Ecosystem element cycling can be driven by an organism's need for growth, or by an organism's need for energy.

N cycling:

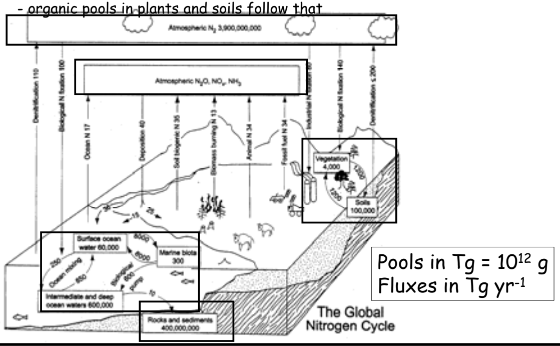
-Nitrogen is essential for life

-Necessary for growth, e.g. synthesis of nucleic acids, proteins

-N_2 comprises 78% of the atmosphere; yet it is limiting - most life forms cannot use it directly from the atmosphere

Global Pools of N:

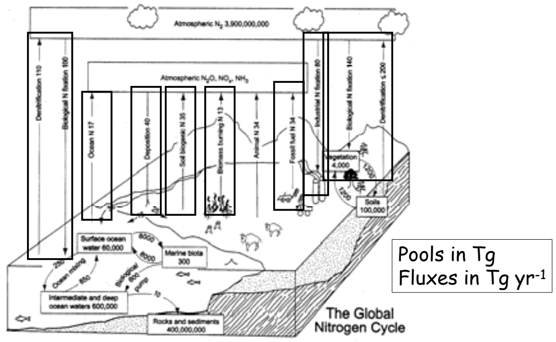
- most in the atmosphere, but not biologically available
- reactive N in atmosphere: trace gases
- lots in sediments and rocks , but not available
- inorganic N in ocean is next largest
- organic pools in plants and soils follow that



Pools in Tg = 10^{12} g
Fluxes in Tg yr^{-1}

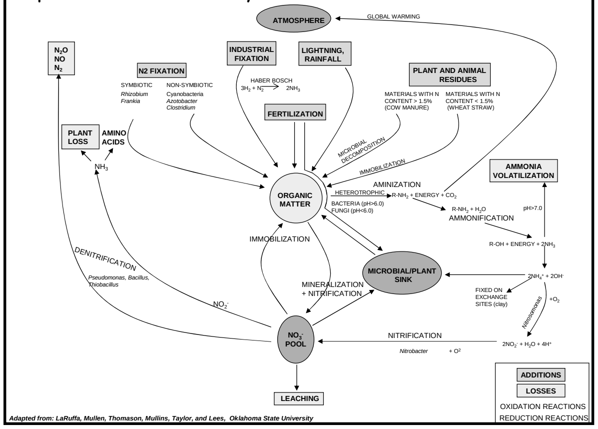
Fluxes between biosphere-atmosphere:

- biological: fixation, denitrification, nitrification
- abiotic: industrial fixation, lightning fixation, fossil fuel and biomass burning, deposition



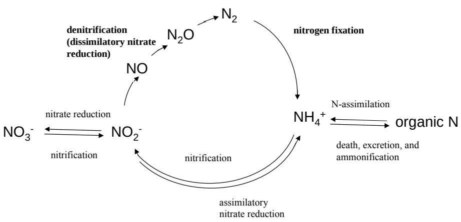
Pools in Tg
Fluxes in Tg yr^{-1}

Comprehensive biotic/abiotic N cycle



Adapted from: LaRuffa, Mullen, Thomason, Mullins, Taylor, and Lees, Oklahoma State University

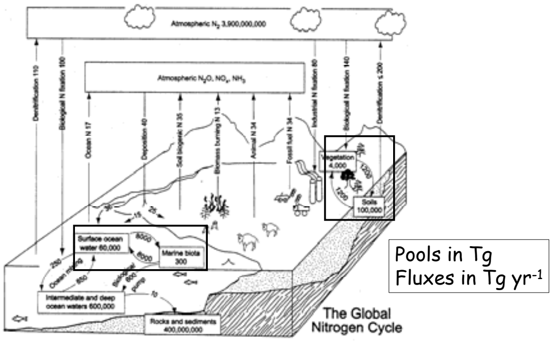
Microbial enzymatic processes drive biological N cycle



