Environmental Microbiology

5 Biochemical Cycles – I
Carbon and Nitrogen

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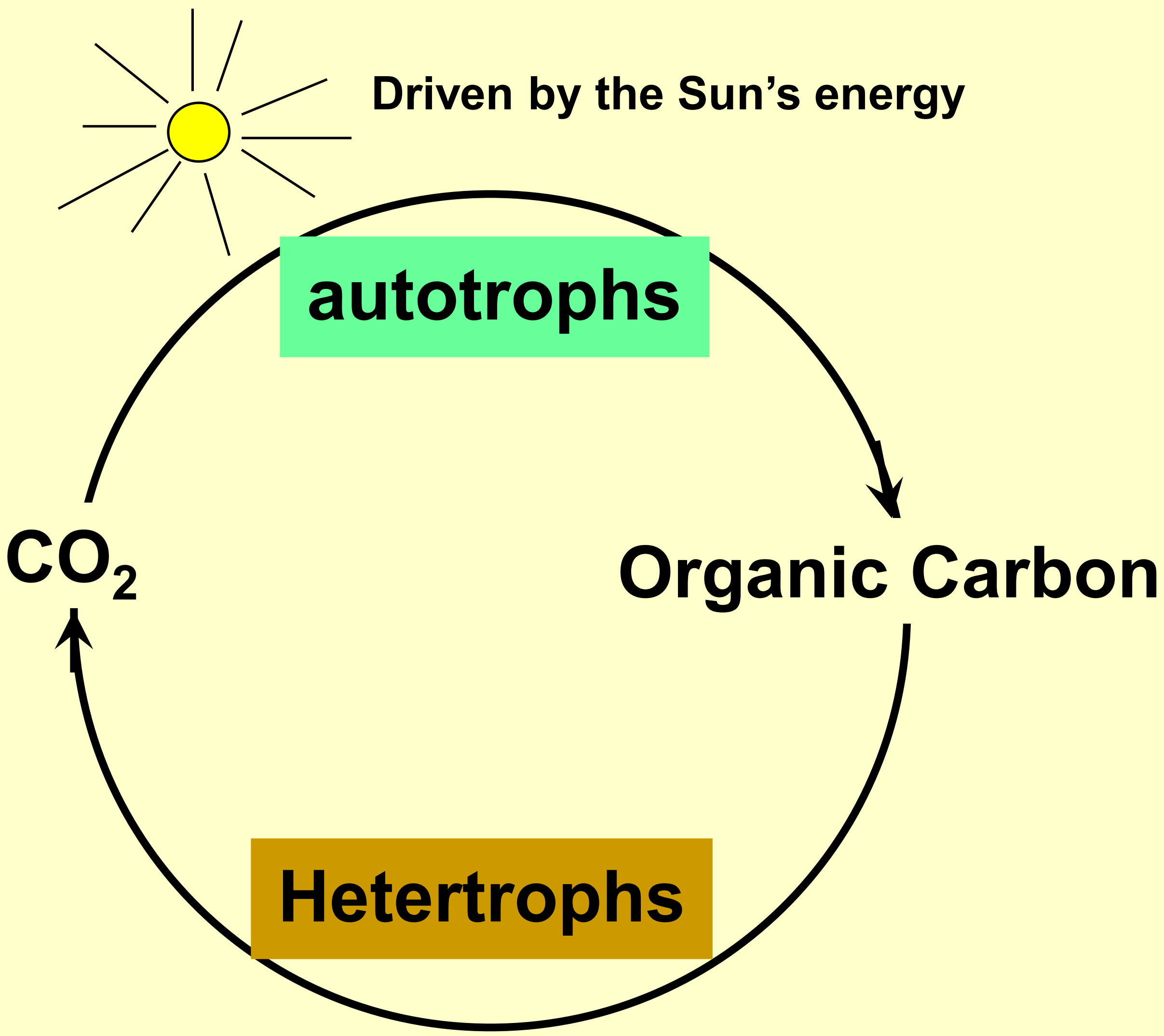
Microbes contribute significantly on cycling of elements

1. Carbon Cycle
2. Nitrogen Cycle
3. Phosphate Cycle
4. Sulfur Cycle
5. Iron Cycle

Part I

Part II

1. Global Carbon Cycle



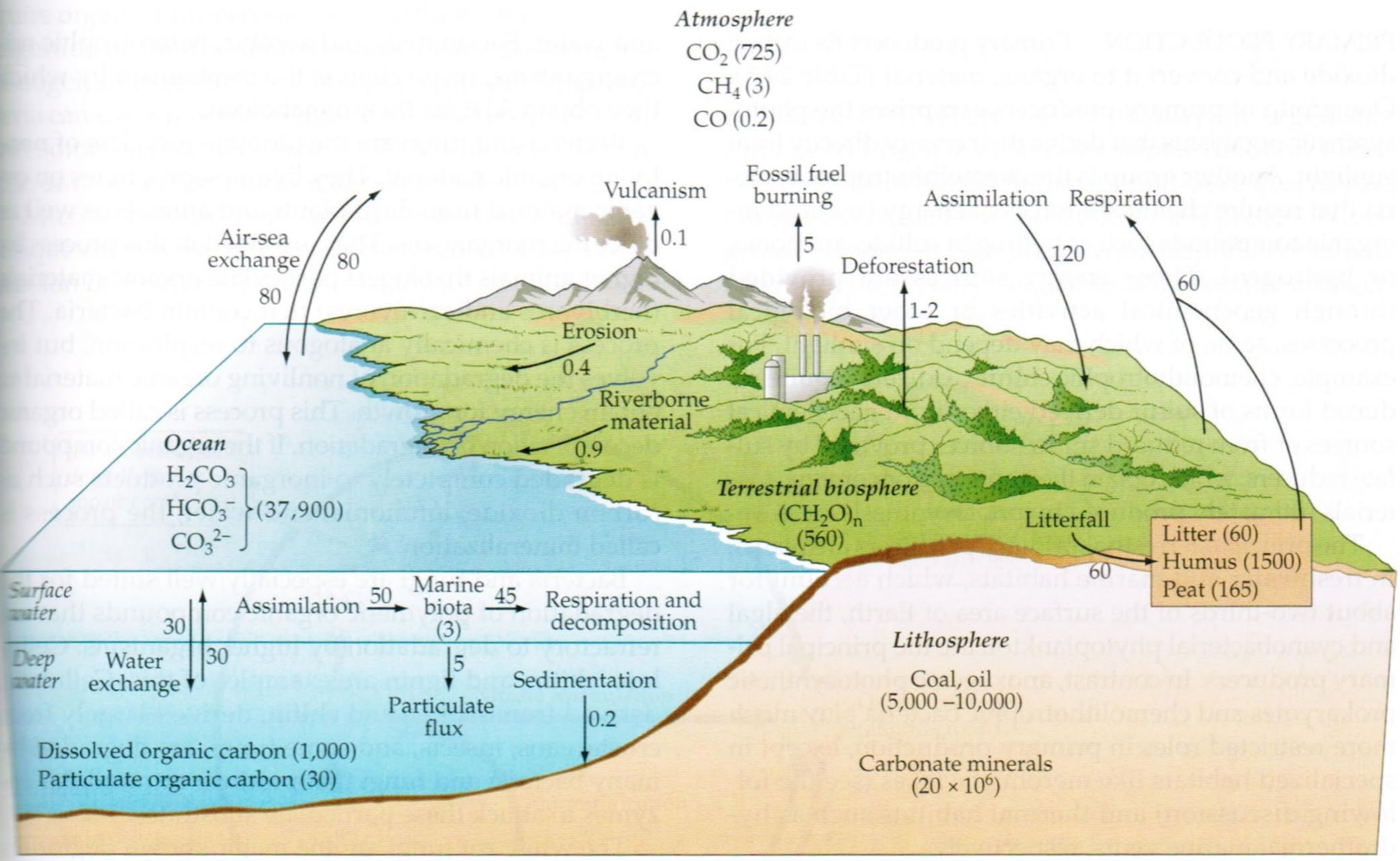


Figure 24.23 Global carbon cycle
Cycling of carbon through the atmosphere, biosphere, oceans, and lithosphere. Reservoir amounts are in pentagrams of carbon (PgC) and fluxes (shown by arrows) are in PgC per year. Freshwater environments are included in the terrestrial compartment. Adapted from K. Holmén, *Global Biogeochemical Cycles*, Academic Press.

1 Pg = 10^{15} g

(1)Global warming

Global Warming

Carbon Cycle

A) Carbon reservoirs (Cells are ~50% C)

TABLE 14.3 Global Carbon Reservoirs

Carbon reservoir	Metric tons carbon	Actively cycled
Atmosphere		
CO_2	6.7×10^{11}	Yes
Ocean		
Biomass	4.0×10^{9}	No
Carbonates	3.8×10^{13}	No
Dissolved and particulate organics	2.1×10^{12}	Yes
Land		
Biota	5.0×10^{11}	Yes
Humus	1.2×10^{12}	Yes
Fossil fuel	1.0×10^{13}	Yes
Earth's crust[a]	1.2×10^{17}	No

[a] This reservoir includes the entire lithosphere found in either terrestrial or ocean environments. (Data from Dobrovolsky, 1994.)

