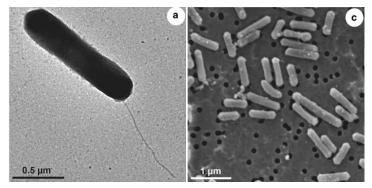


#### 1. Introduction. Growth strategies

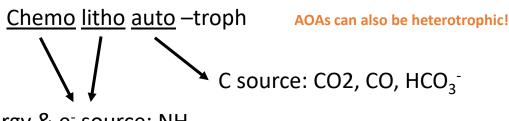
#### Main resources

- Energy (photons, e⁻ donors) → Photo- & Chemo- trophy
- e⁻ and H⁺ donors (organic, inorganic) → Organo- & Litho- trophy
- Carbon (organic, inorganic) → Hetero- & Auto- trophy



Nitrosopumilus adriaticus NF5. Bayer B., et al. 2015

- Ammonia oxidising archaea (AOA). Class Nitrososphaeria.
- AOAs are among the most abundant organisms on Earth!
- Major contributors of the greenhouse gas nitrous oxide N₂O.



Energy & e<sup>-</sup> source: NH<sub>3</sub>

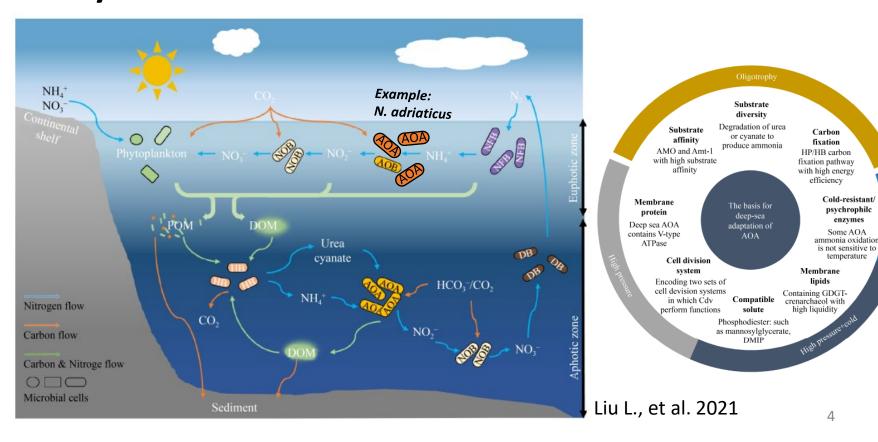
#### 1. Introduction. Growth components

- Carbon source
- Nitrogen source
- Other macronutrients: S, P, K, Mg.
- Micronutrients: Fe, Mn, Co, Cu, Zn, Ni, Na.
- e donors:
  - Inorganic (H<sub>2</sub>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, H<sub>2</sub>S, Fe<sup>2+</sup>)
  - Organic (glucose, acetate, propionate, chitin, lignin, proteins...)
- e<sup>-</sup> acceptors: O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Fe<sup>3+</sup>.

#### 1. Introduction. Growth conditions

Microbial life can thrive in an extremely wide range of conditions, some of the main **community drivers** are:

- Temperature
- Water activity
- pH
- O<sub>2</sub>
- Light
- Salinity

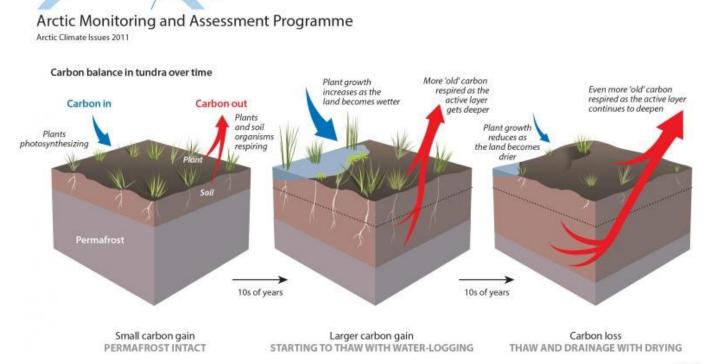


#### 1. Introduction. Biogeochemical systems

- Microorganisms are the backbone of all ecosystems.
  - Microbial processes are critical to maintain a balanced ecosystem by facilitating nutrient cycling.
  - Many ecosystems function without macroorganisms, but none do without microorganisms.
- Microorganisms play a critical role in energy transformations and biogeochemical processes that result in the recycling of elements.
  - Biogeochemical cycles (incl. nutrient cycles) are the cyclic transformation of chemicals between their chemical forms. Often via *redox* reactions.

#### 1. Introduction. Sinks and sources

- Sinks are anything that absorbs more than emits any given nutrient.
- Sources are anything that emits more than absorbs any given nutrient.



#### 2. Key microbial processes in the environment

#### **Carbon cycling**

- Flow of carbon through all of Earth's major reservoirs.
  - Atmosphere, land, oceans, freshwater, sediments, Earth's crust, rocks, biomass.
  - Reservoir size and turnover time are important in understanding the cycles.

All nutrient cycles are linked to the carbon cycle, but the nitrogen (N) link is particularly strong.

C, N and H<sub>2</sub>O constitute the bulk of living organisms.