Nutrient cycles are not necessarily continuous in space or time

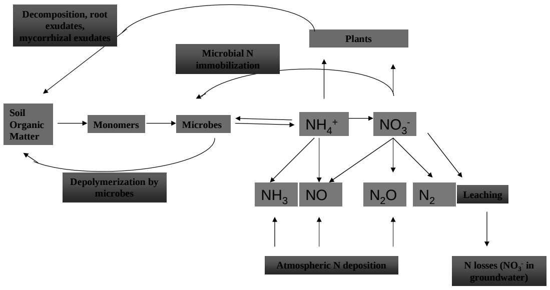
Nutrient cycle processes do not evolve to "help" the ecosystem – they are selected by survival of organisms within niches.

Biological cycling within systems greatly outweighs inputs/outputs – not very "open"

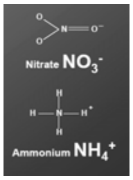


Pools in Tg
Fluxes in Tg yr^{-1}

The Soil N Cycle: microbes are central to turnover



NO_3^- and NH_4^+ are used by plants and microbes for protein synthesis.



Plants have high-affinity transporters for NO_3^- and NH_4^+
Ammonium preferred; nitrate subject to leaching in runoff

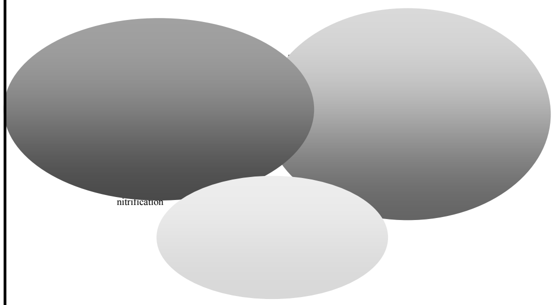
NO, N_2O, N_2 are gases that are lost from ecosystems to the atmosphere

Nitrous oxide (N_2O = depletes ozone; greenhouse gas, 4th behind CO2 because of longevity, stable)

Nitric oxide (NO) – interacts with organic compounds to form ozone, nitric acid if mixed with rain, unstable, free radical

At high pH (>8), NH_3 can also escape as gas

Balance among major processes determines net N available in ecosystem



Nitrogen fixation is the chemical transformation of N_2 to NH_3

Industrial: Haber-Bosch process used to make fertilizer and explosives.

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad -16.6\ \text{kJ/mol}$$

Exergonic but lacks catalyst to proceed spontaneously at RT
Industrial: 400°C-600°C; 100-200 atm.

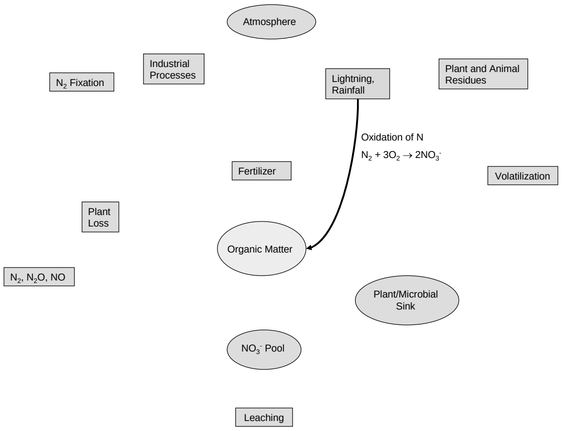
Biological: mutualistic relationship between legumes and *Rhizobium* or related genera; OR done by free living Bacteria and Archaea (e.g. *Azotobacter*).

