MICROBES RESPONSIBLE FOR NITROGEN TRANSFORMATIONS

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THE INFORMATION PRESENTED REPRESENTS THE AUTHOR'S THOUGHTS AND OPINIONS, AND DO NOT REFLECT THE OPINIONS OF NOWRA

Onsite wastewater is a crossroad discipline

- Blend of Biology, Chemistry and Physics concepts
- One can enter the onsite wastewater field from many starting points
- Resolve to learn something new every day
- Advanced treatment wastewater technologies focus on nitrogen

Biology basics

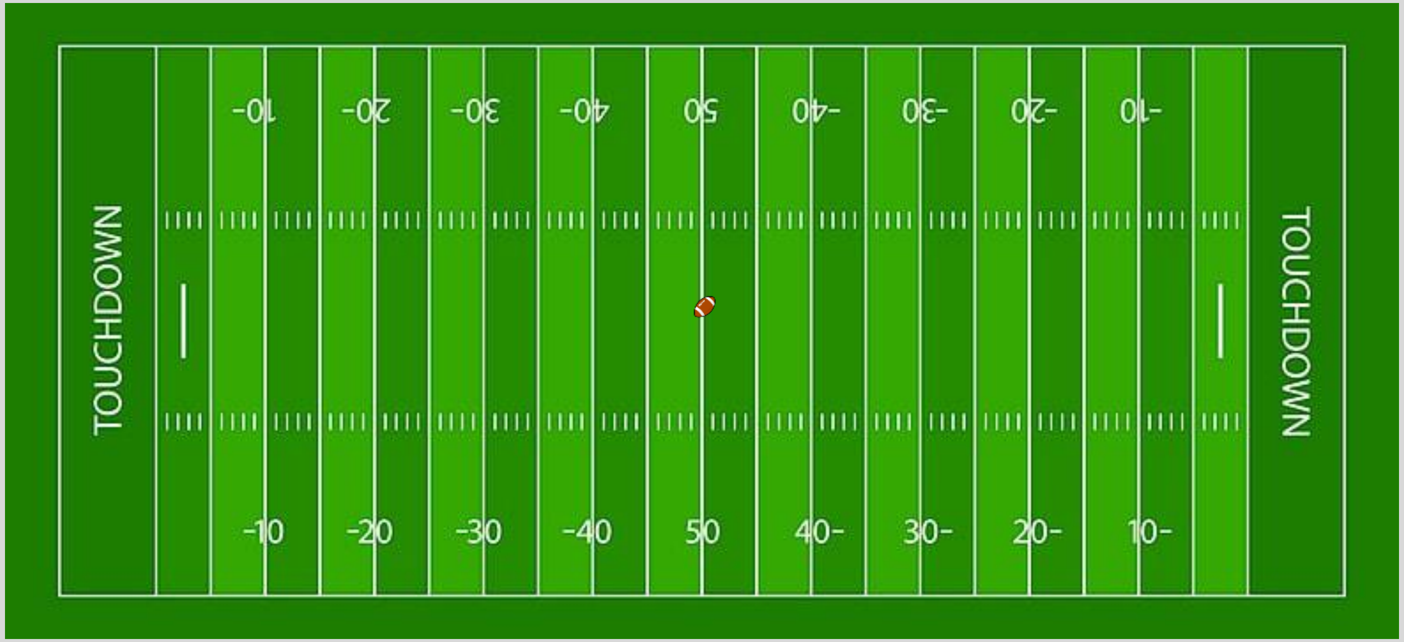
- Taxonomy – The science of naming, describing and classifying living things
- Three Domains (Archaea, Bacteria, Eukarya)
- Next smaller groups Kingdom, Phylum, Class, Order, Family
- Last two groups genus and species - Both are used to name an organism
- Genus name is written beginning with a capital letter
- Species name is written in all lowercase letters
- Both genus and species names are formatted *in italics*
- Multiple species of the same genus has the abbreviation spp.
- More than one genus is referred to as genera

Biology basics - bacteria

- Bacteria and virus - how close are they in size?

If a virus was the size of a football…

A bacteria would be the size of a football field!



More Biology basics - Bacteria

- Bacterial Generation Times
- Bacterial Genetic Material coded on Ribonucleic Acids (RNA)
- Major defining criteria of bacteria is how they respond to oxygen

Oxygen Classifications for Bacteria

Class name	Definition	Environment(s) where found	Example species
Obligate(strict) aerobe	Require abundant (22%) oxygen to thrive	Animal skin fast flowing streams	*Neisseria meningitides* *Bacillus subtilis* *Pseudomonas aeruginosa*
Microaerophile	Require a minimum of oxygen (1 – 10%) to grow	Animal gut	*Campylobacter jejuni*
Aerotolerant anaerobe	Indifferent to the presence of oxygen. Not used in its metabolism	Drains, marshlands, swamps	*Streptococcus* spp. *Lactobacillus* spp.
Facultative aerobe / anaerobe	Have dual metabolic pathways. Can switch between them	Fluctuating aerobic – anoxic - anaerobic	*Escherichia coli* *Staphylococcus aureus*
Strict (obligate) anaerobe	Exposure to oxygen causes instant death	Decaying matter Petroleum seeps Animal gut	*Clostridium perfringens* *Bacteroides* spp. *Methanobrevibacter smithii*

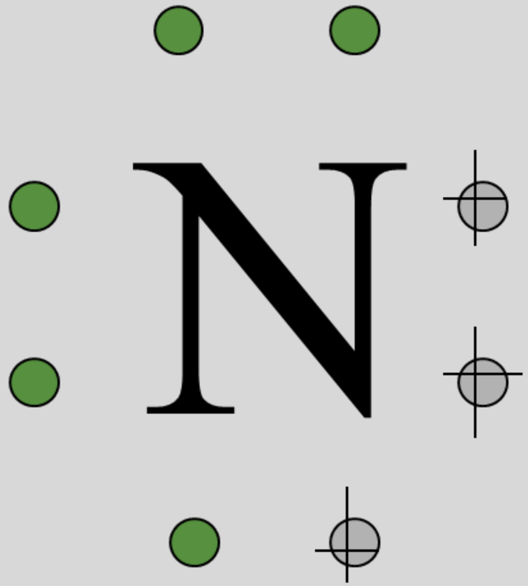
Microbial Dark Matter

The bacteria we know of are culturable. We are unaware many more types of bacteria simply because we cannot culture them yet. This statement is particularly true of obligate anaerobic bacteria. As we perfect techniques to culture expect many more species to be discovered

The atom Nitrogen & its chemistry

- Atomic number (number of Protons) of 7
- Atomic weight (Protons + Neutrons) 14.0067
- Electrons inner shell full (2)
- Second shell (d) 5 of possible 8 spots filled