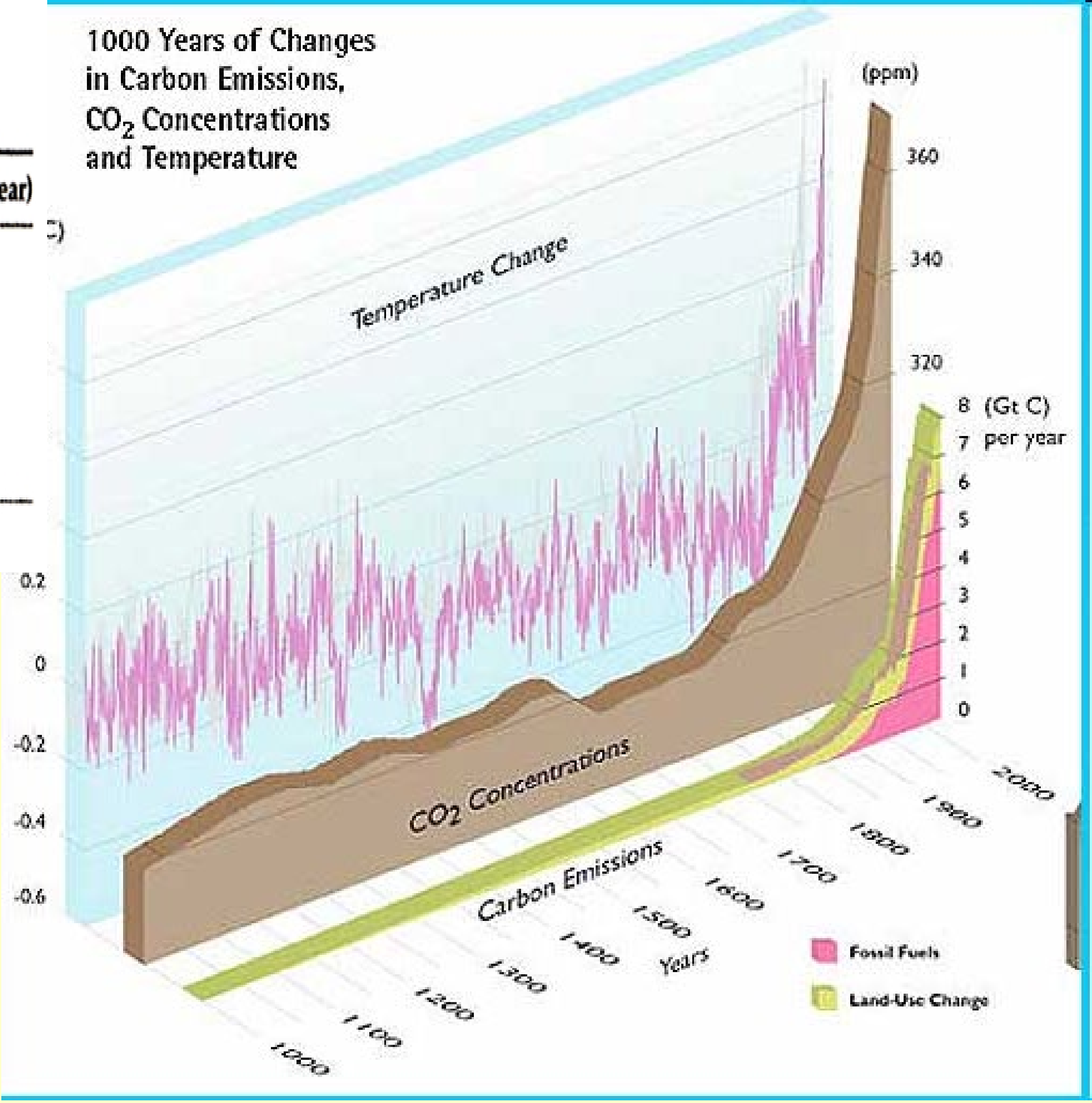
*

Ocean and soil act as CO_2 buffer

Global warming ("Greenhouse" effect) due to CO_2 ,CH_4 ,CFC, N_2O

TABLE 14.4 Net Carbon Flux between Selected Carbon Reservoirs

Carbon source	Flux (metric tons carbon/year)
Release by fossil fuel combustion	7×10^9
Land clearing	3×10^9
Forest harvest and decay	6×10^9
Forest regrowth	-4×10^9
Net uptake by oceans (diffusion)	-3×10^9
Annual flux	9×10^9



(2)Humus Production

Carbon Cycle in Soil – the production of Humus

C. **Carbon Respiration** (organic-C metabolism to CO_2)

*if C-respiration were inhibited, it would take 30-300y for primary producers to consume CO_2(**why?**)

Organic polymers used for C-respiration:

- Cellulose纤维素(most abundant),15-60% plant matter, degraded by bacteria/fungi
  α-1,4 glucose linkage(1000-10000 units)
  extracellular enzymes
- Hemicellulose半纤维素(10-30%plant matter)
- Starch 淀粉
- Chitin 壳质, 角素= β-1,4 n-acetylglucosamine乙酰氨基葡萄糖
  (fungal cell walls,insect exoskel
- Peptidoglycan (bact. cell walls=n-acetylglucosamine/n-acetylmuramic acid)
- Lignin木质素(500-600units), phenylpropane polymer, strengthen
  plant cell walls; relatively resistant to degradation (many mos.)
  H_2O_2-dependent lignin peroxidase(forms free-radicals)

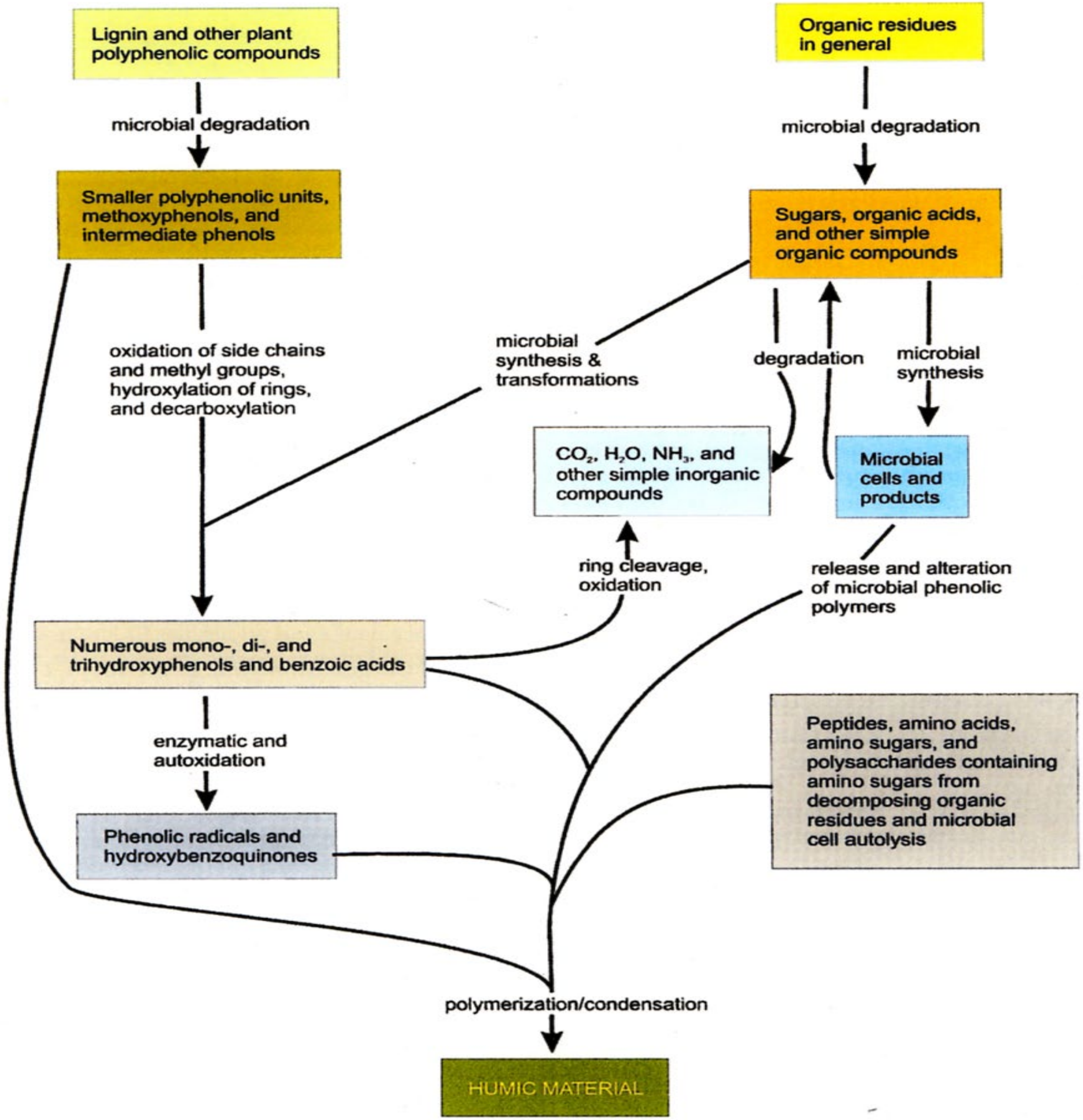
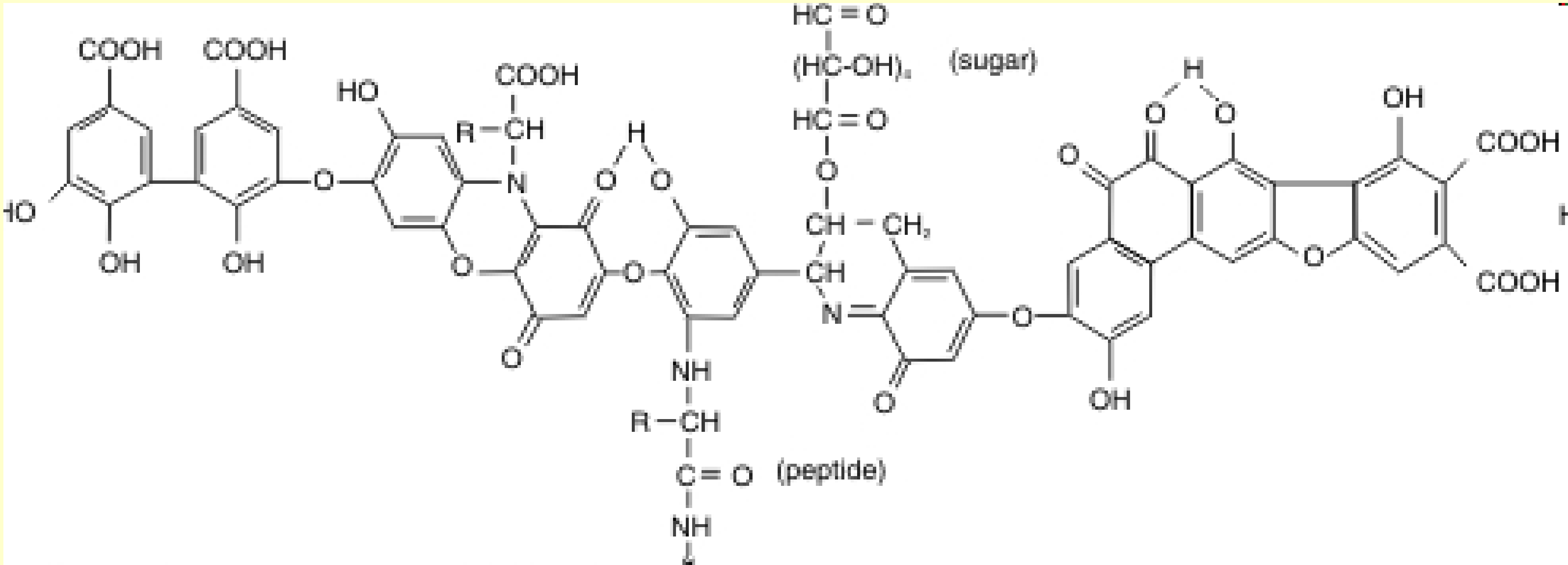


FIGURE 14.9 Possible pathways for the formation of soil humus. (Adapted with permission from Wagner and Wolf, 1998.)

Humus腐殖质- formed from the polymerization of biodegradation products of plant material (cellulose/lignin) *degradation very slow, 2-5%/y. They form the dark brown organic part of most soil.