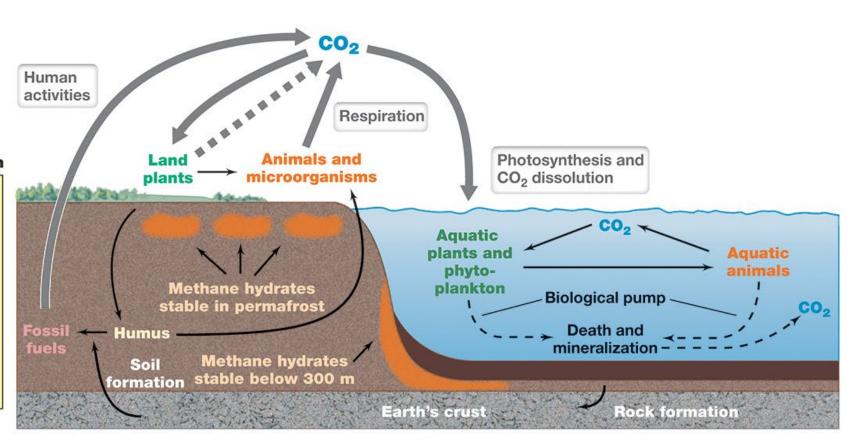


## 2. The Carbon cycle I

#### **Major Carbon Reservoirs on Earth**

Reservoir	Percent of Total <sup>a</sup>
Rocks and sediments	99.5 <sup>b</sup>
Oceans	0.05
Methane hydrates	0.014
Fossil fuels	0.006
Terrestrial biosphere	0.003
Aquatic biosphere	0.000002

<sup>&</sup>lt;sup>a</sup>Total carbon, 76 × 10<sup>15</sup> tons <sup>b</sup>80% inorganic



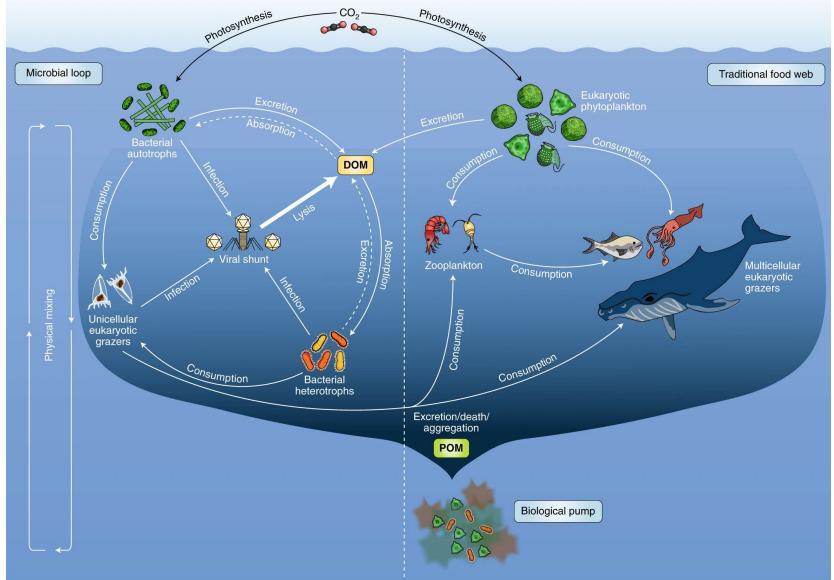
#### 2. The Carbon cycle II

#### **Carbon reservoirs**

- Earth's crust, rocks and sediments are the largest C reservoir. However,  $CO_2$  in the atmosphere is the most rapidly transferred one.
- CO<sub>2</sub> is removed (mobilised) from the atmosphere by photosynthesis.

- Necromass is the total mass of dead organic material. Microbial necromass contributes significantly to soil organic matter (SOM) and accelerates C and N cycling. Necromass contains more C than the living organisms of a region.
- The **viral shunt** is the process that funnels dissolved organic matter (DOM) from viral cell lysis of host cells directly into the microbiome.

2. The Carbon cycle III - Viral Shunt



Breitbart M., et al. 2018

Other recommended reading:

Gao Y., et al. 2022



#### 2. The Carbon cycle IV – carbon turnover

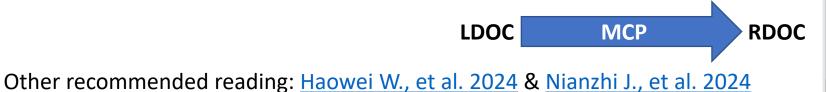
• CO<sub>2</sub> is returned to the atmosphere by respiration and decomposition as well as by human-related (anthropogenic) activities.

• Since the Industrial Revolution, human (anthropogenic) activities have increased

atmospheric carbon by 40 percent.

Microbial decomposition is the largest source of CO<sub>2</sub>
 released to the atmosphere.

Microbial activity also generates the **Microbial Carbon Pump** (MCP) "microbial transformation of organic carbon from labile to refractory states"



Wagner S., et al. 2020

"Labile"

#### 2. The Carbon cycle V

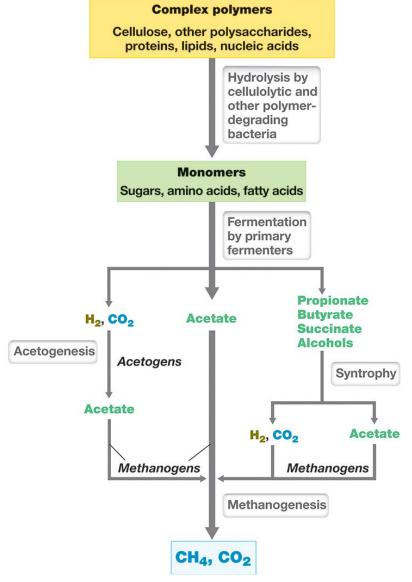
- The rise in carbon dioxide has led to steadily increasing temperatures worldwide (global warming) because CO<sub>2</sub> is a **greenhouse gas** (GHG).
- Phototrophic organisms produce organic or fixed carbon and reduce the level of CO<sub>2</sub> in the atmosphere
  - Oxygenic phototrophic organisms can be divided into two groups: plants and microorganisms
    - Plants dominate terrestrial environments.
    - Microorganisms dominate aquatic environments.