7
N
14.01



Periodic Table of the Elements

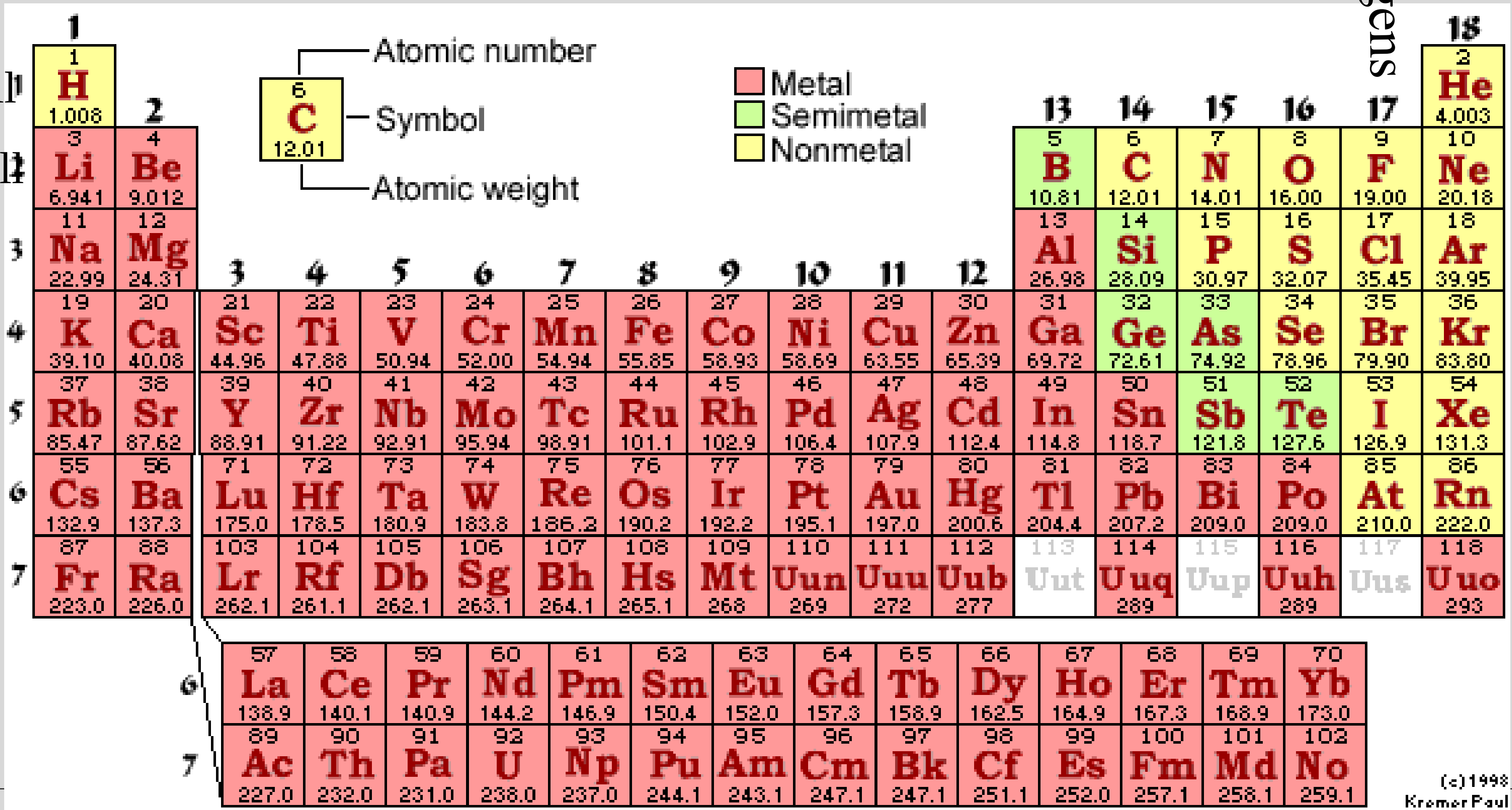
Alkali Metals

Halogens

Noble Gasses

S shell

D shell



Goal: to reach the stability of a Noble Gas

- Add three electrons - Neon (atomic number 10)
- Loose 5 electrons - Helium (atomic number 2)

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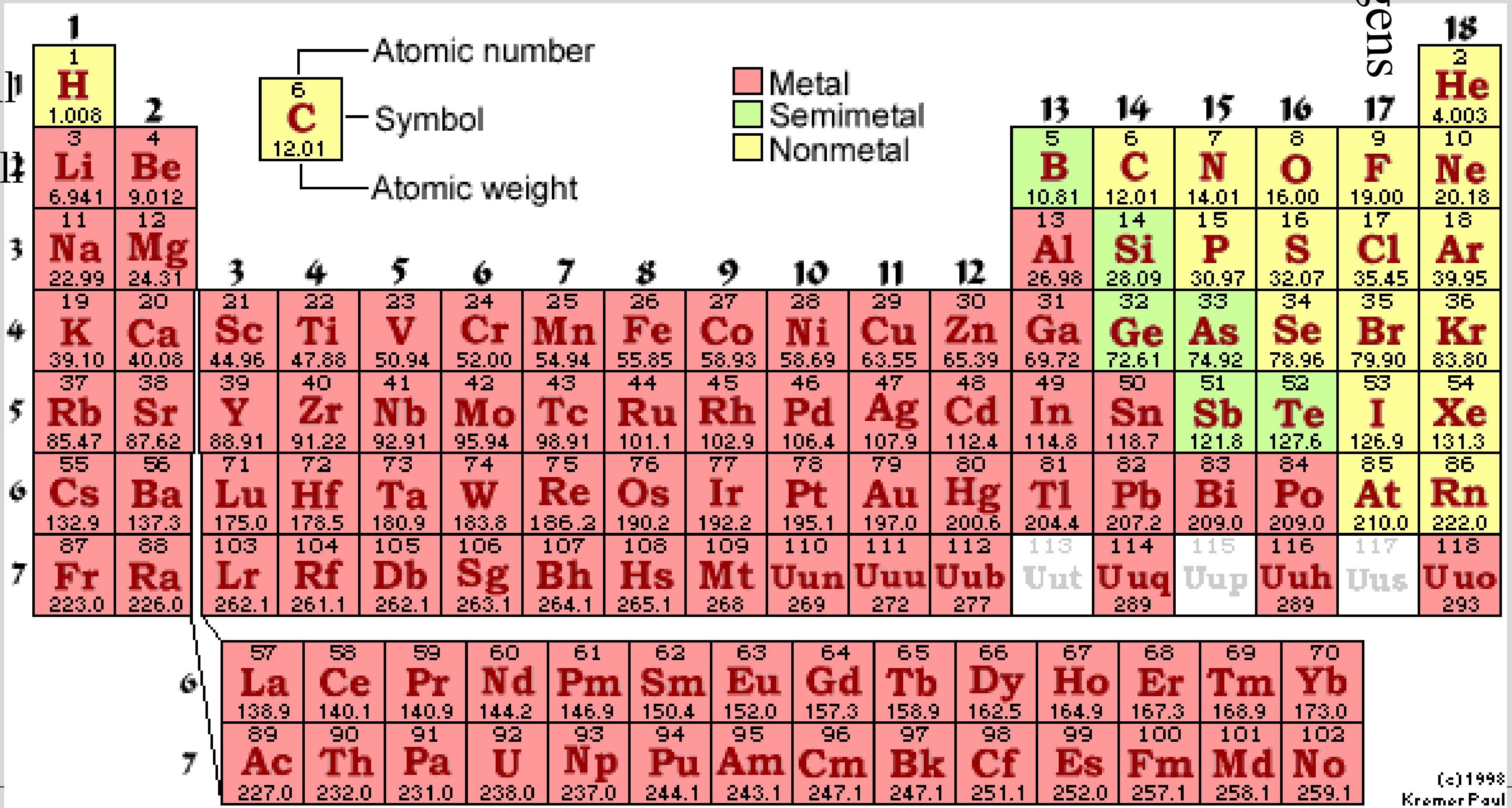
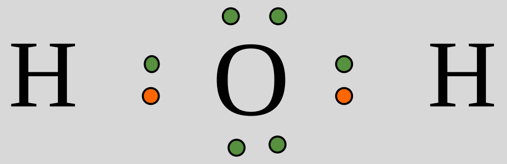