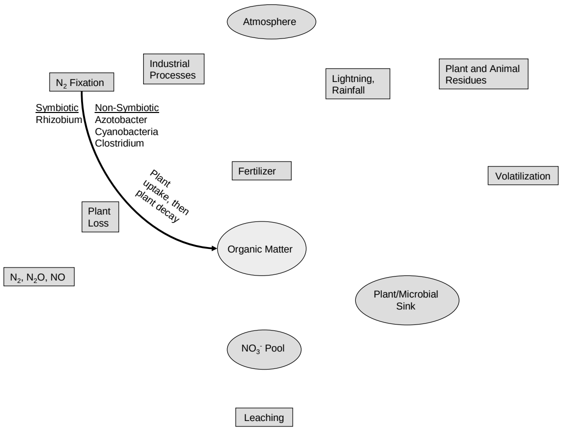
$$N_2 + 10H^+ + 8e^- \rightarrow 2NH_4^+ + H_2$$

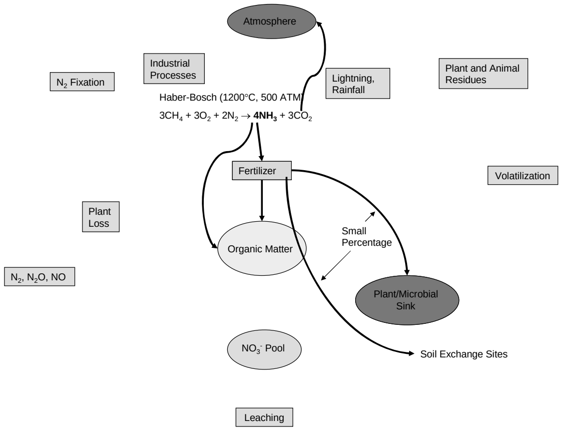
Also exergonic, but requires input of 16 ATP per molecule of N_2 reduced.
Requires e^- source. H_2 is "by-product"
… in what way might this be useful to humans??

Combustion of fossil fuels: combustion engines and thermal power plants release various nitrogen oxides (NO_x).

Other processes: formation of NO from N_2 and O_2 due to photons and lightning are important for atmospheric chemistry, although minor contributors to terrestrial or aquatic N pools.







Atmosphere

Industrial Processes

Lightning, Rainfall

Plant and Animal Residues

N_2 Fixation

Haber-Bosch (1200°C, 500 ATM)

$3CH_4 + 3O_2 + 2N_2 \rightarrow \mathbf{4NH_3} + 3CO_2$

Fertilizer

Volatilization

Plant Loss

Small Percentage

Organic Matter

N_2, N_2O, NO

Plant/Microbial Sink

NO_3^- Pool

Soil Exchange Sites

Leaching

Nodules on plant roots

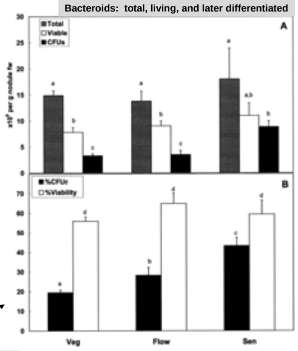
Mutualism… really??

Senescing bacteroids have potential to re-differentiate into free-living rhizobia

Number of bacteroids in *L. japonicus* root nodules and their potential to redifferentiate into growing bacteria, grouped according to the developmental status of the plant.

L. japonicus was inoculated with *Rhizobium*, grown 11 weeks, and grown in darkness 1 week (senescence induction)

Bacteroids: total, living, and later differentiated



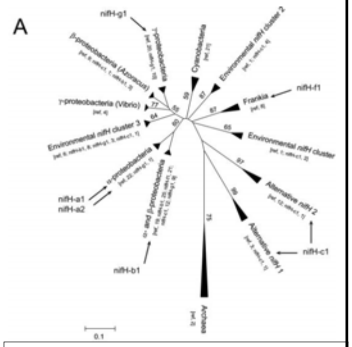
% total bacteroids living and later differentiated

Müller et al. 2001. J. Exp. Bot. 52: 2181

Free-living diazotrophs

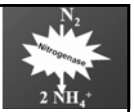
Autotrophs, heterotrophs, anaerobes, aerobes, facultatives.

CFUs per gram of soil in different crop rhizospheres varied from 1 × 10^4 to 8 × 10^5.



Bürgmann et al., 2004. Appl. Env. Microbiol. 70: 240.