Model structure of humic acid (Stevenson 1982)

The impact of Humus compounds in the environment

1. In the soil environment:
    - They help soil to retun water
    - Keep the soil microorganisms alive
    - Absorb organic pollutants keep them from migrating away
    - Complex metal ions. Make them available for plant growth
2. In the water environment, the water soluble organic material are called humic acids or fulvic acids. In clean natural water their concentration range from < 1 mg/L C up to 10 mg/L C.
    - These acids are not harmful to human health.
    - They will interfere water treatment processes – reduce calcium or metal precipitation efficiency.
    - Form THMs (trihilomethanes) in chlorination processes – cancer causing chemicals
    - Form easily degradable organics after ozonation – treated water becomes bio-active.

(3)Methane formation

Carbonaceous gases Methane (CH_4) formation

TABLE 14.8 Estimates of Methane Released into the Atmosphere

Source	Methane emission (10^6 metric tons/year)	
	Million metric tons/y	
Biogenic		
Ruminants	80–100	
Termites	25–150	
Paddy fields	70–120	
Natural wetlands	120–200	
Landfills	5–70	
Oceans and lakes	1–20	
Tundra	1–5	
Abiogenic		
Coal mining	10–35	
Natural gas flaring and venting	10–35	
Industrial and pipeline losses	15–45	
Biomass burning	10–40	
Methane hydrates	2–4	
Volcanoes	0.5	
Automobiles	0.5	
Total	350–820	
Total biogenic	302–665	81–86% of total
Total abiogenic	48–155	13–19% of total

Adapted from Madigan *et al.* (1997).

Methanogens

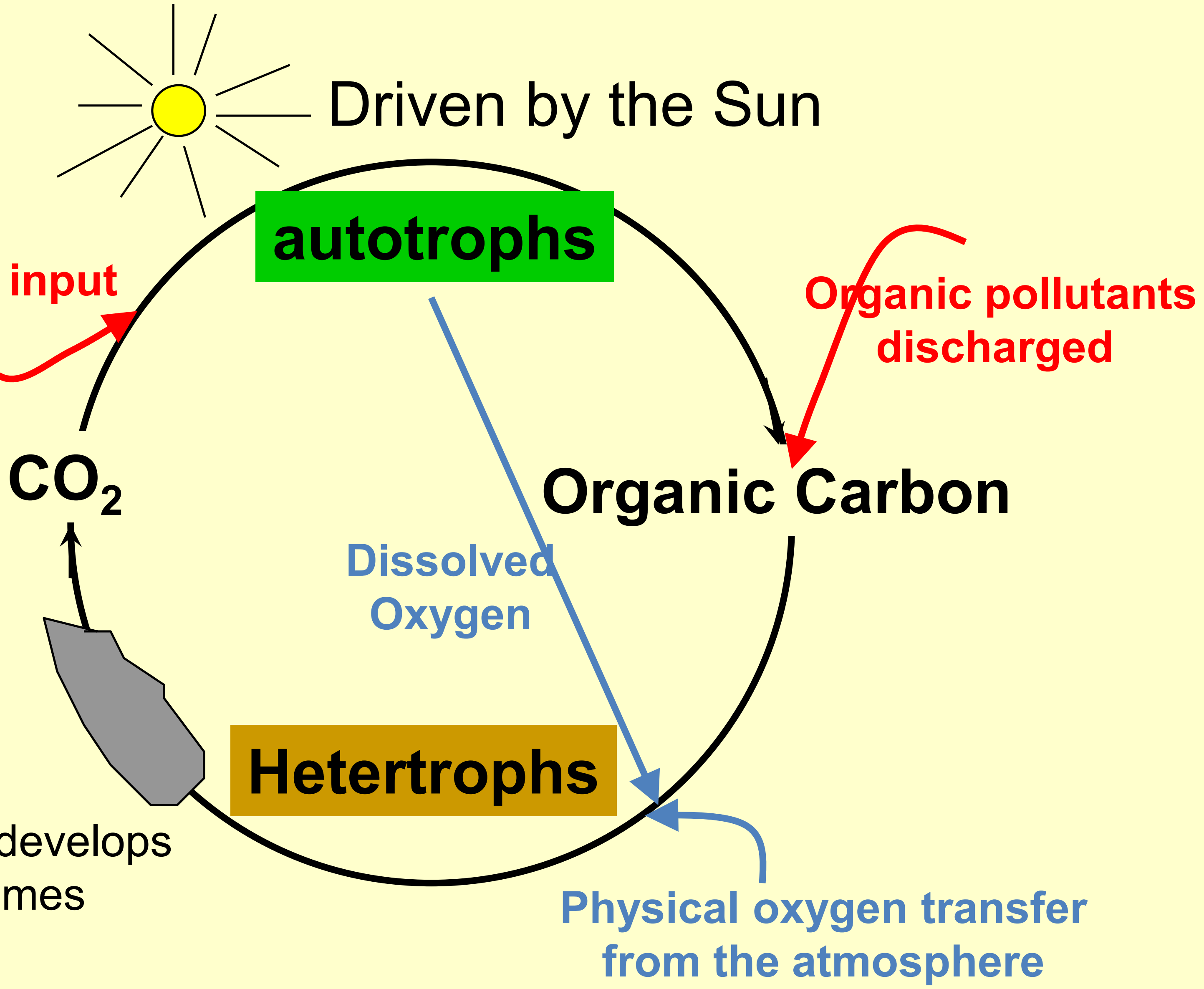
$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$

*predominately CO_2 reduction (also acetate,MeOH,formate)

*depends on metabolism of other anaerobic heterotrophs异养生物

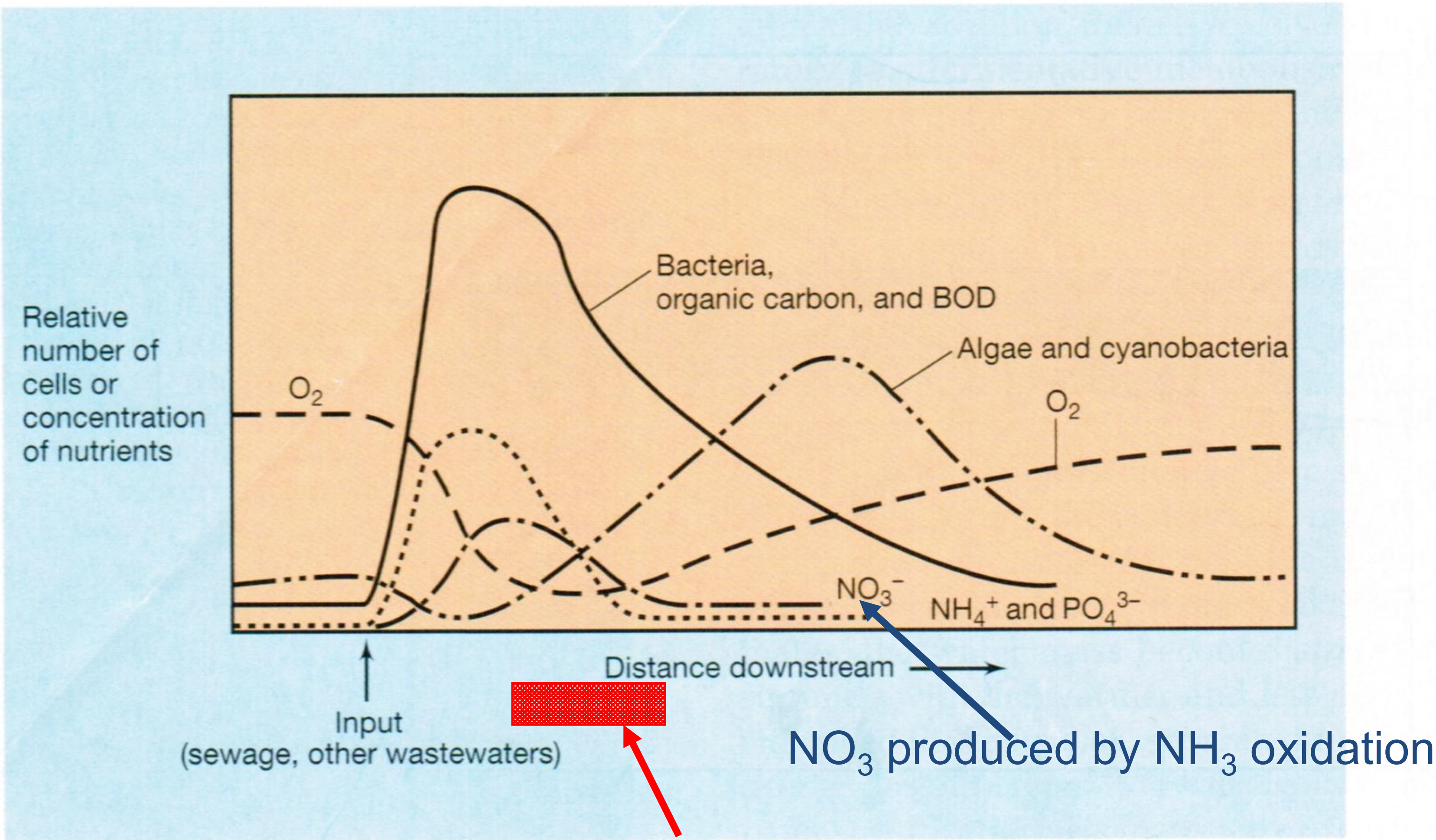
(4)Local unbalanced carbon cycles Impact on water pollution

Localized Unbalanced Carbon Cycle



Hypoxic condition develops when system becomes unbalanced

River Pollution by discharging organic material exceed the self cleaning capacity of the river



DO too low to sustain fish life (they depend on dissolved oxygen for breath),
Fish kill events can happen

(a)