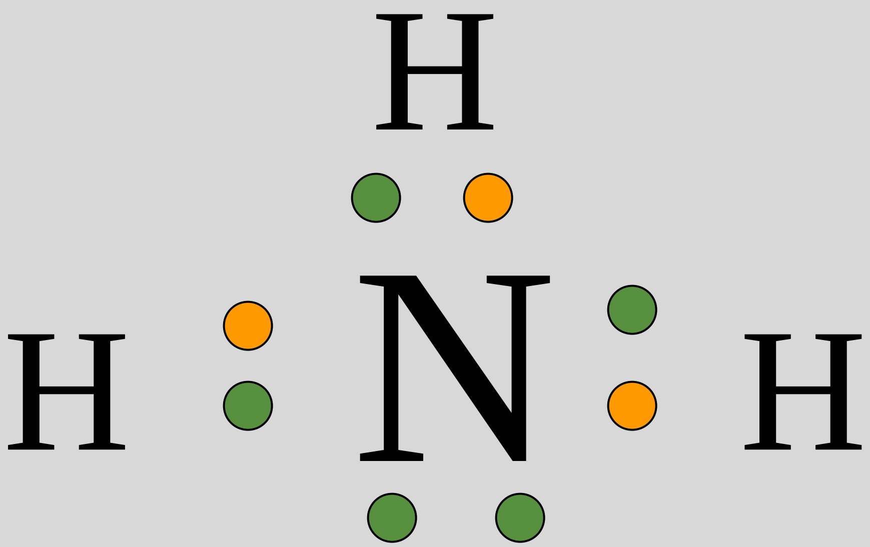
Nitrogen is found in 7 oxidation states

- Depending on the other elements it is combined with (compound), can be stable (inert) or reactive (explosive)
- In a compound, the subscript after the element is the number of them eg: H_2O



When adding 3 electrons

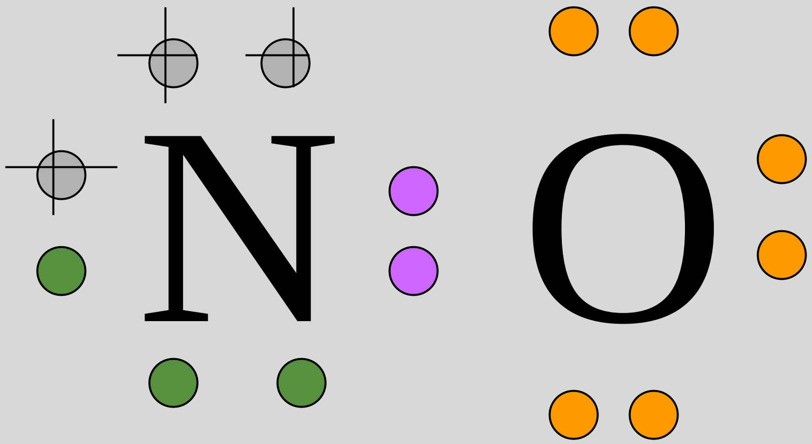
- Oxidation state -3
- Organic Nitrogen (proteins & amino acids)
- Ammonia NH_3



= electron taken permanently from Hydrogen (ionic bond)