Nitrogenase:



-3 main types
Molybdenum, vanadium, or iron cofactors

Limitations: cold, O_2, limiting cofactors Fe, P (ATP) or Mo

Gets around O_2 toxicity by day/night (non-cyst forming cyanos), crowding, symbiosis, compartmentalization (cyst cyanos)

Found widely: invertebrate guts, soils, plants, bioreactors, lakes, rivers, and the open ocean

But highly conserved: early origin (then retention and loss), or HGT??

nifH has become one of the largest non-ribosomal gene datasets on uncultivated microorganisms

Not likely via HGT – phylogenies align fairly well with 16S so far

---

Fig. 3. Comparison of *nifH* (A) and 16S rRNA (B) phylogeny for cultivated microorganisms. *nifH* and 16S rRNA sequences were aligned and analysed by neighbour-joining using ARB. Analysis was bootstrapped and bootstrap values greater than 50% are indicated at respective nodes. Numbers in brackets indicate number of sequences in each cluster. *nifH* cluster names refer to cluster designations shown in Figs 1 and 2.

Zehr et al., 2003. Env. Microbiol. 5: 539

Pathways that use N

Assimilatory: incorporation into organic molecules
    Most microbes can take up nitrate and incorporate into organic molecules
    NO_3^- + 8 e^- ➔ NH_3 (nitrate reductase, nitrite reductase)
    NH_3 incorporated into glutamine (glutamine synthetase)
    Glutamine is the amino donor for purine, pyrimidine, amino sugars and glutamate
    Glutamate is the amino donor for amino acid synthesis

Dissimilatory: electron acceptor (respiration; redox balance)

Oxidative: electron source

Pathways that use N

Assimilatory: incorporation into organic molecules

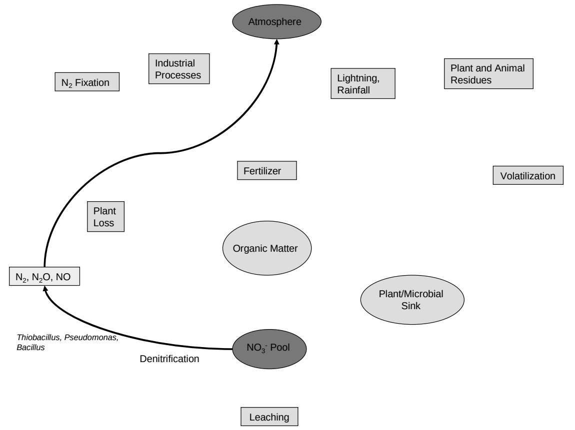
Oxidative: electron source

Dissimilatory: electron acceptor (respiration; redox balance)
    a.k.a denitrification
    Many microbes, e.g. *Alcaligenes, Pseudomonas, Bacillus, Thiobacillus, Paracococcus*
    NO_3^- + e^- → NO_2^-, NO, N_2O, N_2, NH_3
    Membrane-associated enzymes
    Usually facultative, occurs as substitute for aerobic respiration when O_2 low
    Sites of anoxia: waterlogged soils, composting, sludge digestion

Denitrification is the reduction of NO_3^- to nitric oxide (NO), to nitrous oxide (N_2O), and finally to molecular nitrogen (N_2)

NO_3^- ➔ NO ➔ N_2O ➔ N_2

Denitrification occurs when oxygen is not present and nitrate acts as an electron acceptor



Atmosphere

Industrial Processes

N_2 Fixation

Lightning, Rainfall

Plant and Animal Residues

Fertilizer

Volatilization

Plant Loss

Organic Matter

N_2, N_2O, NO

Plant/Microbial Sink

Thiobacillus, Pseudomonas, Bacillus

NO_3^- Pool

Denitrification

Leaching

Nitrate reduction/denitrification

BUT: nitrate reduction can be dissimilatory OR assimilatory.