#### 2. The Carbon cycle VI

- Photosynthesis and respiration are part of redox cycle.
  - Photosynthesis reduces inorganic CO<sub>2</sub> to organic carbohydrates C(H<sub>2</sub>O)  $CO_2 + H_2O \rightarrow (CH_2O) + O_2$
  - Respiration oxidises organic carbohydrates to inorganic CO<sub>2</sub>  $(CH_2O) + O_2 \rightarrow CO_2 + H_2O$
- The two major end products of decomposition are methane (CH<sub>4</sub>) and carbon dioxide  $(CO_2)$ .
  - $CH_{\Delta}$  is a potent greenhouse gas and is produced anoxically (or oxygen-free).
  - Most methane is converted to CO<sub>2</sub> by **methanotrophs**; however, some enters the atmosphere. Methane is a more potent GHG than CO<sub>2</sub>.
  - Most **methanogens** use CO<sub>2</sub> as e<sup>-</sup> acceptor and H<sub>2</sub> as donor. The reduction of CO<sub>2</sub> with H<sub>2</sub> yields CH<sub>4</sub>. Some methanogens use other substrates (e. g., acetate)



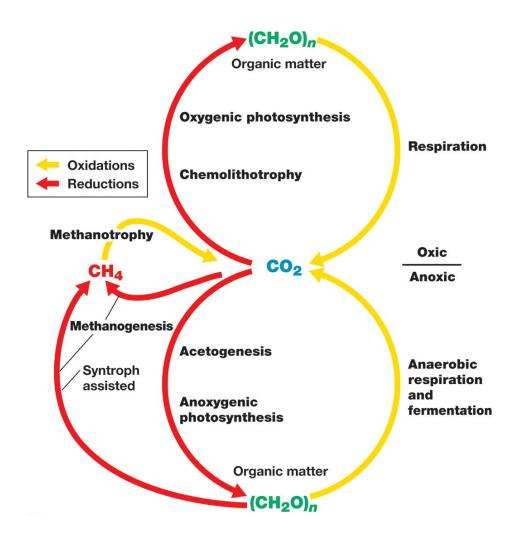
2. The Carbon cycle – Anoxic decomposition



**Syntrophy**: Two or more microorganisms cooperate to perform a metabolism that they can't do on their own (e. g. sharing intermediates, H<sub>2</sub>, e<sup>-</sup>, etc.)

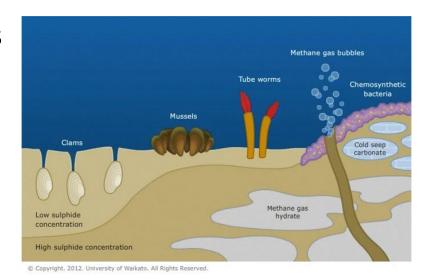


### 2. The Carbon cycle VIII – redox of cycle for C



### 2. The Carbon cycle IX – Methane hydrates

- Methane hydrates form when high levels of methane are under high pressure and low temperature.
- Huge amounts of methane are trapped underground as methane hydrates.
  - e.g., beneath the permafrost in the Arctic and in marine sediments.
- Methane hydrates can absorb and release methane.
  - Methane hydrates fuel deep-sea ecosystems called cold seeps.



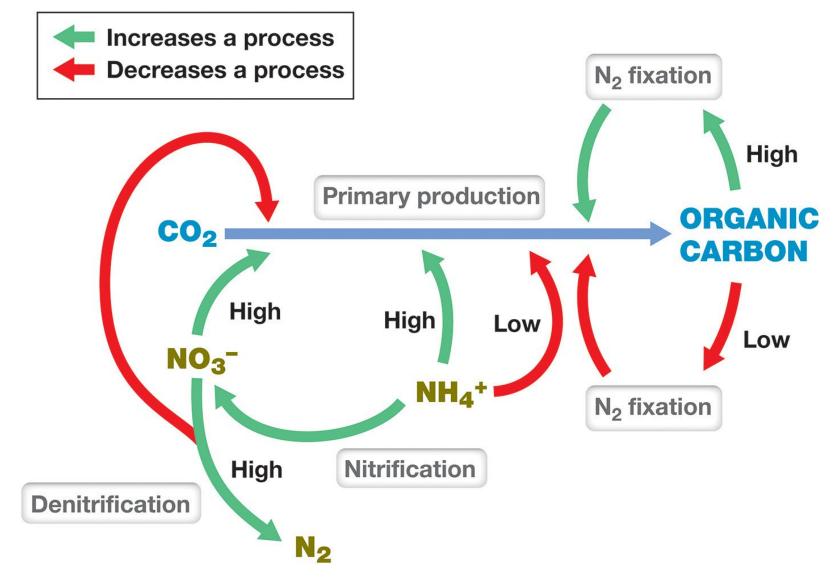
Ward C. H., et al. 2027

#### 3. Coupled cycles I

- In nature, nutrient cycles are interconnected and feed back upon one another.
- Major changes in one cycle affect the functioning of other cycles.
- For example, the rate of carbon fixation and plant growth is often limited by the available nitrogen. This is why adding nitrogen to farm fields will increase yield.
- The carbon cycle and the nitrogen cycle are very closely coupled.