When giving up 2 electrons

- Oxidation state +2
- Nitric Oxide NO

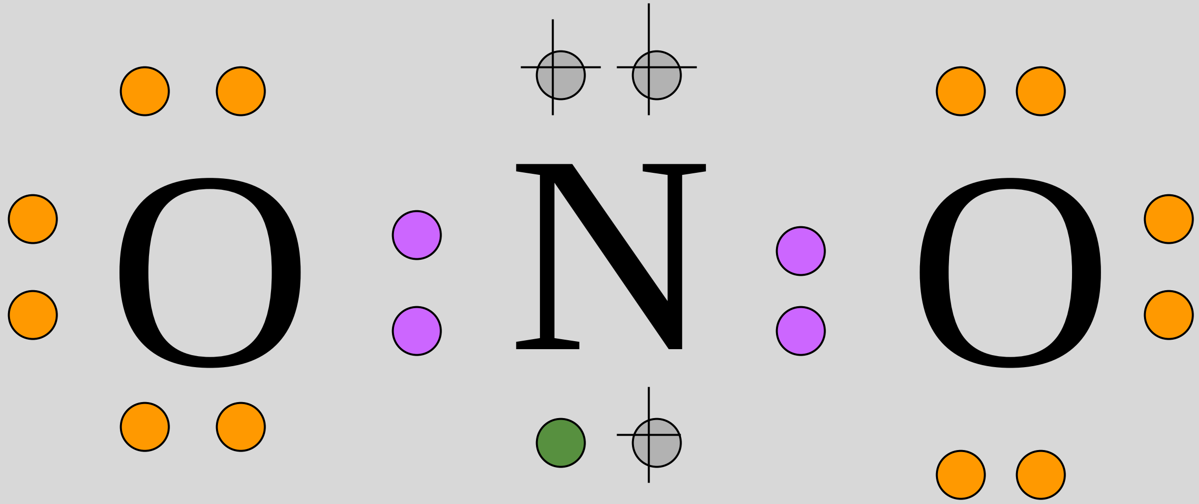


= electron permanently given to oxygen

= empty spot

When giving up 4 electrons

- Oxidation state +4
- Nitrite NO_2^-

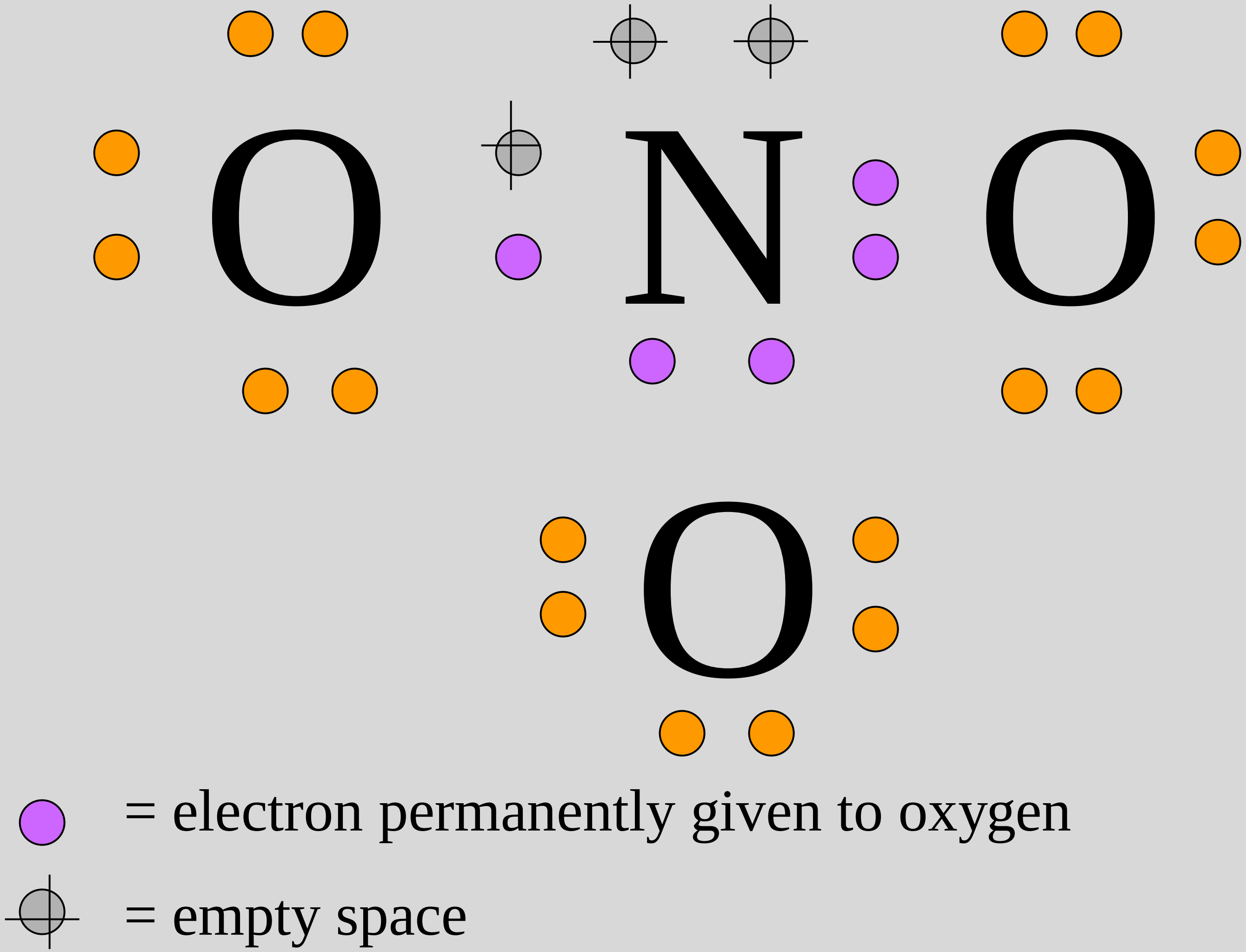


= electron permanently given to oxygen

= empty space

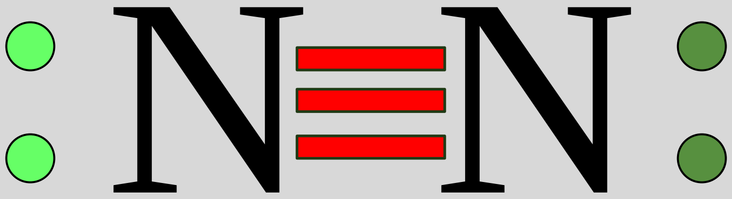
When giving up 5 electrons

- Oxidation state +5
- Nitrate NO_3^-


O
N
O
O
= electron permanently given to oxygen