Separate genes, enzymes for each process; different enzyme localization

Cytoplasmic assimilatory (Nas): requires ABC transport protein to get NO_3^- into cell

Membrane-bound respiratory (Nar) - generates a transmembrane proton motive force (PMF) allowing ATP synthesis

Periplasmic dissimilatory (Nap) nitrate reductases: redox balance (excess reductant)

All systems – highly regulated gene expression

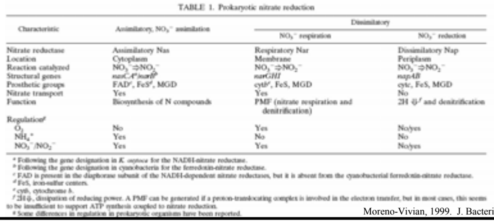


TABLE 1. Prokaryotic nitrate reduction

Characteristic	Assimilatory, NO_3^- assimilation	Dissimilatory	
		NO_3^- respiration	NO_3^- reduction
Nitrate reductase	Assimilatory Nas	Respiratory Nar	Dissimilatory Nap
Location	Cytoplasm	Membrane	Periplasm
Reaction catalyzed	$NO_3^- \Rightarrow NO_2^-$	$NO_3^- \Rightarrow NO_2^-$	$NO_3^- \Rightarrow NO_2^-$
Structural genes	*nasCA*[a]/*narB*[b]	*narGHI*	*napAB*
Prosthetic groups	FAD[c], FeS[d], MGD	cyt*b*[e], FeS, MGD	cyt*c*, FeS, MGD
Nitrate transport	Yes	Yes	No
Function	Biosynthesis of N compounds	PMF (nitrate respiration and denitrification)	2H ⇓[f] and denitrification
Regulation[g]			
O_2	No	Yes	No/yes
NH_4^+	Yes	No	No
NO_3^-/NO_2^-	Yes	Yes	No/yes

[a] Following the gene designation in *K. oxytoca* for the NADH-nitrate reductase.
[b] Following the gene designation in cyanobacteria for the ferredoxin-nitrate reductase.
[c] FAD is present in the diaphorase subunit of the NADH-dependent nitrate reductases, but it is absent from the cyanobacterial ferredoxin-nitrate reductase.
[d] FeS, iron-sulfur centers.
[e] cyt*b*, cytochrome *b*.
[f] 2H ⇓, dissipation of reducing power. A PMF can be generated if a proton-translocating complex is involved in the electron transfer, but in most cases, this seems to be insufficient to support ATP synthesis coupled to nitrate reduction.
[g] Some differences in regulation in prokaryotic organisms have been reported.

Moreno-Vivian, 1999. J. Bacteriol. 181: 6573

Nitrate reduction/denitrification

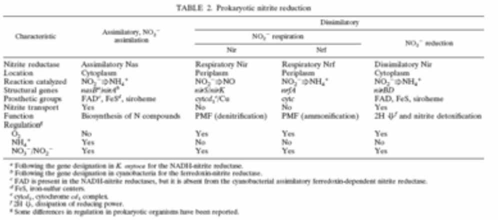


TABLE 2. Prokaryotic nitrite reduction

Characteristic	Assimilatory, NO_2^- assimilation	Dissimilatory		
		NO_2^- respiration		NO_2^- reduction
		Nir	Nrf	
Nitrite reductase	Assimilatory Nas	Respiratory Nir	Respiratory Nrf	Dissimilatory Nir
Location	Cytoplasm	Periplasm	Periplasm	Cytoplasm
Reaction catalyzed	$NO_2^- \Rightarrow NH_4^+$	$NO_2^- \Rightarrow NO$	$NO_2^- \Rightarrow NH_4^+$	$NO_2^- \Rightarrow NH_4^+$
Structural genes	*nasB*[a]/*nirA*[b]	*nirS*/*nirK*	*nrfA*	*nirBD*
Prosthetic groups	FAD[c], FeS[d], siroheme	cytcd₁[e]/Cu	cyt*c*	FAD, FeS, siroheme
Nitrite transport	Yes	No	No	Yes
Function	Biosynthesis of N compounds	PMF (denitrification)	PMF (ammonification)	2H ⇓[f] and nitrite detoxification
Regulation[g]				
O_2	No	Yes	Yes	Yes
NH_4^+	Yes	No	No	No
NO_3^-/NO_2^-	Yes	Yes	Yes	Yes

[a] Following the gene designation in *K. oxytoca* for the NADH-nitrite reductase.
[b] Following the gene designation in cyanobacteria for the ferredoxin-nitrite reductase.
[c] FAD is present in the NADH-nitrite reductases, but it is absent from the cyanobacterial assimilatory ferredoxin-dependent nitrite reductase.
[d] FeS, iron-sulfur centers.
[e] cytcd₁, cytochrome *cd*₁ complex.
[f] 2H ⇓, dissipation of reducing power.
[g] Some differences in regulation in prokaryotic organisms have been reported.