## 3. Couple cycles II



### 4. The Nitrogen cycle I

- Nitrogen is a key component of cells.
- Nitrogen has a wide range of oxidation states (-3 to +5).
- There are four major nitrogen transformations.
  - Nitrification

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

Nitrogen Fixing Bacteria / Archaea

Denitrification

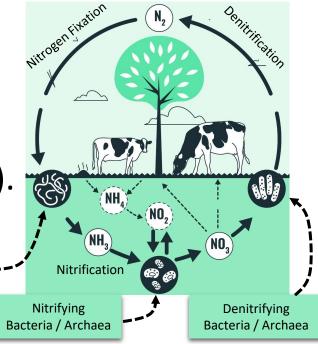
$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Anammox

$$NH_4^+ + NO_2^- \to N_2 + 2H_2O$$

Nitrogen fixation

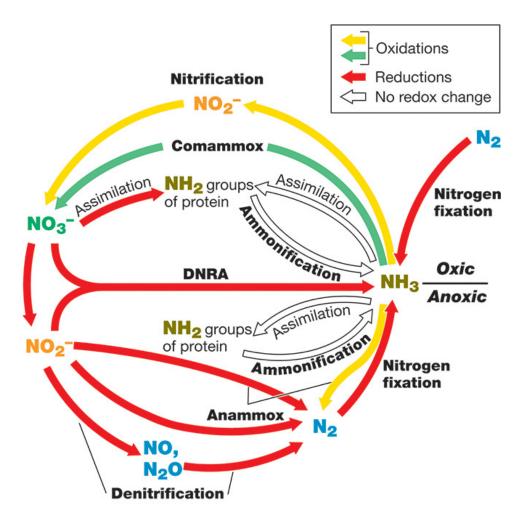
$$N_2 + 16ATP + 16H_2O + 8e^- + 8H^+ \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$





# 4. The Nitrogen cycle II – Redox cycle of nitrogen

Key Processes and Microbes in the Nitrogen Cycle		
Processes	Example organisms	
Nitrification (NH <sub>4</sub> <sup>+</sup> $\rightarrow$ NO <sub>3</sub> <sup>-</sup> )		
$NH_4^+ \longrightarrow NO_3^-$	Comammox (Nitrospira species)	
$NH_4^+ \longrightarrow NO_2^-$	Nitrosomonas, Nitrosopumilus (Archaea)	
$NO_2^- \longrightarrow NO_3^-$	Nitrobacter	
<b>Denitrification</b> $(NO_3^- \longrightarrow N_2)$	Bacillus, Paracoccus, Pseudomonas	
$N_2$ Fixation ( $N_2 + 8 \text{ H} \rightarrow 2 \text{ NH}_3 + \text{H}_3$	2)	
Free-living		
Aerobic	Azotobacter	
	Cyanobacteria	
Anaerobic	Clostridium, purple and	
	green phototrophic bacteria	
	Methanobacterium (Archaea)	
Symbiotic	Rhizobium	
	Bradyrhizobium	
	Frankia	
<b>Ammonification</b> (organic-N $\rightarrow$ NH <sub>4</sub> <sup>+</sup> )		
	Many organisms can do this	
Anammox $(NO_2^- + NH_3 \rightarrow 2 N_2)$	Brocadia	





## 4. The Nitrogen cycle III – Reservoirs & mobility

- N<sub>2</sub> is the most stable form of nitrogen and is a major reservoir. It constitutes approximately 70% of Earth's air.
  - Only a few prokaryotes can use  $N_2$  as a nitrogen source. They convert inorganic  $N_2$  to organic nitrogen through **nitrogen fixation** (energy-intensive).

$$N_2 + 16ATP + 16H_2O + 8e^- + 8H^+ \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

• **Denitrification** reduces nitrate  $(NO_3^-)$  to gaseous nitrogen products  $(N_2)$  and is the primary biological  $N_2$  production mechanism.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

• Ammonia (NH<sub>3</sub>) produced by nitrogen fixation or ammonification can be assimilated into organic matter or oxidised to nitrite (NO<sub>2</sub>-), and then nitrate (NO<sub>3</sub>-).