= empty space

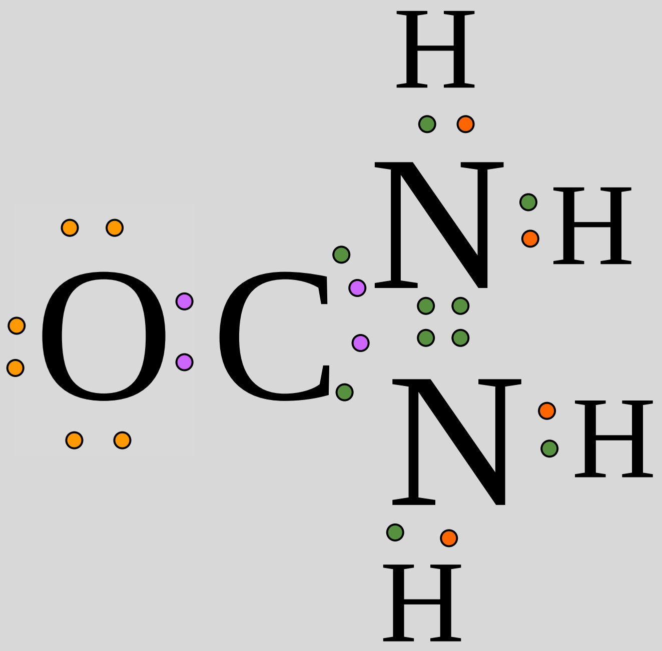
When no change in oxidation state

- Nitrogen gas
- N_2
- Very stable ~ 78% of earth's atmosphere



 = triple bond

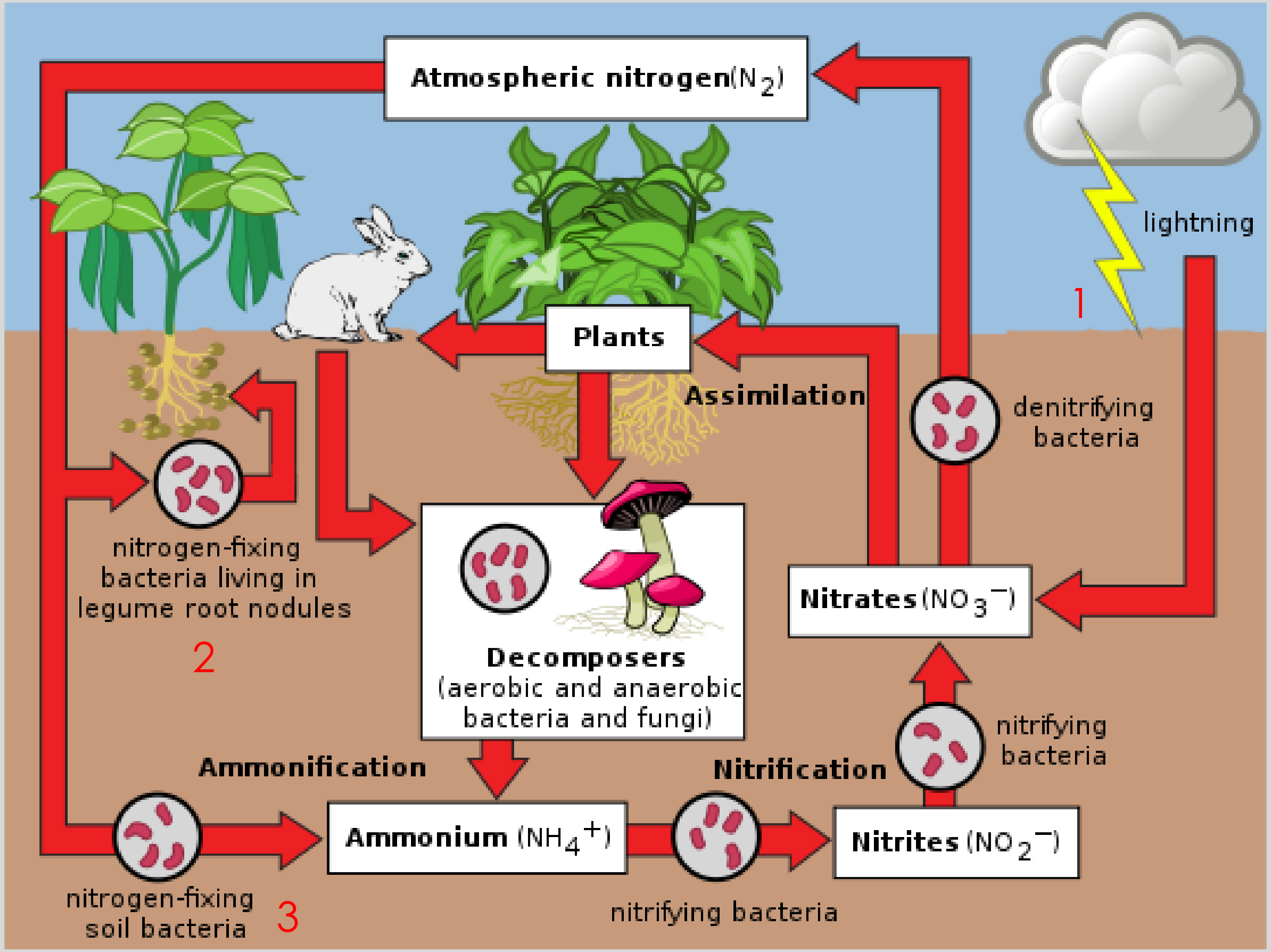
Urea CH_4ON_2



Common Forms of Nitrogen

◦ In tissues	amino acids & proteins
◦ In urine	urea
◦ Reduced forms	NH_3 (ammonia)
	NH_4^+ (ammonium ion in water)
◦ Oxidized forms	NO_2^- (Nitrite)
	NO_3^- (Nitrate)
◦ In Air	N_2 (78% by weight)

