

- The plan for today:
  - The global nitrogen cycle
  - N<sub>2</sub> fixation – general considerations
  - Plant microbial symbioses – Rhizobium, Agrobacterium
  
- Key processes and prokaryotes in the nitrogen cycle
  - Organisms that reduce nitrate and nitrite instead of oxygen
  - Nitrogen fixation
    - This is how new nitrogen gets into the atmosphere
    - There are a lot of organisms that are capable of this
    - It takes a lot of energy
  - All of these processes are mostly bacterial – if you took away the bugs, the nitrogen cycle wouldn't turn
  - Cation exchange in the soil
    - We dump urea and nitrogen in the soil – this eventually causes greater nitrogen loss
  - Forest soils versus cut soils
    - In the forest soil, more recycling goes on
    - In a clearcut forest, the ammonia stops getting taken up by the system
    - With lots of ammonia but no plants to take it up, the bacteria eats it all up, and the soil loses nitrogen
  - In aquaria, nitrifiers are essential – the fish couldn't stay alive otherwise
  - What happens when you dump lots of phosphate in a lake?
    - You get big blooms of nitrogen-fixing cyanobacteria
    - This is why phosphates aren't around in detergents anymore – they're a bit problem
    - Phosphate is almost the only other thing that these bacteria need to grow, so they really take off – this leads to pollution problems – these bacteria create a lot of toxins
    - You decrease the nitrogen to phosphate ratio
  - There are other organisms that can fix nitrogen – autotrophs, some archaea, some proteobacteria
  - Energetics – you have to burn about 16 ATP for each nitrogen that gets reduced – you have to be producing a lot of energy
  
- The Global Nitrogen Cycle
  - You're looking at different fluxes from each activity
  - Remember, the only things that can fix nitrogen and denitrify are microbes
  - Nitrogen is very important to us, particularly in terms of agriculture
  - Around 1900, they thought the world was running out of nitrogen

- Then came the Haber process for the production of ammonia – now people could produce their own fertilizer
- The production of ammonia since 1900 mirrors the takeoff in human population growth – it has allowed the food production necessary for so many people
- We've basically doubled the amount of nitrogen in the biosphere – we're now fixing more nitrogen than all biological nitrogen fixation combined
- Some plants, such as soybeans, have relationships with nitrogen microbes that allow them to grow in very poor soil – this is why crop rotation is important
- Nitrogenase, Nitrogenase enzyme complex
  - Doesn't work in the presence of oxygen
  - However, there's one other type of nitrogenase that does work in the presence of oxygen – there are different types
  - Most of these microbes have to keep the oxygen away from the nitrogenase, so that it doesn't stop working
  - *Anabaena* forms filaments – chains of cells
    - The cells then segregate and specialize, so that the cells that do nitrogen fixation have an oxygen-reduced environment
    - This is an unusual example of microbial cell differentiation
- Remember, every organism really needs nitrogen to grow
  - There are some really interesting associations that form as a result
  - Diagram: N<sub>2</sub> fixing symbiont inside a diatom
  - Ship worms, which eat up wood really quickly so it doesn't just float around the ocean for long.
    - They chew up wood, eating cellulose.
    - This is all carbohydrates – there's no nitrogen
    - The relationship that forms is similar to that of the aphids
    - They have nitrogen-fixing bacteria that provide nitrogen, while the ship worm gives the bacteria a place to live.
  - Nitrogen-fixing bacteria in soya plant root nodules
    - They have symbionts pulling in nitrogen from the atmosphere, so they can grow in really poor soils
    - These relationships involve interspecies crosstalk between bacteria and plants
    - *Rhizobia* wouldn't ordinarily fix nitrogen if they were just living in the soil by themselves – they wouldn't need to
    - But when they live on the plant's root nodules, they start fixing nitrogen
    - There's a particular host recognition and host specificity
    - The *Rhizobia* enters a plant cell via an infection thread – it's an intricate process, that involves the plant sending out signals and the bacteria responding
    - This is species specific – a particular kind of *Rhizobium* invades a particular kind of plant
    - Development of the root nodule

- Flavinoid compounds, which the bacteria senses – interacts with *nod* genes – they make a polysaccharide called a Nod factor
- The Nod factor causes the plant's root hair to curl, causing the infection thread that allows the *Rhizobia* to enter.
- The plant makes this compound called leghemoglobin, which binds oxygen really tightly, so that the plant can provide the bacteria with bound oxygen that won't float around and interfere with the nitrogen fixation
- The plant provides the bacteria with glucose (carbon), and the bacteria provide the plant with nitrogen in return
- This is a remarkably complicated symbiosis
- Agrobacteria causes plant diseases, with galls (tumors) that look a lot like the nodules created by *Rhizobia*.
  - However, this is parasitism, not mutual symbiosis
  - The agrobacteria causes a kind of genetic engineering, inserting its own DNA and changing the plant development
  - This inserted DNA includes oncogenes
  - The DNA also includes genes for opine synthesis, which the bacteria get the plant to produce and feed to them
  - The bacteria can thus naturally transform the plant cells to turn them into an opine factory for themselves
- People use this same method (Ti plasmids, plus some intermediate factors) for genetic engineering in plants
  - Transgene delivery system based on the Ti plasmid
  - Segregation of the transgene – Mendelian
  - Then you do standard genetic crossings
  - Cells from a huge variety of species can be modified in this way – plant, fungal, even human
- A lot of people have problems with this kind of genetic modification, but there are also a lot of benefits
  - Most of the corn grown in the US is genetically modified
  - However, once you get these genetically-modified plants out into the field, it's hard to keep them contained – the pollen spreads from farm to farm
  - The effects of this on plant diversity and gene transfer could be worrisome
  - There are intellectual property issues involved – what happens when a company produces a genetically-modified plant and then it turns up on other farms via pollen
  - Also other controversies of ethics, labeling, society...