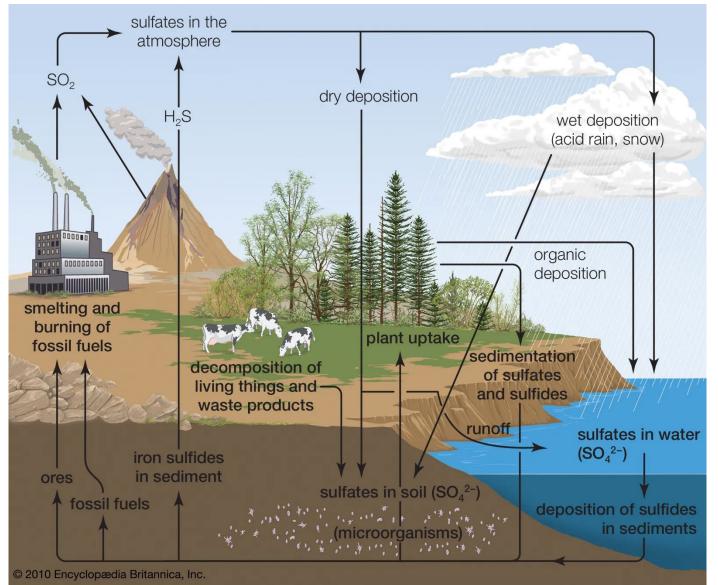


#### 5. The Sulphur cycle I

- Microbial Sulphur transformations are complex but critical to determine the phase and ultimate fate of sulphur in the environment.
  - Sulphur is an important e<sup>-</sup> donor, a component of amino acids and cofactors.
- The bulk of sulphur on Earth is found in sediments and rocks as sulphate and sulphide minerals.
- Oceans are the most significant reservoir of sulphur (as sulphate SO<sub>4</sub><sup>2-</sup>).
- Significant S, particularly  $SO_2$  gas, enters the cycle from the combustion of fossil fuels, especially coal.



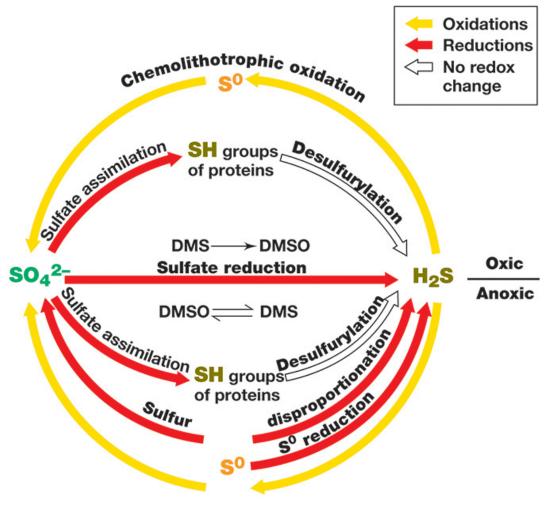
### 5. The Sulphur cycle II





## 5. The Sulphur cycle III

Key Processes and Microbes in the Sulfur Cycle		
Process	Example organisms	
Sulfide/sulfur oxidation ( $H_2S \longrightarrow S^0 \longrightarrow SO_4^{2-}$ )		
Aerobic	Sulfur chemolithotrophs	
	(Thiobacillus, Beggiatoa, many others)	
Anaerobic	Purple and green phototrophic	
	bacteria, some chemolithotrophs	
Sulfate reduction (anaerobic) ( $SO_4^{2-} \longrightarrow H_2S$ )		
,	Desulfovibrio, Desulfobacter	
	Archaeoglobus (Archaea)	
Sulfur reduction (anaerobic) ( $S^0 \longrightarrow H_2S$ )		
· · · · · · · · · · · · · · · · · · ·	Desulfuromonas, many	
	hyperthermophilic Archaea	
Sulfur disproportionation $(S_2O_3^{2-} \rightarrow H_2S + SO_4^{2-})$		
	Desulfovibrio, and others	
Organic sulfur compound oxidation or reduction ( $CH_3SH \rightarrow CO_2 + H_2S$ ) (DMSO $\rightarrow$ DMS)		
	Many organisms can do this	
<b>Desulfurylation</b> (organic–S $\longrightarrow$ H <sub>2</sub> S)		
	Many organisms can do this	



Sulphate  $(SO_4^{2-})$ , sulphite  $(SO_3^{3-})$ , Thiosulphate  $(S_2O_3^{2-})$ , Sulphide  $(S^{2-})$ , elemental Sulphur  $(S^0)$ , Hydrogen sulphide  $(H_2S)$ 

#### 5. The Sulphur cycle IV – Inorganic sulphur

- Hydrogen sulphide (H<sub>2</sub>S) is a major volatile sulphur gas.
  - produced by bacteria via sulphate reduction or emitted from geochemical sources.
- Sulphide (S<sup>2-</sup>) is toxic to many plants and animals and reacts with numerous metals.
  - commonly detoxified in nature by combination with iron, forming the insoluble minerals FeS (pyrrhotite) and FeS<sub>2</sub> (pyrite).
- Sulphur-oxidizing chemolithotrophs can oxidize sulphide and elemental sulphur at oxic/anoxic interfaces.

## 5. The Sulphur cycle V — Organic sulphur

- Organic sulphur compounds can also be metabolized by microorganisms.
- The most abundant organic sulphur compound in nature is dimethyl sulphide (DMS).
  - produced primarily in marine environments as a degradation product of dimethylsulfoniopropionate (an algal osmolyte).
- DMS can be transformed via a number of microbial processes.