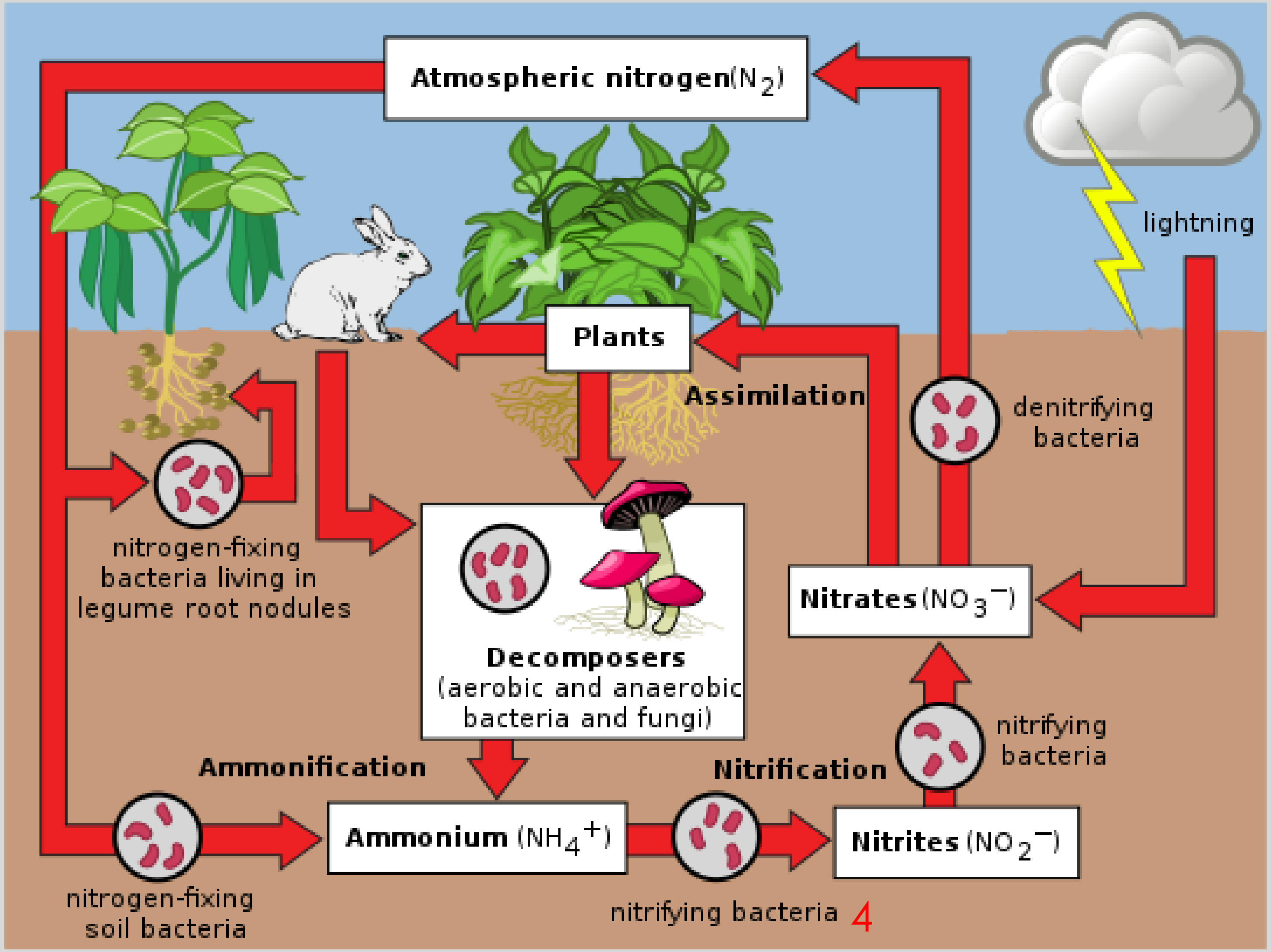
Nitrogen Cycle – emphasis on bacteria



Nitrogen Fixing Bacteria

- Heterotrophic bacteria decomposing organic matter
- Convert Nitrogen gas (N_2) to ammonium (NH_4^+)
- *Azotobacter* spp.
- *Bacillus* spp.
- *Clostridium perfringens* →
- *Klebsiella* spp.
- Form nodules in association with grasses and legumes
- Industrial fertilizer production predicted to exceed natural Nitrogen fixation levels by 2030's

Nitrogen Cycle – emphasis on bacteria



Nitrifying bacteria (step one)

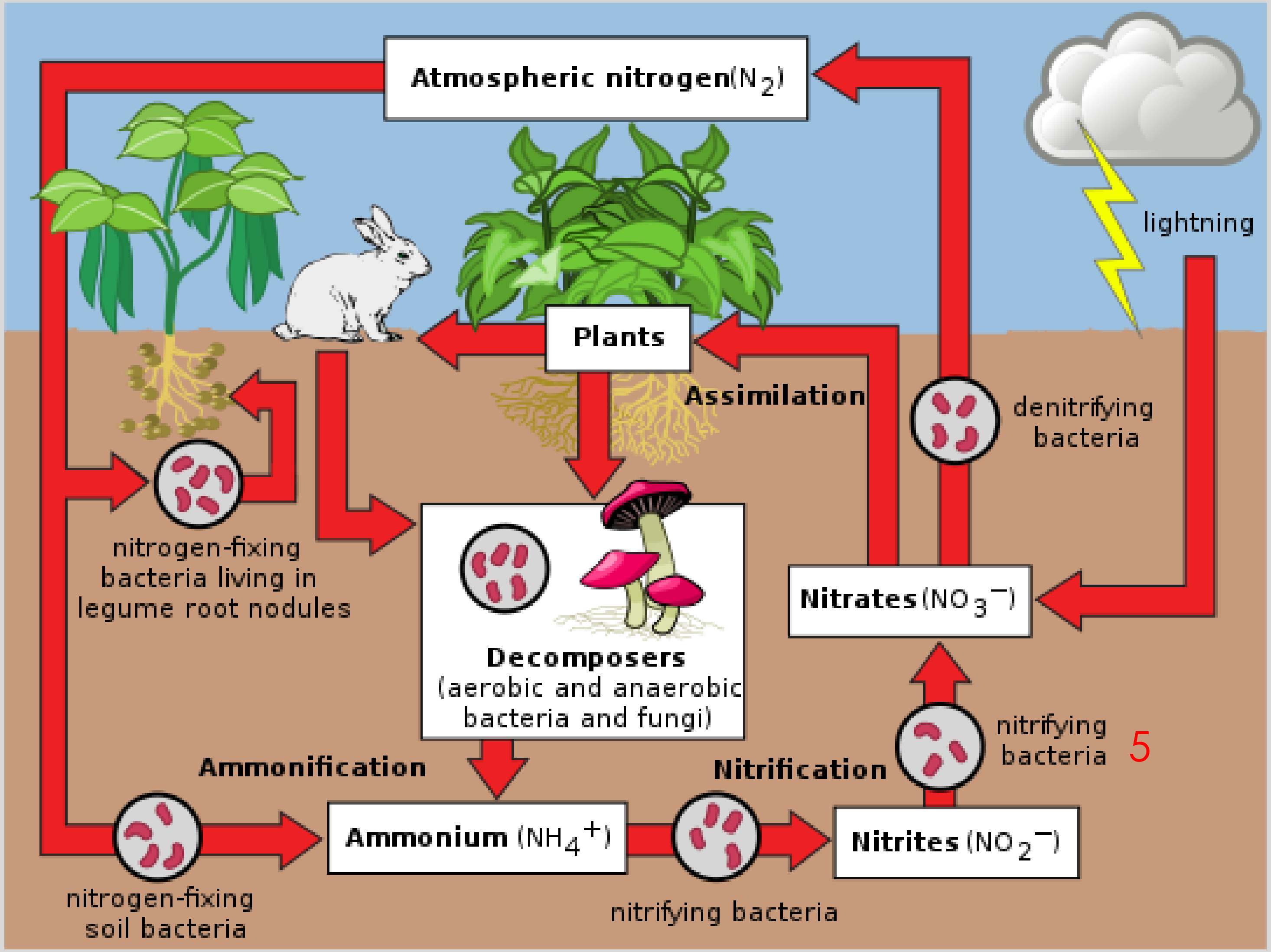
- Performed by *Nitrosomonas* spp.
- Uses ammonia as an energy source
- Uses carbon dioxide (CO_2) as a carbon source
- Genus is chemoautotrophic - it gains its sustenance by capturing energy from chemical reaction inside cell
- $NH_3 + 3/2O_2 \rightarrow NO_2 + 2H^+ + H_2O$ + energy
- Converts ammonia to Nitrite

More Nitrifying bacteria (step one)

- Obligate (strict) aerobe
- Requires temperatures 20-30^{o}C+, pH 6-9, high alkalinity
- Dislikes direct sunlight and organic carbon, killed by organic solvents (acetone)

Species name	Preferences or environment	Motile or sedentary	Has genome been sequenced?
N. aestuarii	Requires salt, uses urea		
N. communis	Soils		
N. europaea	Soils and fresh water		yes
N. eutropha	High ammonia tolerant	Motile through flagellum	
N. halophila	Requires salt	Motile through flagellum	
N. marina	Requires salt, uses urea		
N. nitrosa	Requires low ammonia, uses urea		
N. oligotropha	Requires low ammonia, uses urea		
N. stercoris	Composted cattle manure		
N. ureae	Uses urea		yes

Nitrogen Cycle – emphasis on bacteria



Nitrifying bacteria (step two)

- Performed by the genera *Nitrobacter*, *Nitrococcus*, *Nitrospina*, *Nitrospira*, *Nitrosospina* and *Nitrosococcus*
- Chemoautotrophic with heterotrophic possible
- $NO_2^- \quad + \quad 1/2O_2 \quad \rightarrow \quad NO_3^- +$ energy
- Converts Nitrite to Nitrate
- Obligate (Strict) Aerobe
- Less finicky than *Nitrosomonas*, but success depends on nitrite availability

Have you ever heard of a slinky?