Moreno-Vivian, 1999. J. Bacteriol. 181: 6573

Pathways that use N

Assimilatory: incorporation into organic molecules
Dissimilatory: electron acceptor (respiration; redox balance)

Oxidative: electron source
Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria
a.k.a. nitrifiers ➔ aerobic, obligate chemolithoautotrophs (except *Nitrobacter* – facultative)
NH_3 from denitrification diffuses from anoxic niche to **aerobic** niche
Nitrifiers often found at oxic/anoxic boundary

Oxidation under anoxic conditions: an oxymoron?
anammox: $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

Nitrification is the oxidation of ammonium:

ammonium (NH_4^+) to nitrite (NO_2^-)
nitrite (NO_2^-) to nitrate (NO_3^-)

Sources of ammonia:

Nitrogen fixation
Nitrate reduction
Ammonification (conversion of organics, e.g. amino acids, to NH_3)

Nitrification (oxidative N utilization)

Nitrification = 2-step process

1. Ammonia oxidation
Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, Nitrosovibrio
(terrestrials = β-proteobacteria; marine = γ-proteobacteria)

1a. ammonia monooxygenase, AMO, cell membrane

$$2H^+ + NH_3 + 2e^- + O_2 \rightarrow NH_2OH + H_2O$$

1b. hydroxylamine oxidoreductase, HAO, periplasm

$$NH_2OH + H_2O \rightarrow HONO + 4\ e^- + 4H^+$$

4 e^- to electron transport chain; sole source of energy for *Nitrosomonas*

2. Nitrite oxidation
Nitrobacter, Nitrococcus, Nitrospina, Nitrospira

electrons flow from nitrite to oxygen via reversed electron flow in membrane

---

Old paradigm: **Nitrification** is an **aerobic** process.
NH_3 from denitrification diffuses from anoxic niche to **aerobic** niche
Nitrifiers often found at **oxic/anoxic** boundary

Oxidation under anoxic conditions: an oxymoron?

anammox: $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

Major contribution to cycling inorganic N in oceans
Nitrite can even come from denitrification (dissimilatory nitrate reduction) in same cells

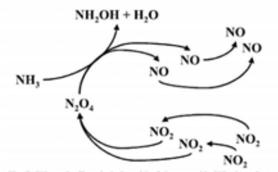
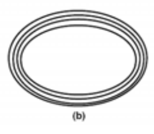


Fig. 3. NO_x cycle. Hypothetical model of the anaerobic NO_2-dependent ammonia oxidation by *Nitrosomonas*. N_2O_4 is the oxidant for the ammonia oxidation.


(b)

Anaerobic:
AMO down, membranes up

Schmidt et al., 2001. FEMS Microbiol. Ecol. 39: 175

Niftrik et al., 2004. FEMS Microbiol. Lett. 233: 7

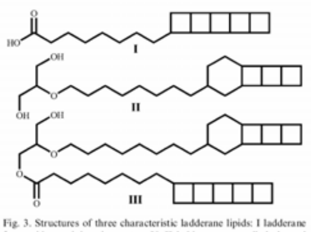


Fig. 3. Structures of three characteristic ladderane lipids: I ladderane fatty acid-containing ring-system Y. II ladderane monoalkyl glycerol ether-containing ring-system X. III ladderane glycerol ether/ester containing both ring-systems, X and Y. Lipids containing ladderane moieties X and Y are abundant membrane lipids in anammox bacteria. Adapted with permission from Jetten et al. [29].