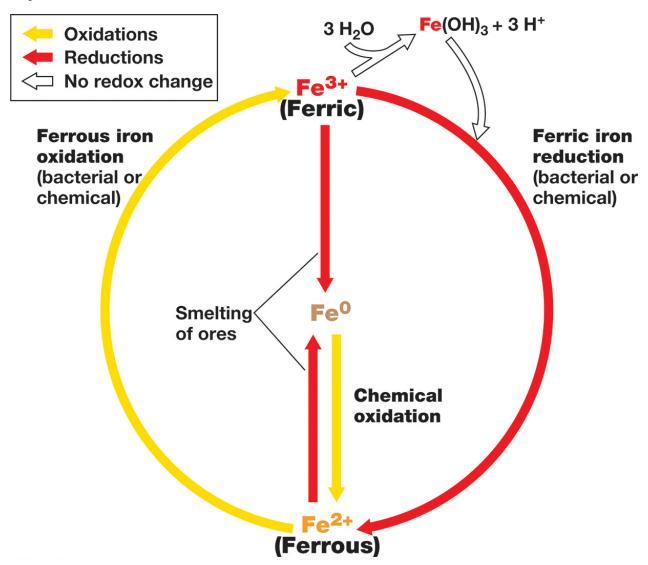


#### 6. Iron and Manganese cycles: Redox cycles

- Iron is one of the most abundant elements in Earth's crust but often a limiting nutrient for microbial growth.
  - The abundance of iron and its ease of switching valence states (3+ ≤ 2+) make it a very important component of cellular physiology.
  - On Earth's surface, iron exists naturally in two oxidation states:
    - Ferrous (Fe<sup>2+</sup>) and ferric (Fe<sup>3+</sup>)
- Manganese (M n) is also present on Earth's surface
  - Manganese exists mainly in two oxidation states: Mn<sup>2+</sup> and Mn<sup>4+</sup>
  - The reactivity of Manganese also make it relevant for cellular physiology, specially as cofactor and in the protection against reactive oxygen species (ROS).
- In aquatic ecosystems, Manganese and iron cycle between oxidised and reduced states.

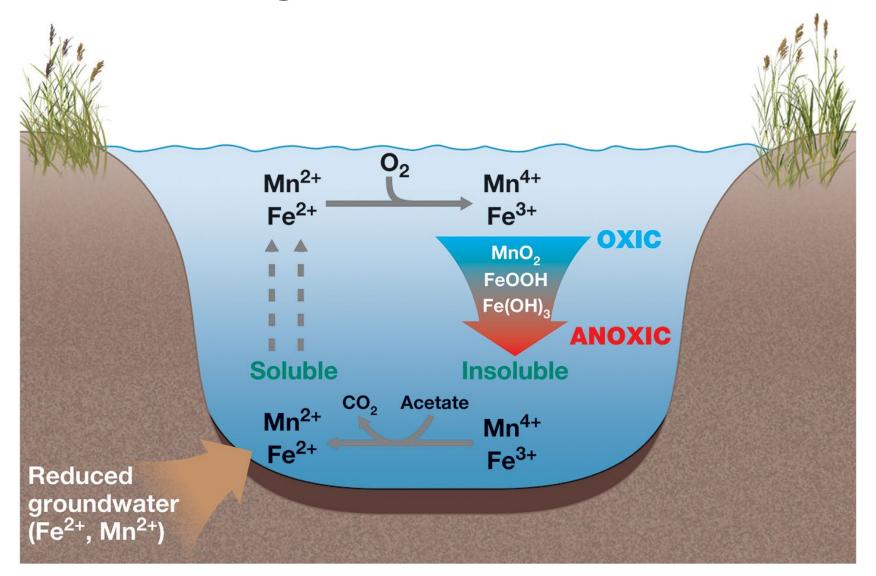


# 6. Redox cycle for Iron





#### 6. Iron and Manganese redox in freshwater



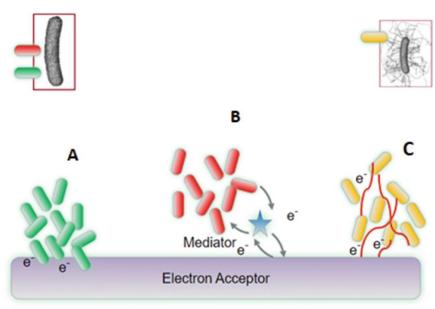
#### 6. Iron and Manganese cycles – Electrogens

#### Long Range Electron Transfer:

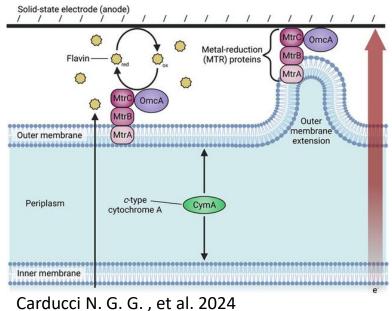
- Many bacteria reduce ferric iron (Fe<sup>3+</sup>) under anoxic conditions, including the obligate anaerobe *Geobacter sulfurreducens* and the facultative aerobe *Shewanella oneidensis*.
- Fe<sup>3+</sup> is typically present in nature as an insoluble mineral and thus the reduction of Fe<sup>3+</sup> must occur outside the cell.
  - **Geobacter** forms direct electrical connections with insoluble materials via cytochromes along pili that are generally 10–20 micrometres long.
- Electrogenic bacteria are capable of transferring electrons to extracellular acceptors. Aka exoelectrogens.



### 6. Electrogenic bacteria



Ilshadsabah A. & Suchithra T. V. 2019



G. sulfurreducens
Reguera G., et al. 2005

#### 6. Iron Bacteria and Archaea

- Fe<sup>3+</sup> can be used by some microorganisms as an e<sup>-</sup> acceptor in aerobic or anaerobic respiration. Iron oxidising Bacteria (IOB) Iron oxidizing Archaea (IOA).
- In nature, humic substances can serve as an electron shuttle (donor) to reduce Fe<sup>3+</sup>.
  - Humic substances are the recalcitrant products of organic matter decomposition.
- The oxidation of ferrous iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>) yields very little energy, so bacteria with this type of metabolism need to oxidize large amounts of ferrous iron to fuel their growth.



Wingecarribee shire, AUS.