- As slinky moves down stairs, what is happening?
- Converting potential energy (elevation) to kinetic (motion) energy
- What would happen if slinky moves down basement stairs -1 ft below floor -2 ft below floor etc.?
- Would it move any differently?

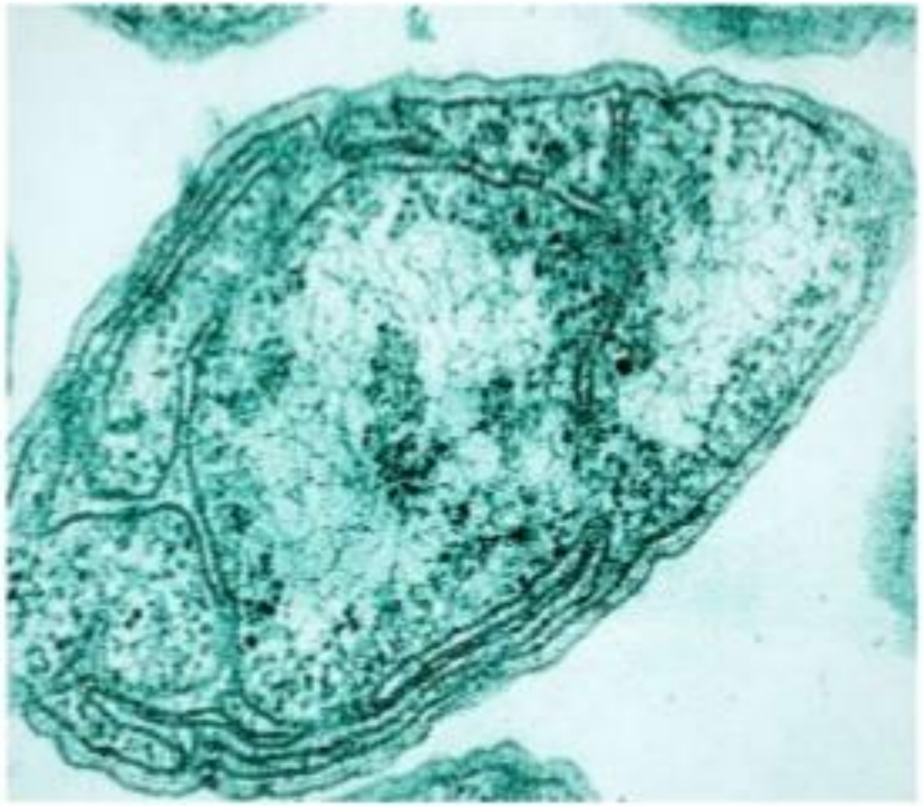
Nitrifying Bacteria are doing something similar with nitrogen containing chemicals

- Chemicals have a measurable quantity of energy associated with them
- Chemical reactions from one compound to another will either require energy or release energy
- Gibbs Free Energy of Ammonia is –16.4 kJ mol–1
- Gibbs Free Energy of Nitrite is –32.2 kJ mol–1
- Gibbs Free Energy of Nitrate is –111.3 kJ mol–1

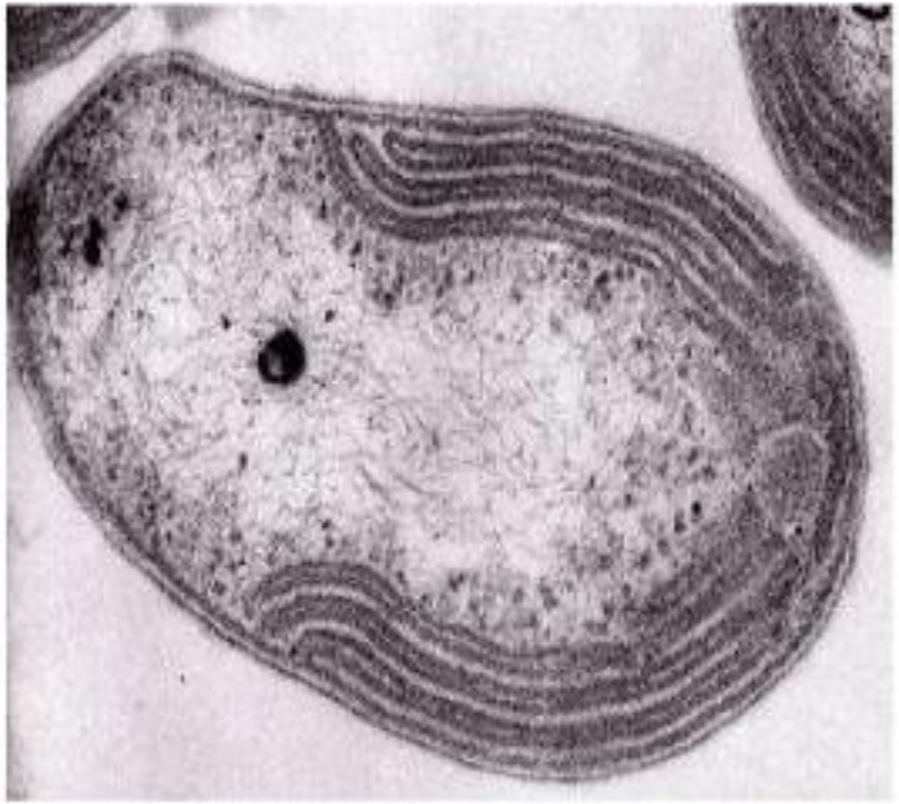
Overall Nitrification Reaction

- $NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O$
- Requires Oxygen (4.6 lb/lbN)
- Uses up alkalinity (7.1 lb/lbN)
- Nitrifying bacteria prefer stable, neutral to basic pH with high alkalinity