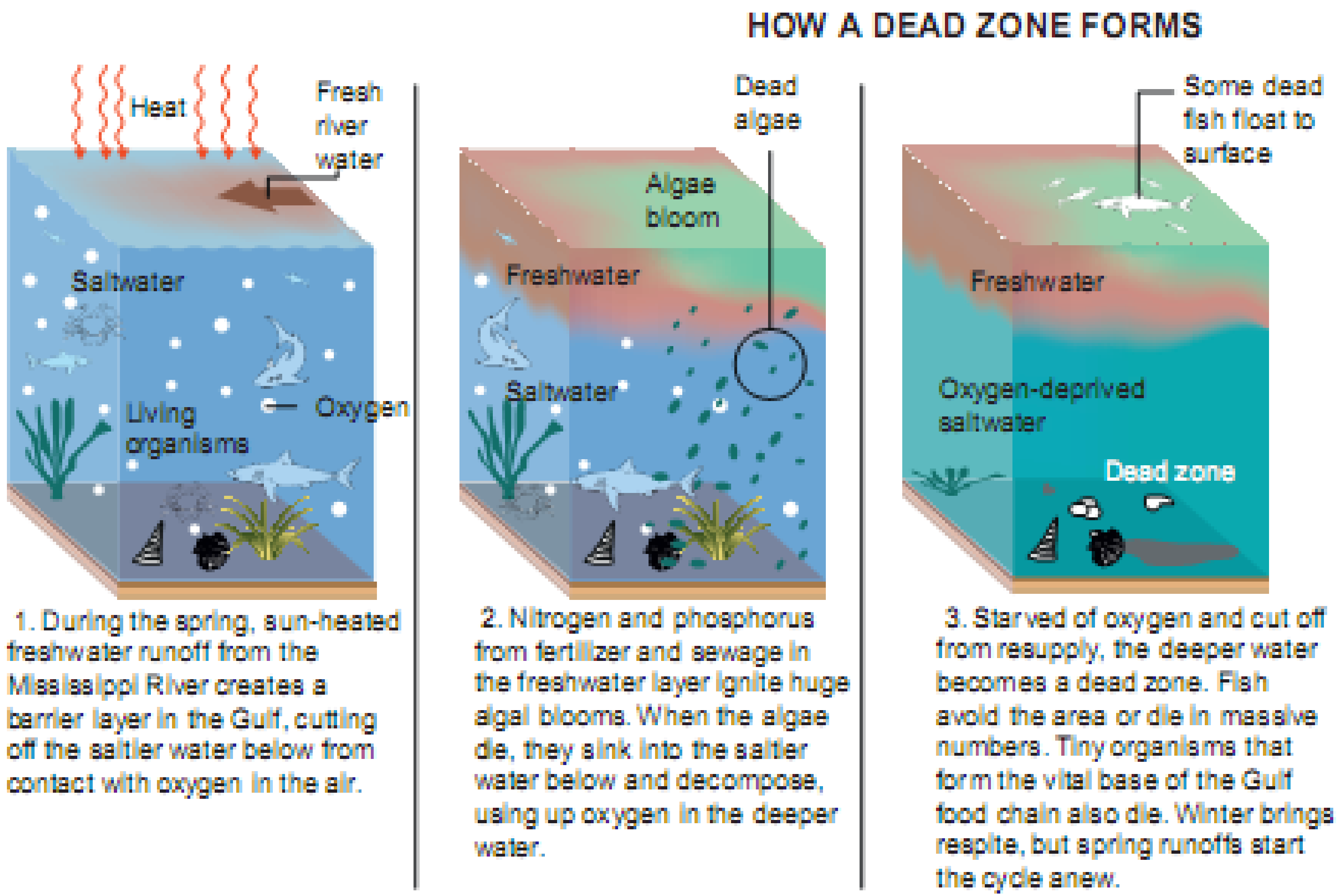
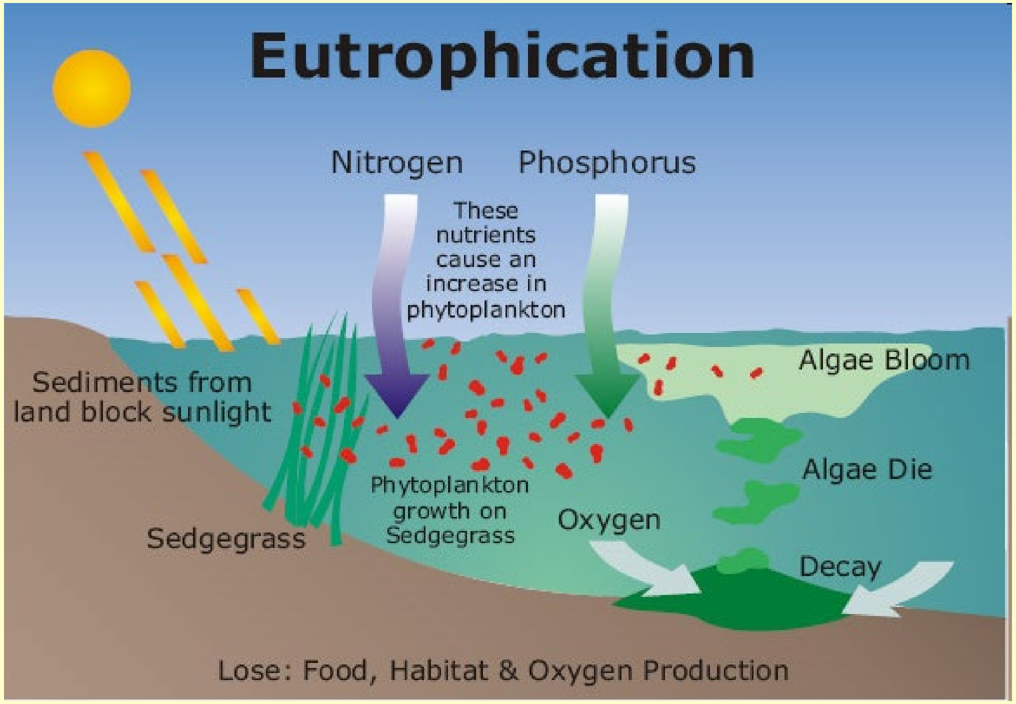


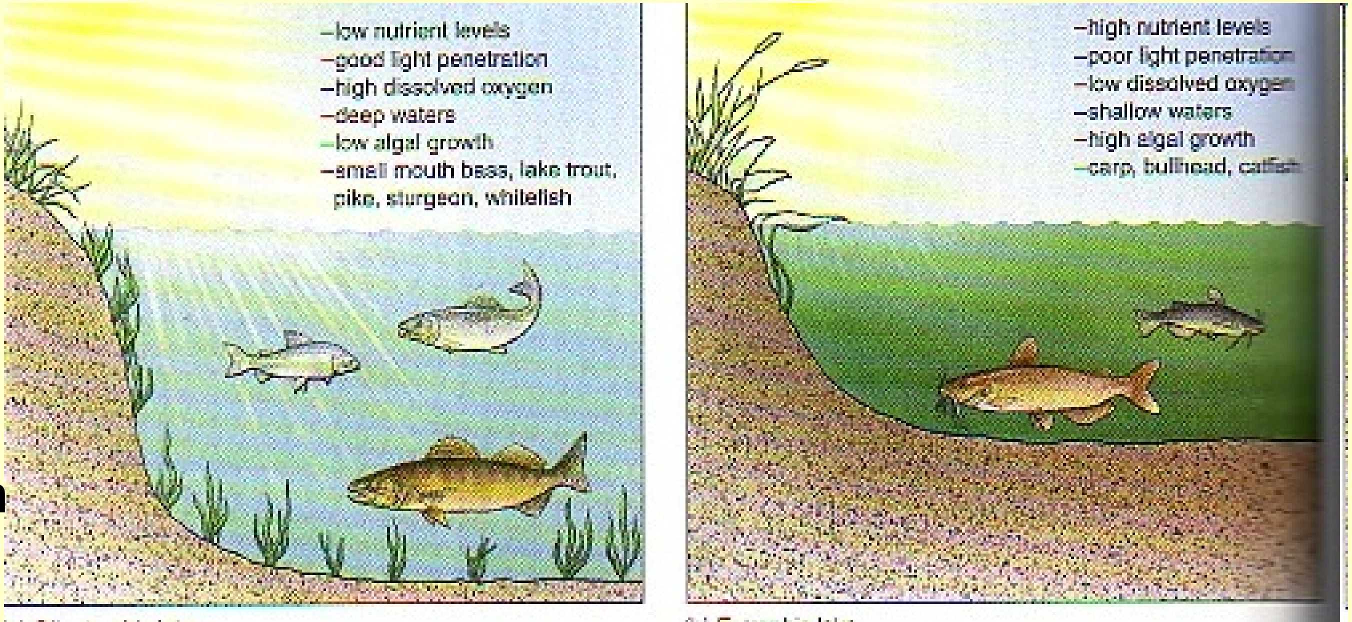
FIGURE 6.11 A so-called Dead Zone, where heterotrophic decomposition has stripped the water of oxygen, leading to large regions of anoxia and sometimes resulting in massive fish die-offs.

Fish kill due to low DO in the river.
Not due to toxicity

Due to the limited capacity of aeration in a lake, the impact of organic material in the lake DO concentration is more severe.

In addition, the unbalanced autotrophic growth due to the input of nutrients, P or N would cause further DO depletion and the accumulation of algal biomass on the bottom of the lake would cause the premature shallowing of the lake





(a) Oligotrophic lake
(b) Eutrophic lake

Lake Eutrophication

Lake stratification in the temperate region. De-stratification will happen in the fall and early winter