Niftrik et al., 2004. FEMS Microbiol. Lett. 233: 7

Domain Bacteria
Phylum Planctomycetes

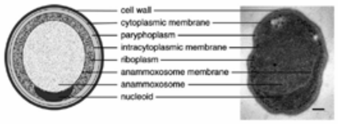


Fig. 2. Cellular compartmentalization in anammox bacteria. Left: schematic drawing, right: thin section of cryosubstituted *Candidatus* "Brocadia anammoxidans" seen via transmission electron microscopy. *Bar* 100 nm.

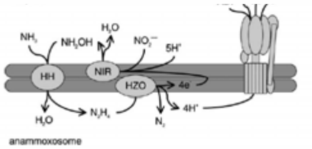


Fig. 4. Postulated anaerobic ammonium oxidation coupled to the anammoxosome membrane in anammox bacteria resulting in a proton motive force and subsequent ATP synthesis via membrane-bound ATPases. HH: hydrazine hydrolase; the hydrazine-forming enzyme, HZO: hydrazine-oxidizing enzyme, NIR: nitrite-reducing enzyme.

Niftrik et al., 2004. FEMS Microbiol. Lett. 233: 7

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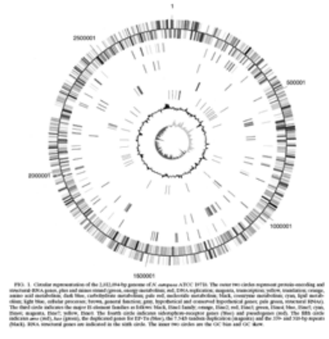
Complete Genome Sequence of the Ammonia-Oxidizing Bacterium and Obligate Chemolithoautotroph *Nitrosomonas europaea*†

Patrick Chain,[1,2] Jane Lamerdin,[1,2] Frank Larimer,[1,3] Warren Regala,[1,2] Victoria Lao,[1,2]
Miriam Land,[1,3] Loren Hauser,[1,3] Alan Hooper,[4] Martin Klotz,[5] Jeanette Norton,[6]
Luis Sayavedra-Soto,[7] Dave Arciero,[4] Norman Hommes,[7]
Mark Whittaker,[4] and Daniel Arp[7]*

TABLE 1. Summary of *N. europaea* genome



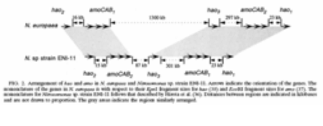
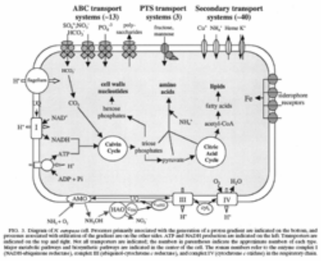
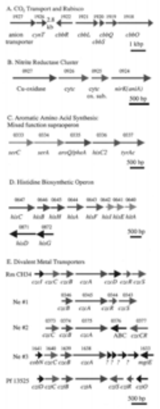
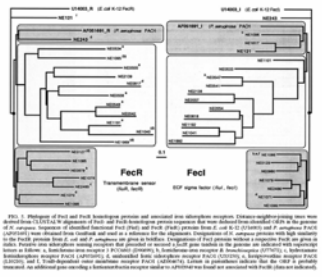


TABLE 2. Complex repetitive sequences







Purple bacteria

Cyclic

NAD(P) reduction requires -0.32 V

NAD(P)H for CO_2 fixation generated by reverse e^- flow (requires energy to push e^-s against potential): further metabolic burden on already energy - poor process

ATP generated by PMF built during electron transport

Anoxygenic photosynthesis

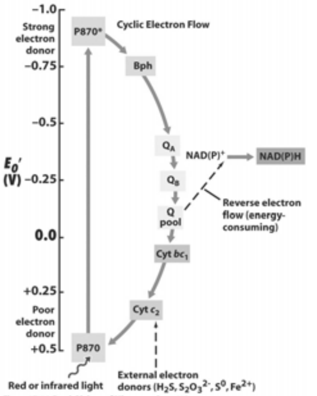


Figure 17-14 Brock Biology of Microorganisms 11/e