Nitrifying bacteria

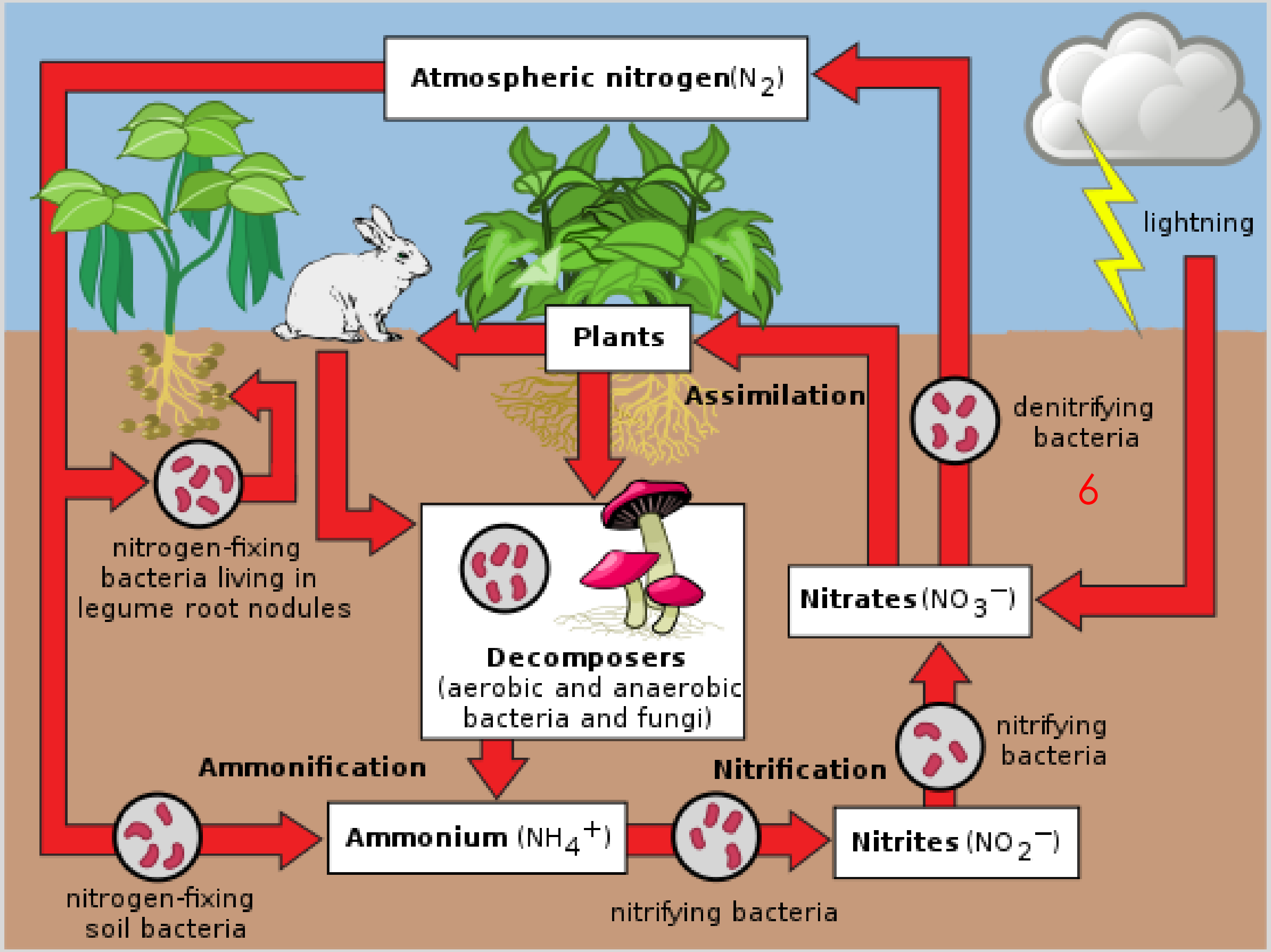


Nitrosomonas



Nitrobacter

Nitrogen Cycle – emphasis on bacteria



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Denitrifying Bacteria

- Bacteria, once again, are responsible for the process
- Conditions must be anoxic (no free O_2)
- Nitrate NO_3 used as the electron acceptor
- More energy released using O_2, so if it is present it will be used instead of nitrate
- Organic matter (external carbon source) must be supplied

Various Heterotrophic Bacteria

$$NO_3^- + \text{Organic Matter} \rightarrow N_2 + CO_2 + OH^- + H_2O$$

Denitrifying Bacteria Details

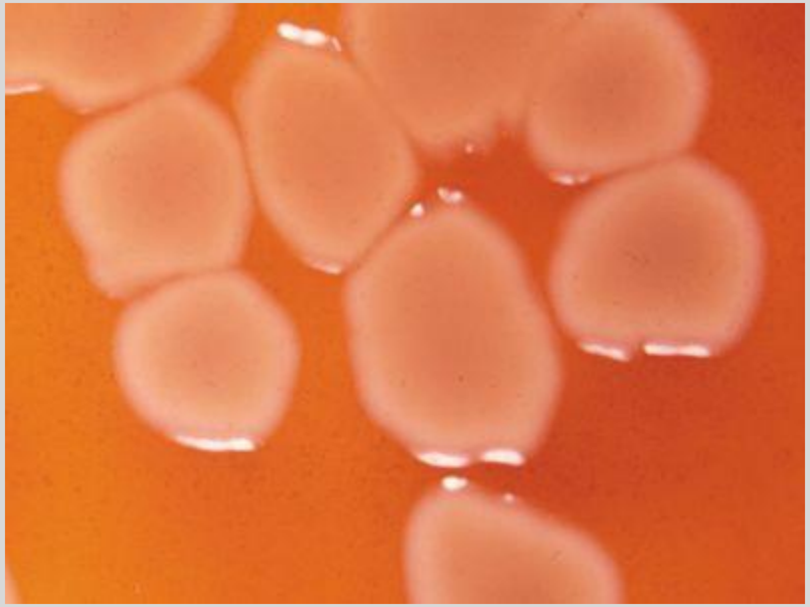
- Denitrifying bacteria are facultative aerobes / anaerobes and can shift between oxygen respiration and fermentation
- They are outcompeted by obligate anaerobes in a septic tank
- They are outcompeted by obligate aerobes in an aerobic treatment unit
- Denitrifiers only thrive in fluctuating aerobic - anaerobic environments, creating anoxic conditions between the two environments

More Denitrifying Bacteria Detail

- carbon source can come from the original wastewater, bacterial cell material, or an external source such as methanol or acetate
- Introduce (or reintroduce) fully nitrified effluent to an anoxic environment with carbon added
- Sequential Nitrification/Denitrification process

Denitrifying Bacteria

- There are over 50 denitrifying bacteria genera with over 124 species
- Examples: *Thiobacillus denitrificans*, *Micrococcus denitrificans*, *Pseudomonas* spp., *Achromobacter* spp.



Pseudomonas aeruginosa

The irony of biological Nitrogen reduction

- Nitrifiers are slow growers
- They are sensitive to inhibitory compounds
- They desire low organic carbon concentrations
- They thrive in high dissolved oxygen concentration environments

- Denitrifiers are fast growers
- They are resilient to inhibitory compounds
- They require high organic carbon concentrations
- They thrive in fluctuating low to absent dissolved oxygen

Understanding and responding to these different requirements is the greatest challenge to successful onsite and decentralized system nitrogen reduction

Questions?

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