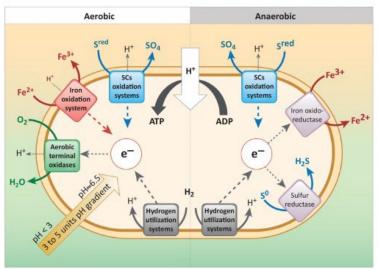
#### 6. Humic substances and indirect metal reduction

• Chemical and organic source of e<sup>-</sup>, organic source of C:

 Chemoorganoheterotroph **Humus reduced** OH Fe<sup>2+</sup> Acetate. Cell Fe oxide **Dissimilatory Iron reducing Humus oxidized** microorganism (DIRM)

#### 6. Iron and Manganese as e⁻ donors

- When reduced, Fe and Mn can also be e<sup>-</sup> donors.
- In acidic, ferrous iron-rich habitats, acidophilic chemolithorophs such as Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans can oxidise ferrous iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>)
  - A. ferrooxidans grows at pH 1-2 and fixes C and N from the atmosphere





#### 6. Microbial oxidation of reduced Manganese

- Some pathogens are able to replace Fe with Mn. Manganese is an important tool for the successful establishment of pathogens in their environment (host).
- Manganese oxidising microorganisms can also be found in almost every environment but their abundance is generally lower than Iron bacteria due to the lower relative abundance of Mn vs Fe and higher redox potential.
- Several genera of Bacteria can oxidize Mn<sup>2+</sup> including Sphaerotilus and Leptothrix—both Betaproteobacteria—and Hyphomicrobium and Pedomicrobium—both Alphaproteobacteria.
- Some *Pseudomonas* species can also oxidize Mn<sup>2+</sup>.

#### 7. The Phosphorus and Calcium cycles

#### Phosphorous cycle

- Organic and inorganic phosphates (PO<sub>4</sub><sup>2-</sup>).
- Phosphorus is a typical limiting nutrient that limits the growth of aquatic photosynthetic autotrophs.
- Alternate forms, such as phosphite (PO<sub>3</sub><sup>-3</sup>) and hypophosphite (H<sub>2</sub>PO<sub>2</sub><sup>-</sup>), rapidly cycle through aquatic ecosystems.

#### Calcium cycle

Reservoirs are rocks and oceans.

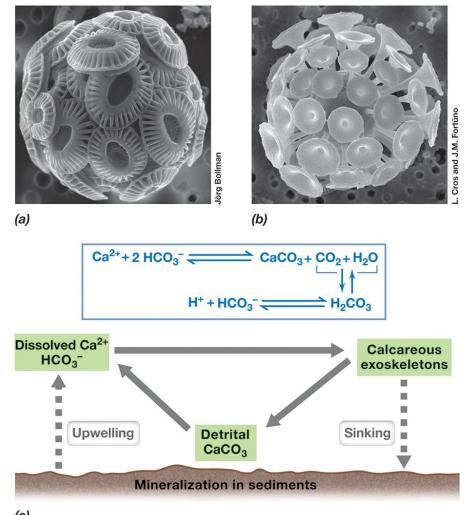
Marine phototrophic microorganisms, such as *foraminifera*, use Ca<sup>2+</sup> to form exoskeleton.

This is what formed a large part of the White Cliffs of Dover.





## 7. The (marine) Calcium cycle



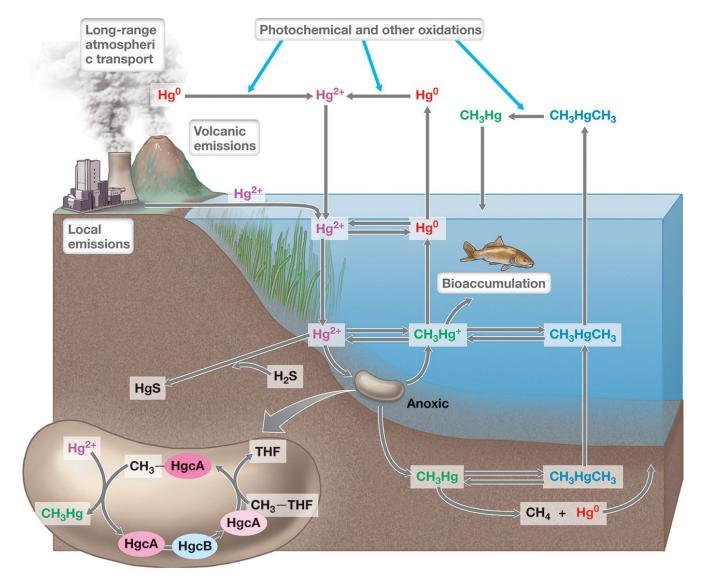
More  $CO_2 \rightarrow$  more carbonic acid  $\rightarrow$  more bicarbonate and  $H^+ \rightarrow$  acidification

#### 8. The Mercury cycle I

- Mercury has a tendency to concentrate in living tissues and is highly toxic.
  - Mercury causes macromolecular structural changes, oxidative stress and DNA damage.
- The major form of mercury in the atmosphere is elemental mercury (Hg<sup>0</sup>), which is volatile and oxidized to mercuric ion (Hg<sup>2+</sup>) photochemically.
- Most mercury enters aquatic environments as Hg<sup>2+</sup>.