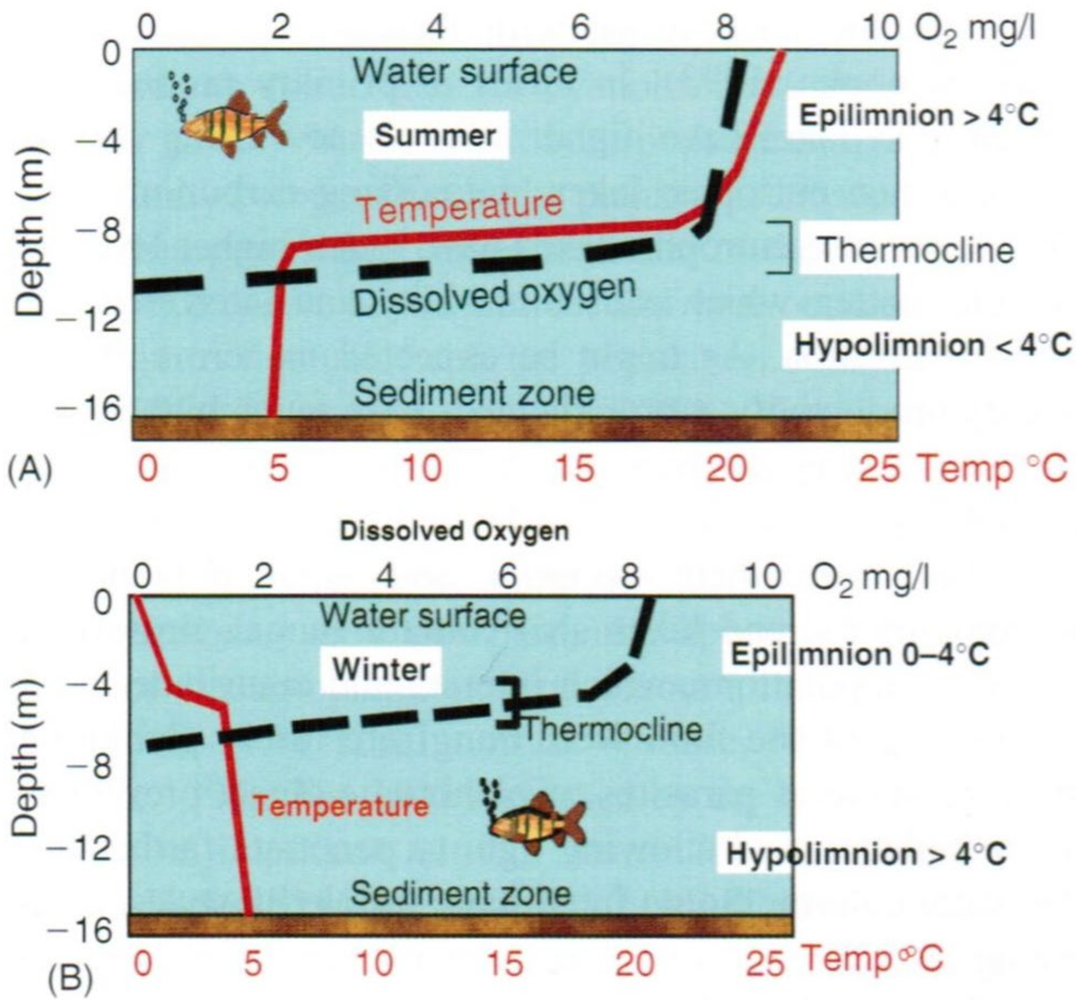
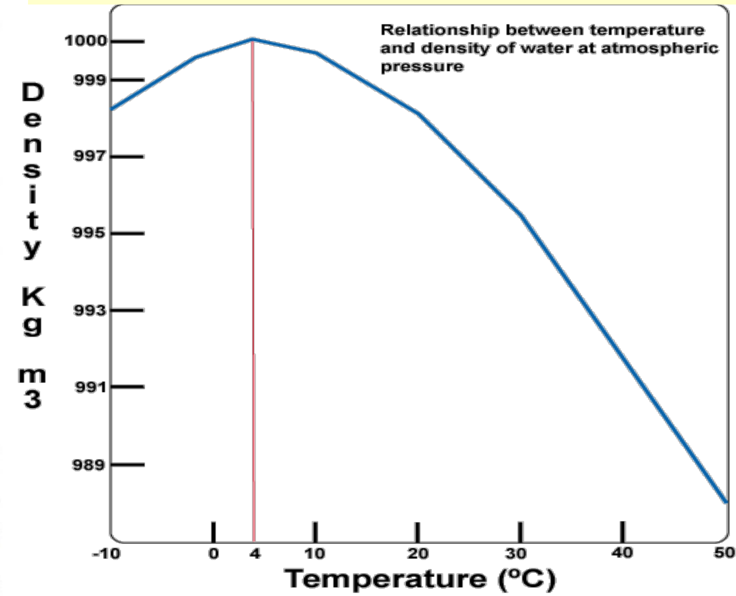
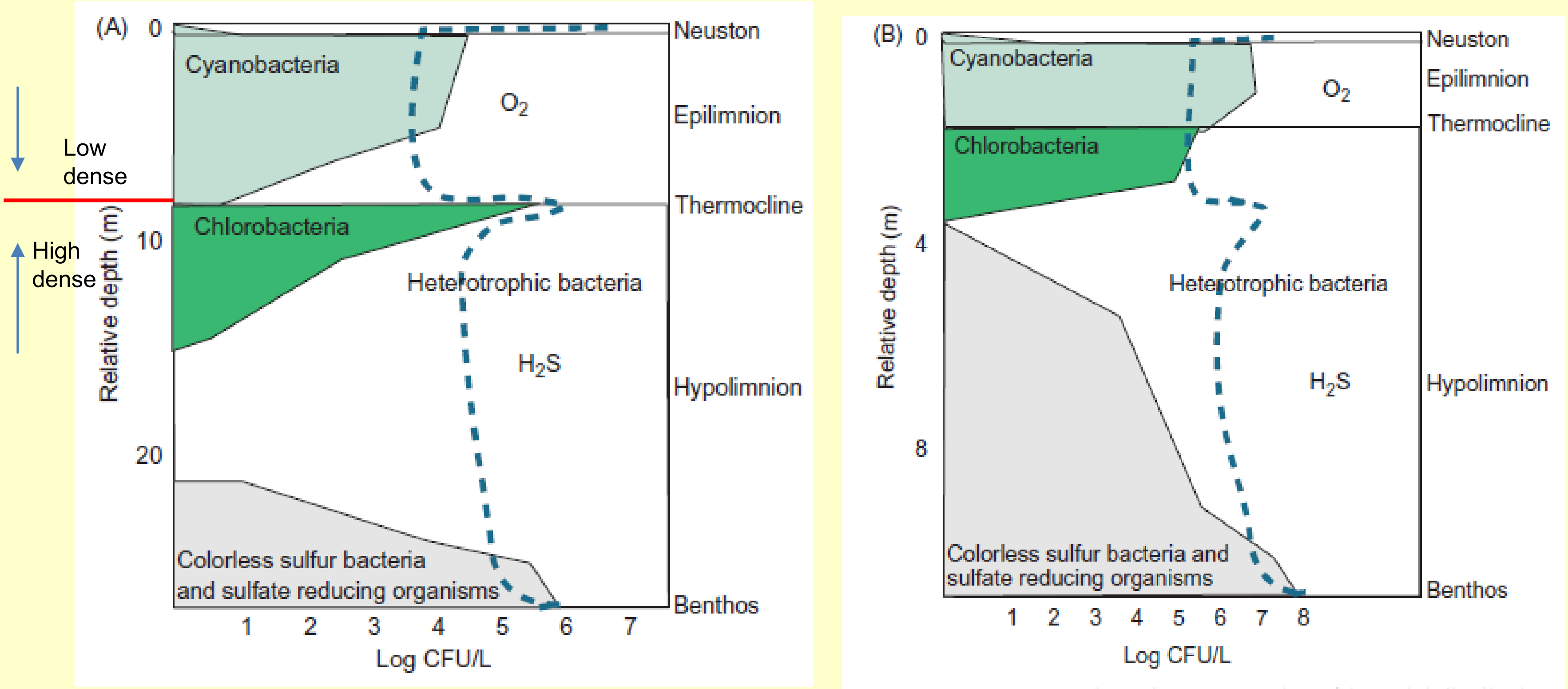


FIGURE 6.10 Idealized profiles of temperature and oxygen in a temperate region, eutrophic lake during the summer (A) and winter (B). Stratification is due to thermal warming of the upper layers in the summer months. Cooling of the upper layer in the fall and early winter breaks the mixing barrier and allows the sediment zone to be reoxygenated. Adapted from Wetzel, 1983.



Lakes are among the most complex of the freshwater environments.



(A) Schematic representation of bacterial distribution in a typical oligotrophic lake. Notice especially the distribution and concentrations of the **photosynthetic populations**. Also note the lower concentration of heterotrophs in the upper zone, **where cyano-bacteria predominate**. The large increase in the heterotrophic population between the epilimnion and the hypolimnion is related to the presence of a zone where organic matter accumulates. This area is known as a thermocline and is a zone where the sunlight-warmed surface water (less dense) and the deeper colder water (more dense) meet, forming a **density gradient** where organic matter accumulates.(B) Schematic representation of a typical eutrophic lake. This figure shows the same groups of organisms as in (A) indicating the localization and relative concentrations throughout the water column. Notice that both the photosynthetic and the heterotrophic populations are considerably higher in a eutrophic lake. Adapted from Rheinheimer (1985).

圖為滇池藍藻 cyanobacteria
暴發後的景象

A algal protoplasm with C:N:P ratio of 101:16:1 implies an increase of over 100 mg/L in algae dry matter for each 1 mg/L of P. N nutrient is usually not a limiting factor in fresh water lakes due to N_2 fixation. Phosphate level as low as 0.01 mg/L P has been reported to cause excessive algal growth in lakes.

Algal bloom can cause significant pH swings in the lake (see next page). This is due to the algae removal of CO_2 from the water during their growth which causes a swift of $H_2CO_3 \rightleftarrows HCO_3^-$ balance.

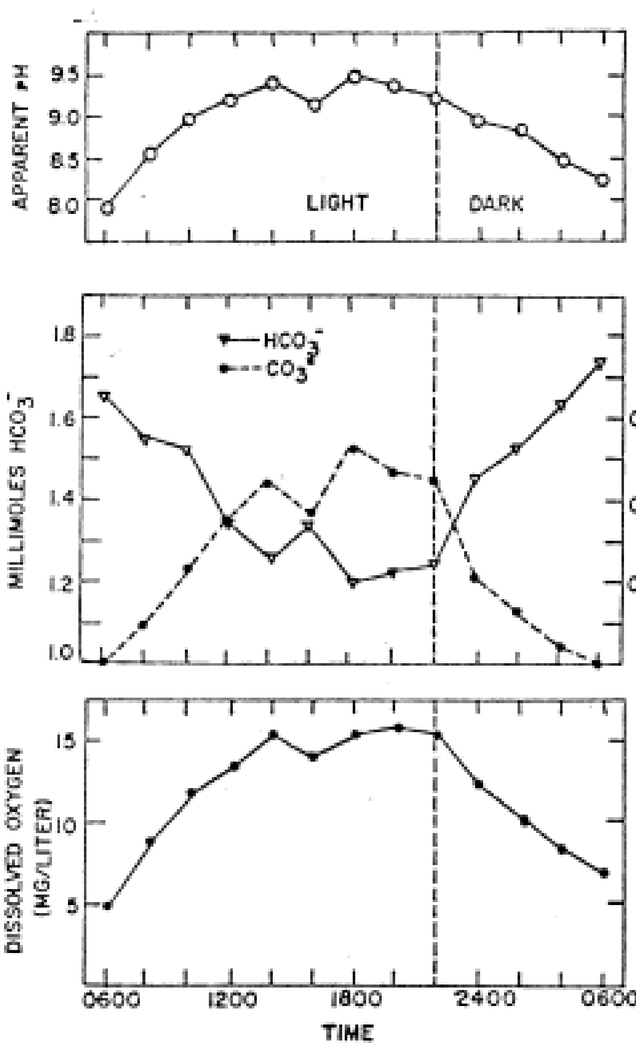
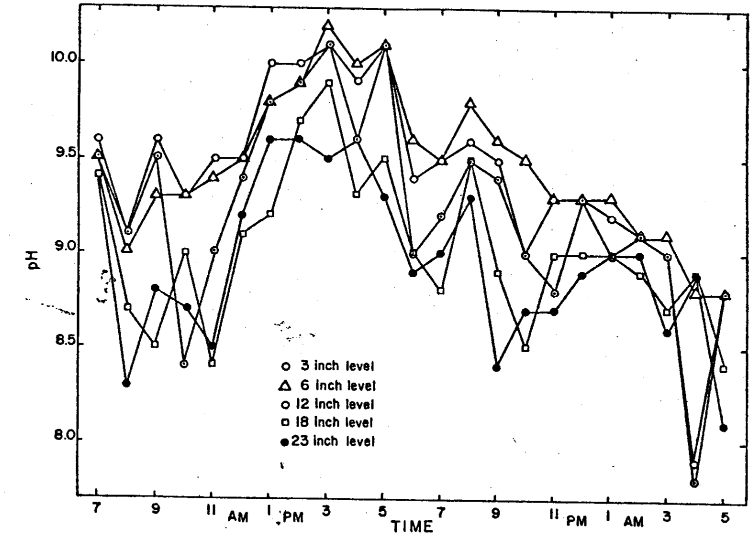
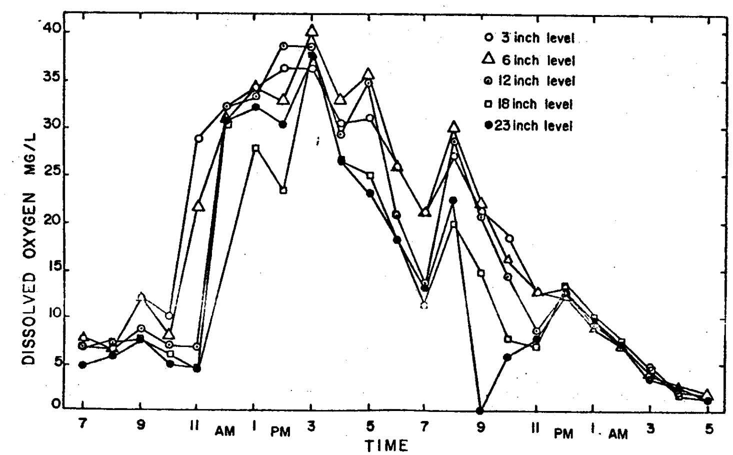


