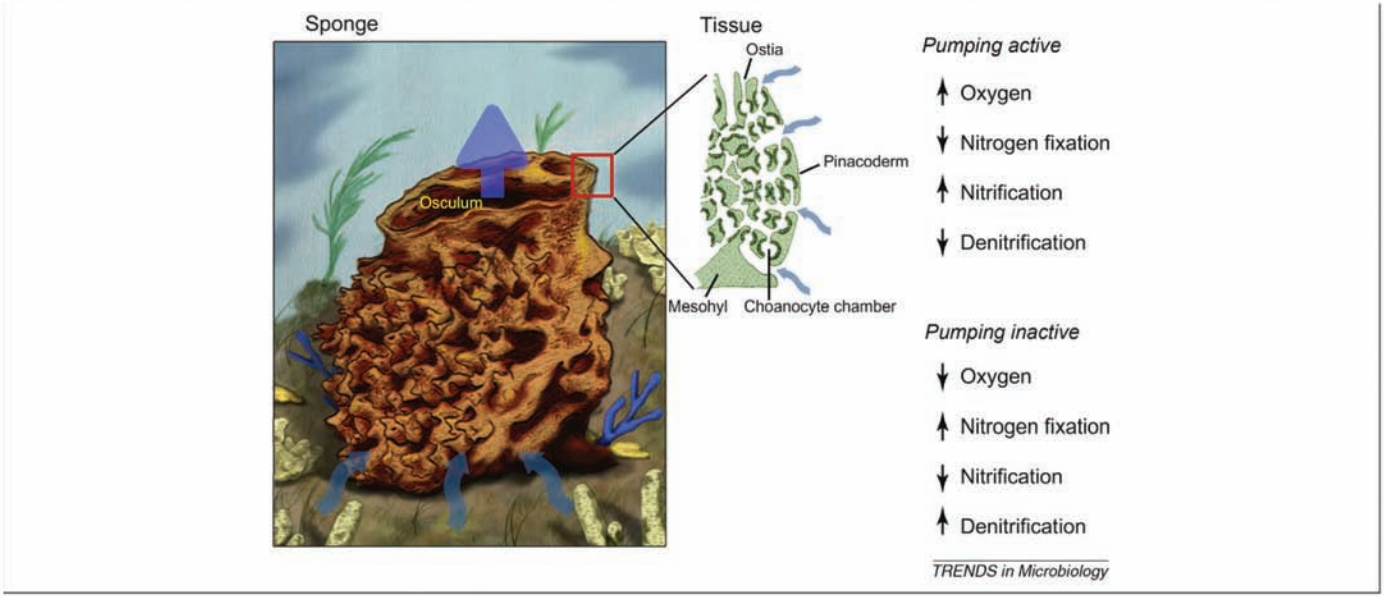


Figure 4. Illustration of a typical sponge with simple cellular grade construction where symbiotic bacteria are located freely and in bacteriocytes of the mesohyl. Both the outer mesohyl and pinacoderm can harbor nitrogen-fixing and nitrifying prokaryotes, whereas the choanocyte chambers contain the cells responsible for driving the pumping action of the sponges. In fact, the most important abiotic factor affecting the internal environment of sponges is their pumping activity. Sponges pump large amounts of water through their tissues (blue arrows) to filter picoplankton and to access oxygen in the water for their respiratory activities. When sponges actively pump the tissues of sponges are oxygenated (normoxia) and if the sponges also contain symbiotic cyanobacteria in the outer mesohyl or pinacoderm, then hyperoxic conditions with strong gradients of oxygen within the tissues can also occur. When sponges stop pumping, the respiratory activities of host and microbes create strong gradients of hypoxia. As in corals, these internal changes make it possible for different functional groups of symbiotic prokaryotes to be both spatially and temporally separated over a range of physiological conditions that are created by the sponge itself.

Fiore et al. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends in Microbiology 18: 455-463.

LETTERS

Reversal of the net dinitrogen gas flux in coastal marine sediments

R. W. Fulweiler[1], S. W. Nixon[1], B. A. Buckley[1] & S. L. Granger[1]

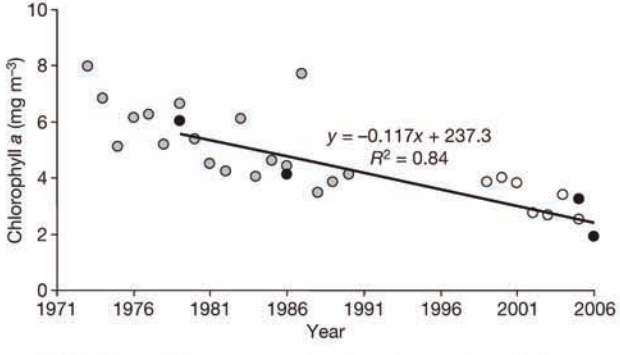


Figure 1 | Multi-decadal mean annual water column chlorophyll *a* concentrations. Mean annual water column (surface and bottom) chlorophyll *a* concentrations in mid-bay over the past three decades. Grey circles are from ref. 6; open and closed circles from the Graduate School of Oceanography plankton monitoring programme (http://www.gso.uri.edu/phytoplankton). Black circles are the mean summer (June, July, August) chlorophyll *a* concentrations shown for the years when N_2 fluxes were measured at the mid-bay station (41° 35.3′, 071° 22.3′). Regression is for the mean summer chlorophyll *a* values.

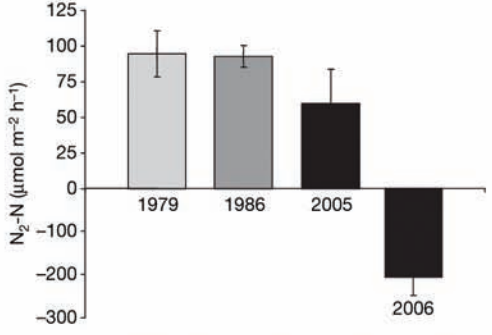


Figure 2 | Mean summer (17–23 °C) N_2 fluxes in mid-bay. Positive rates indicate denitrification, negative rates indicate N-fixation. 1979 data are from ref. 29 (mean ± s.d., $n = 2$), 1985/86 data are from ref. 30 (mean ± s.d., $n = 7$), 2005 data are from ref. 7 (mean ± s.d., $n = 6$), and 2006 data are from this study (mean ± s.d., $n = 6$). Historical denitrification rates are significantly (one-way ANOVA; $P = 0.002$) higher than those measured in 2005/06 (see the Methods).

nd 02882-1197, USA.