## 8. The Mercury cycle II

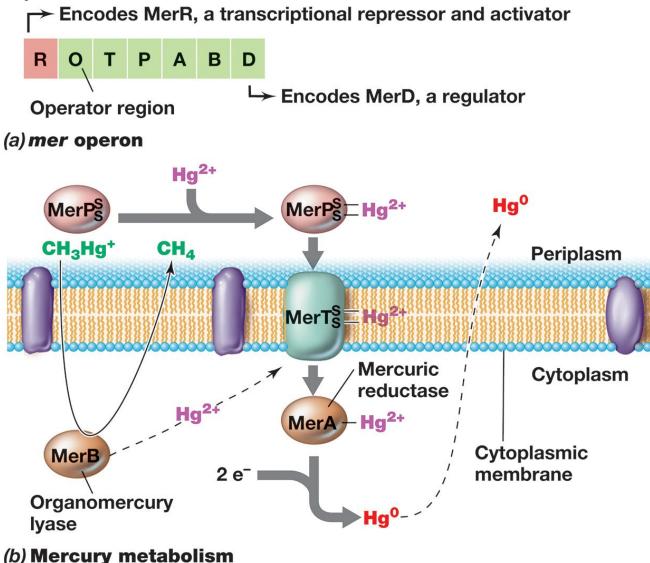


#### 8. Mercury transformations

- Hg<sup>2+</sup> readily adsorbs to particulate matter where it can be metabolized by microorganisms.
- Microorganisms form **methylmercury** (CH<sub>3</sub>Hg<sup>+</sup>), an extremely soluble and toxic compound. Methylmercury is also bioaccumulative.
- Several bacteria can also transform toxic mercury to nontoxic forms.
- Bacterial resistance to mercury is often linked to specific plasmids that encode enzymes capable of detoxifying or pumping out the metals.



#### 8. Mercury transformations and resistance



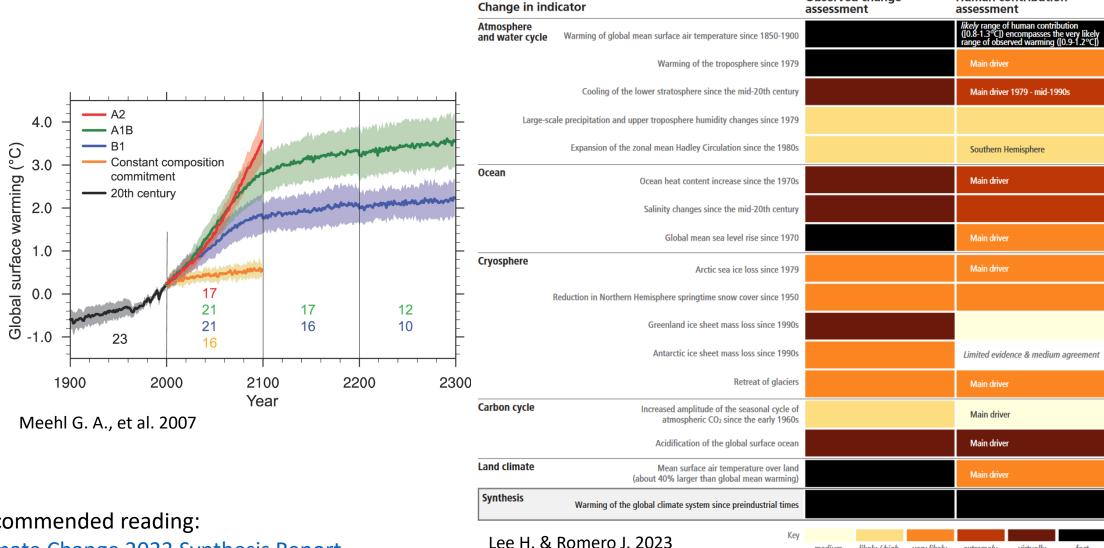


#### 9. Anthropogenic impacts on C and N cycles

- Human activity is believed to have a major impact on the carbon cycle.
  - CO<sub>2</sub> levels have increased more than 40 50 % since the start of the Industrial Revolution and are now higher than they have been in the last 800,000 years.
    - The majority of this 50% increase has been released over the last 70 years.
  - CO<sub>2</sub> is a greenhouse gas that traps long-wave heat waves from the Earth's surface. This phenomenon is called **radiative forcing** (amount of energy that enters Earth is different from amount that leaves).
  - Dissolved CO<sub>2</sub> decreases the pH of the ocean. This acidification endangers coral reefs, which will release calcium carbonate as they die.
  - Air and ocean water temperatures are increasing, which increases the oxygen minimum zones (OMZs) in the ocean.



#### 9. Anthropogenic impact on climate



Observed change

very likely

virtually

certain

fact

likely / high

**Human contribution** 

Recommended reading:

Climate Change 2023 Synthesis Report

#### 9. Human impacts on the Nitrogen cycle

Human activity believed to have a major impact on nitrogen cycle.

- Humans produce large amounts of nitrogenous fertilizers (Haber-Bosch process).
  - Ecological effects of fertilizers are unknown, but the alteration of nitrogen cycles will also change iron availability and the carbon cycle.
- Nutrient cycles are coupled.
  - Change in either nitrogen or carbon cycles will affect other cycles.



#### 9. Nitrogen and humanity

- The Haber-Bosch process has had a monumental impact on humanity.
- Organic, fertiliser-free farming would only support half of the current world population.

#### World population with and without synthetic nitrogen fertilizers



Estimates of the global population reliant on synthetic nitrogenous fertilizers, produced via the Haber-Bosch process for food production. Best estimates project that just over half of the global population could be sustained without reactive nitrogen fertilizer derived from the Haber-Bosch process.