FIGURE 4.—Diurnal variation in pH, bicarbonate-monocarbonate alkalinity, and dissolved oxygen in a microcosm dominated by *Phormidium*.





World impact of in-land eutrophication

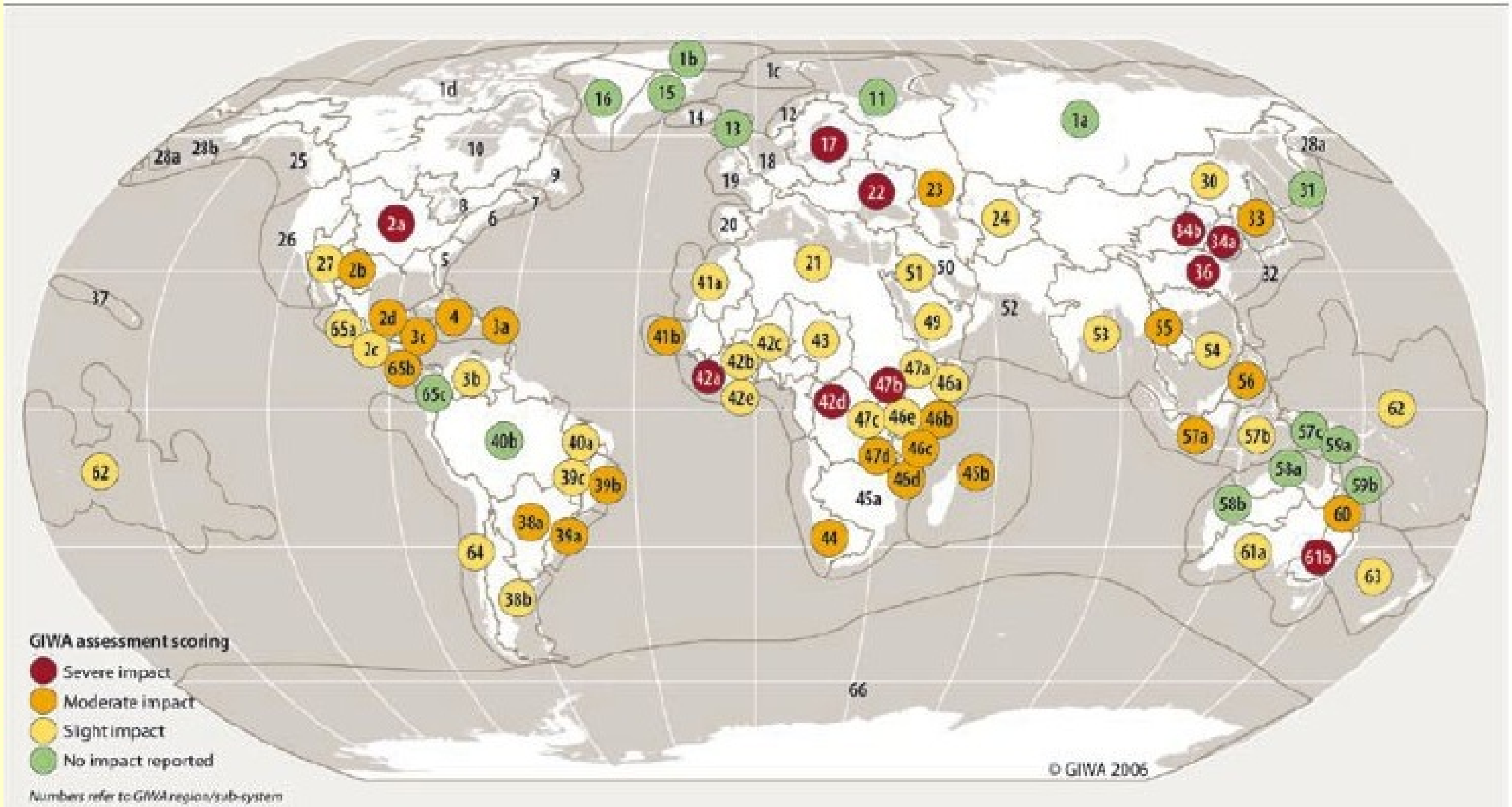
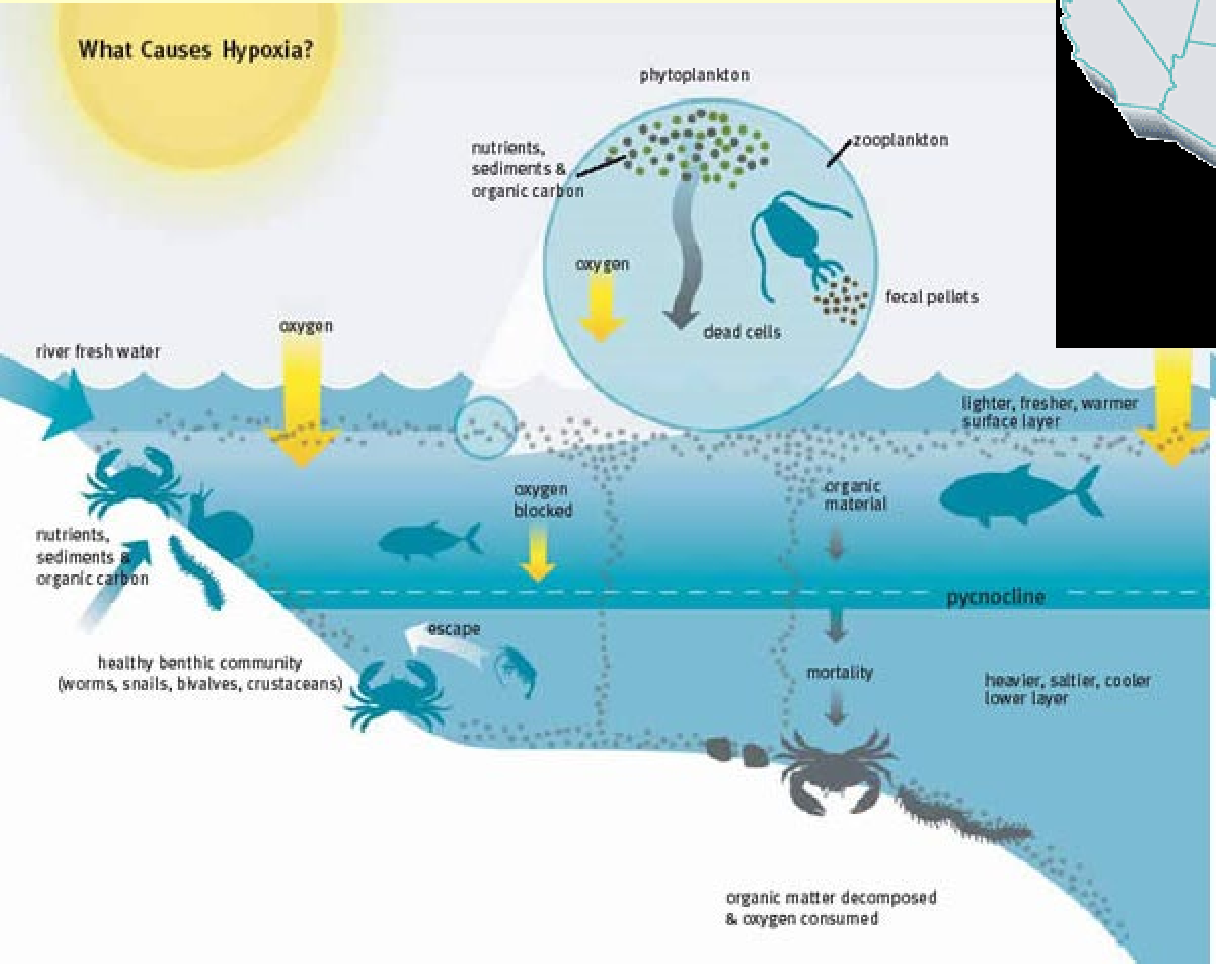
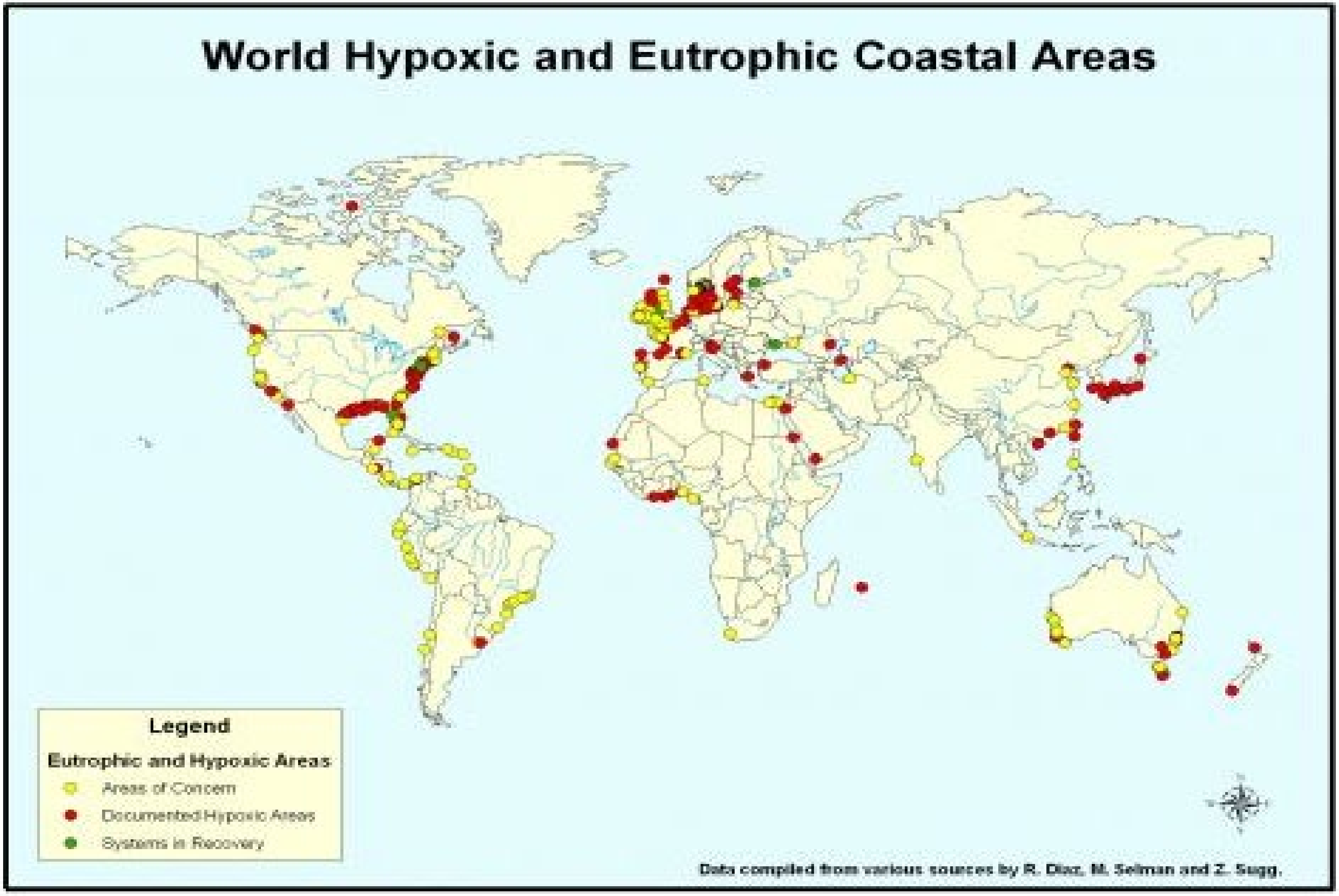


FIGURE I4. IMPACTS OF EUTROPHICATION

Eutrophication of the coastal zones – create dead zones (without fish or bottom feeders). This is called hypoxia.

Rich nutrient → algae growth → algae sink to bottom layer → algal biomass decay → useup all DO → create dead zone




World Hypoxic and Eutrophic Coastal Areas
Legend
Eutrophic and Hypoxic Areas
Areas of Concern
Documented Hypoxic Areas
Systems in Recovery
Data compiled from various sources by R. Diaz, M. Selman and Z. Sugg.

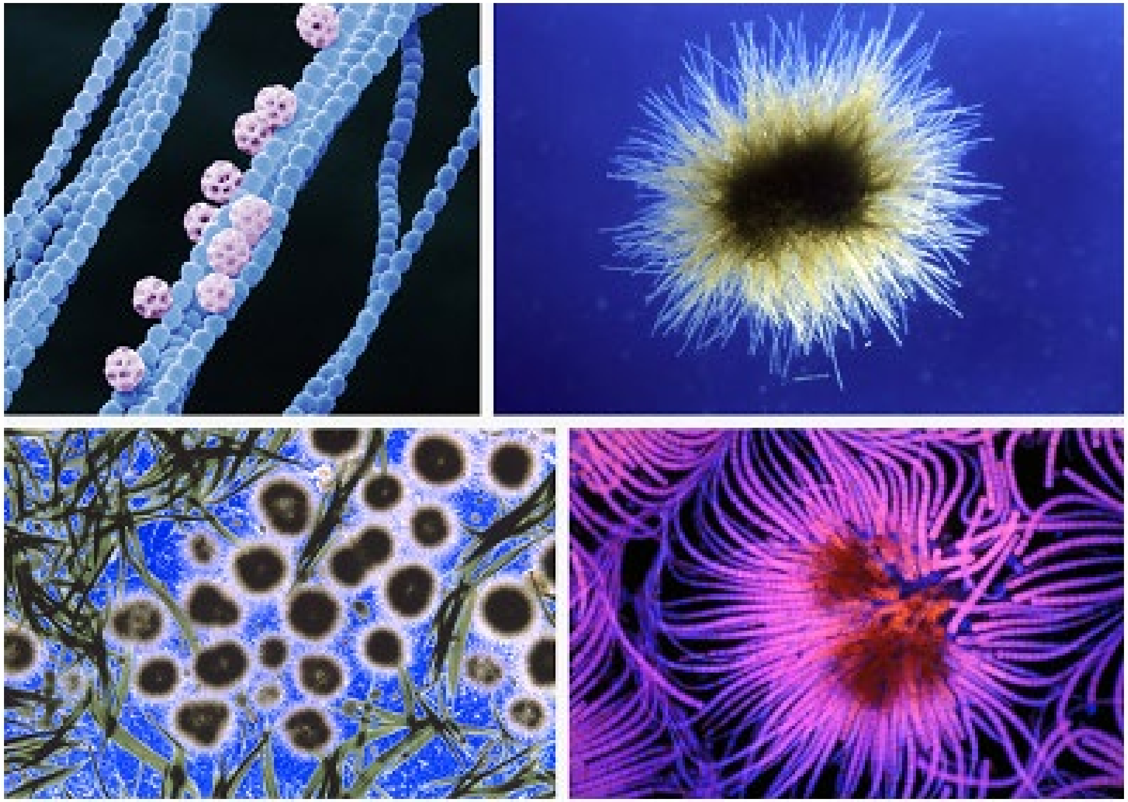


FIGURE 10.9 Various cyanobacteria (blue–green algae).

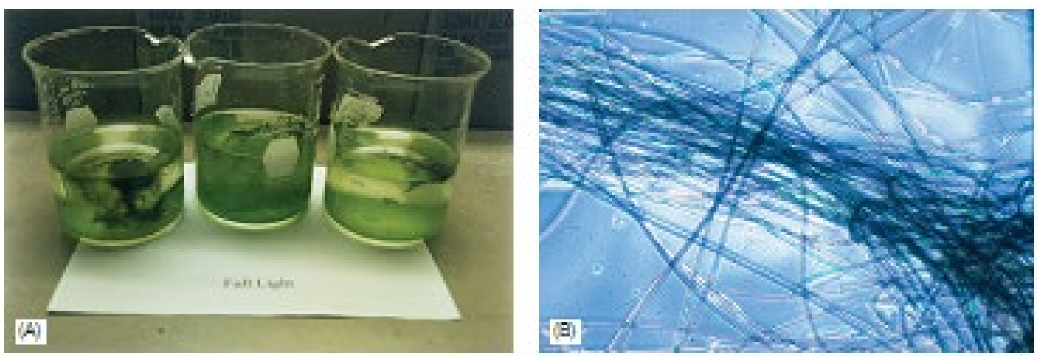
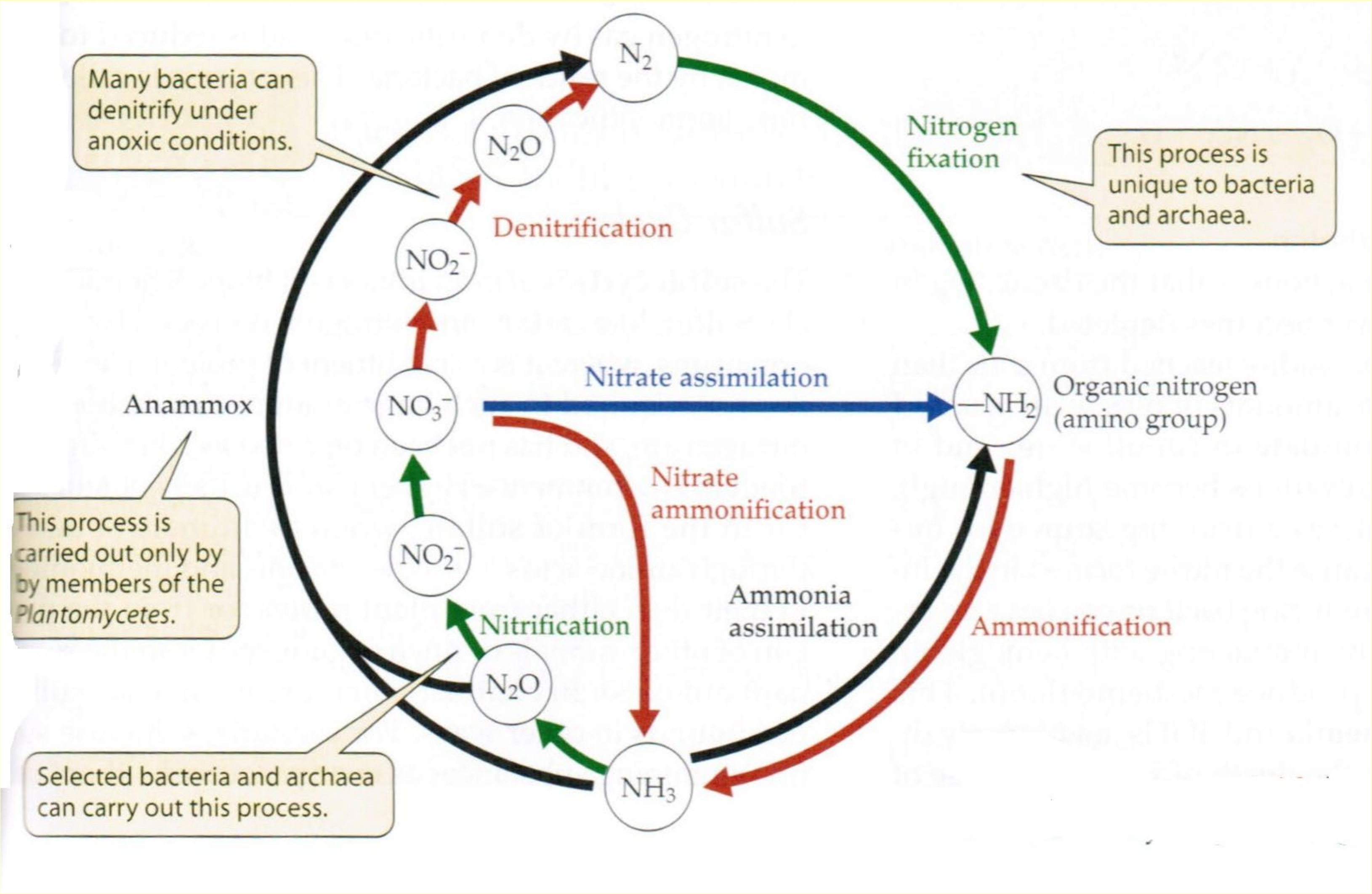


FIGURE 10.10 (A) Cyanobacteria (blue–green algae) grown in full fluorescent light in BG11 broth. (B) An example of a cyanobacterium *Lyngbya* seen using phase contrast microscopy.

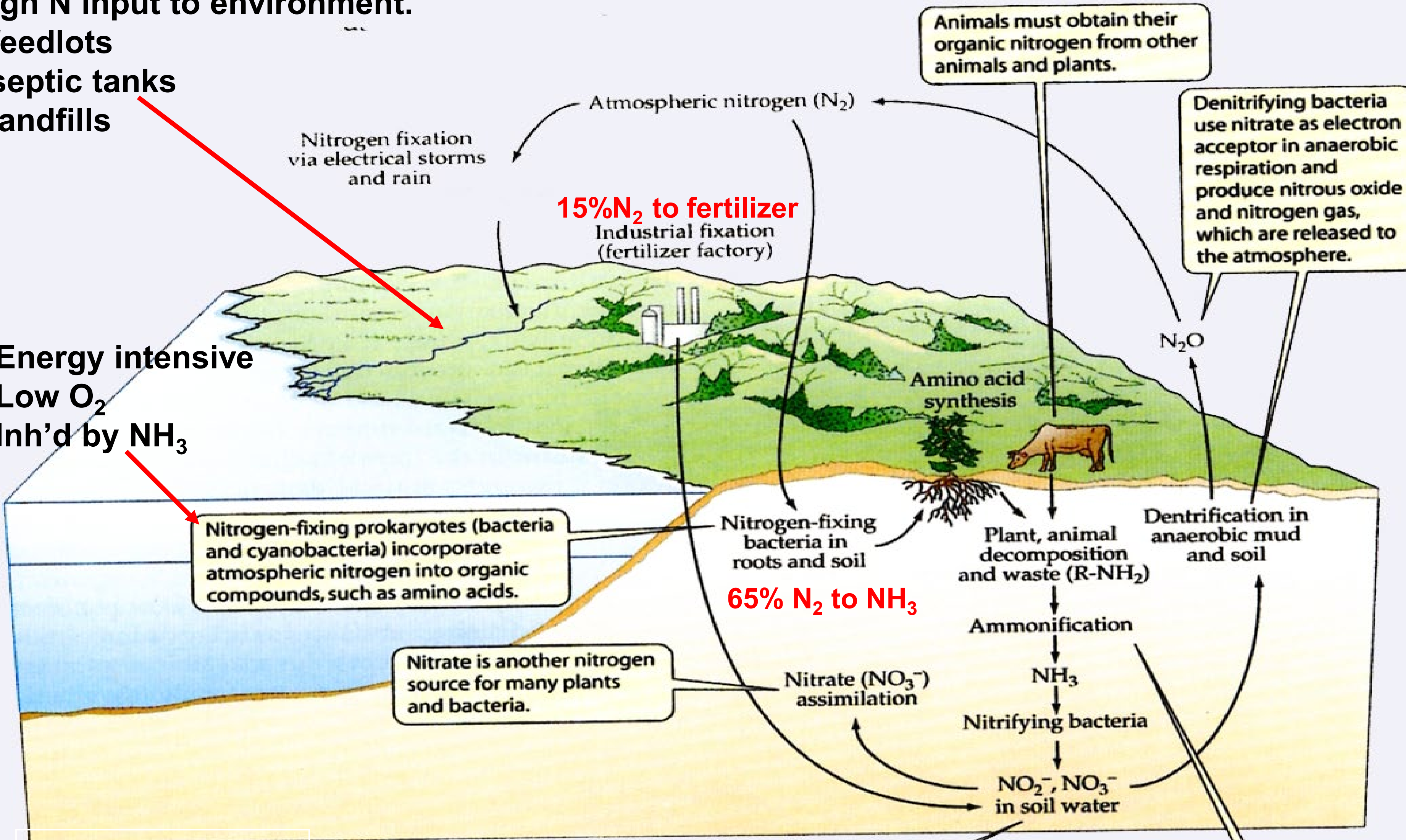
2. Nitrogen Cycle



The impact of ammonia and nitrate species in the aquatic environment:

1. Ammonia toxicity: NH_3 is a toxin for some fish so at higher pH ammonia discharge can create toxicity problem.
2. DO depletion in receiving waters – nitrification will use DO adding oxygen consumption load to the rivers.
3. Eutrification of surface waters – Both NH_3 and nitrate will promote eutrification
4. Ammonia will affect chlorination chemistry due to its reaction with chlorine.
5. Corrosion of copper pipes by ammonia (> 1 mg/L)
6. Nitrate in groundwater will cause “Blue baby syndrome” in infants. The US drinking water standards for nitrate is 10 mg/L as N.

High N input to environment.
*feedlots
*septic tanks
*landfills



*Energy intensive
*Low O_2
*Inh'd by NH_3

(Cells are 14%N)

Figure 24.19 Nitrogen cycle
Cycling of nitrogen through the biosphere.

Org.-N $\rightarrow$NH3
(Aer./Anaer.-deaminases)

Ref.:Perry,Staley and Lory, Microbial Life, 2002, Sinnauer Associates, p591.

1. Nitrogen fixation:

It is an energy demanding process carried out by unique nitrogen fixation bacteria

$$N_2 + 8H^+ + 8e^- = 2NH_3 + H_2$$ Energy required 15 to 20 ATP/ N_2

These bacteria are wide spread in nature

TABLE 14.12 Representative Genera of Free-Living Nitrogen-Fixers

Status with respect to oxygen	Mode of energy generation	Genus
Aerobic	Heterotrophic	*Azotobacter* *Beijerinckia* *Acetobacter* *Pseudomonas*
Facultatively anaerobic	Heterotrophic	*Klebsiella* *Bacillus*
Microaerophilic	Heterotrophic	*Xanthobacter* *Azospirillum*
Strictly anaerobic	Autotrophic Heterotrophic	*Thiobacillus* *Clostridium* *Desulfovibrio*
Aerobic	Phototrophic (cyanobacteria)	*Anabaena* *Nostoc*
Facultatively anaerobic	Phototrophic (bacteria)	*Rhodospirillum*
Strictly anaerobic	Phototrophic (bacteria)	*Chlorobium* *Chromatium*

The most famous ones are symbioses between legume (plants) and bacteria Rhizaobium. Rhizaobium grow in plant roots and the plant supply the bacteria with organics and the bacteria produce ammonium for plant growth.

Ammonia fertilizer can be manufactured from natural gas CH_4. This source of ammonia is about 15% of total nitrogen fixed.

TABLE 14.11 Relative Inputs of Nitrogen Fixation from Biological Sources

Source	Nitrogen fixation (metric tons/year)
Terrestrial	1.35×10^8
Aquatic	4.0×10^7
Fertilizer manufacture	3.0×10^7

- Bacterial nitrogen fixation is inhibited by the presence of ammonium.

- The Nitrognase, the enzyme catalyzes the reaction is very sensitive to oxygen. Fixers required low oxygen tension.

2. Ammonia Assimilation and Ammonification: cycle ammonia between its organic and inorganic forms

$$R\text{-}NH_2 + H_2O \underset{\text{Assimilation}}{\overset{\text{Ammonification}}{\rightleftarrows}} ROH + NH_3 + \text{energy}$$

In nature, assimilation predominates at C:N ratio >30, mineralization predominate at C:N ratio <20.

3. Nitrate assimilation: in the absence of ammonia, bacteria can use nitrate as N nutrient to synthesis protein. Nitrate is first reduced to ammonia. This is an energy demanding process.

$$\underbrace{NO_3^- \xrightarrow{2e^-} NO_2^-}_{\text{Nitrate reductase (NAS)}}$$

$$\underbrace{NO_2^- \xrightarrow{2e^-} HNO \xrightarrow{2e^-} NH_2OH \xrightarrow{2e^-} NH_3}_{\text{Nitrite reductase (NIS)}}$$

4. Nitrification: autotrophic oxidation of ammonia and nitrite. This is a two step process carried out by two different bacteria species.

$$NH_4^+ + O_2 + 2H^+ \longrightarrow NH_2OH + H_2O \longrightarrow NO_2^- + 5H^+ \quad \text{(Nitrosomonas)}$$

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \quad \text{(Nitrobacter)}$$

- **These bacteria are autotrophic, generating biomass from CO_2.**
- **Their growth rate is slow ,0.14 to 0.85 per day and significantly influenced by temperature. Temp < 10 C, not sustainable in activated sludge systems.**
- **Their yield is very low, 0.13 mg of biomass per mg of NH_3-N Nitrogomonas and 0.07 mg of biomass per mg of NO_2-N for Nitorbacter.**
- **Use large amount of oxygen to complete oxidation of NH_3, 4.6 mg of O_2 per mg of NH_3-N**
- **pH sensitive. No growth when pH <6.**
- **Acids are produced so if the natural buffer of the solution is not enough, base may be added to neutralize the acids.**
- **Inhibit by toxic compounds, Ni, Cu, CN, pesticides**

5. Dissimilatory Nitrate Reduction: There are two separate processes:

A. Dissimilatory nitrate reduction to ammonia (DNRA) – this happens in carbon natural environments, such as rumen, intestines and sediments. It is not important in wastewater treatment.

$$NO_3^- + 4H_2 + 2H^+ \longrightarrow NH_4^+ + 3H_2O$$

B. Denitrification: Nitrate is used as electron accepter for oxidizing organics and sulfur.

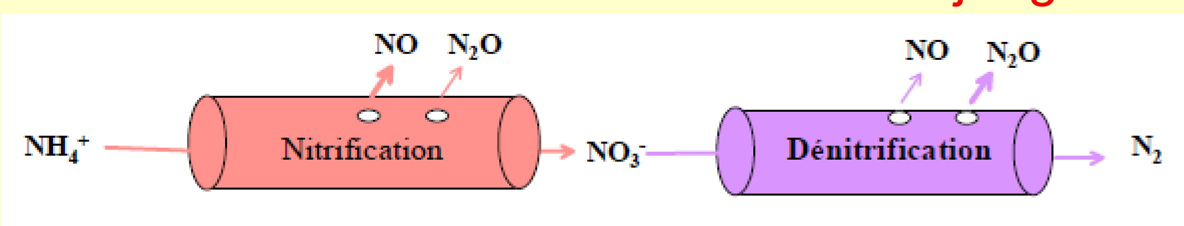
$$6NO_3 + 5CH_3OH \longrightarrow 3N_2 + 5CO_2 + 7H_2O + 6(OH)^-$$

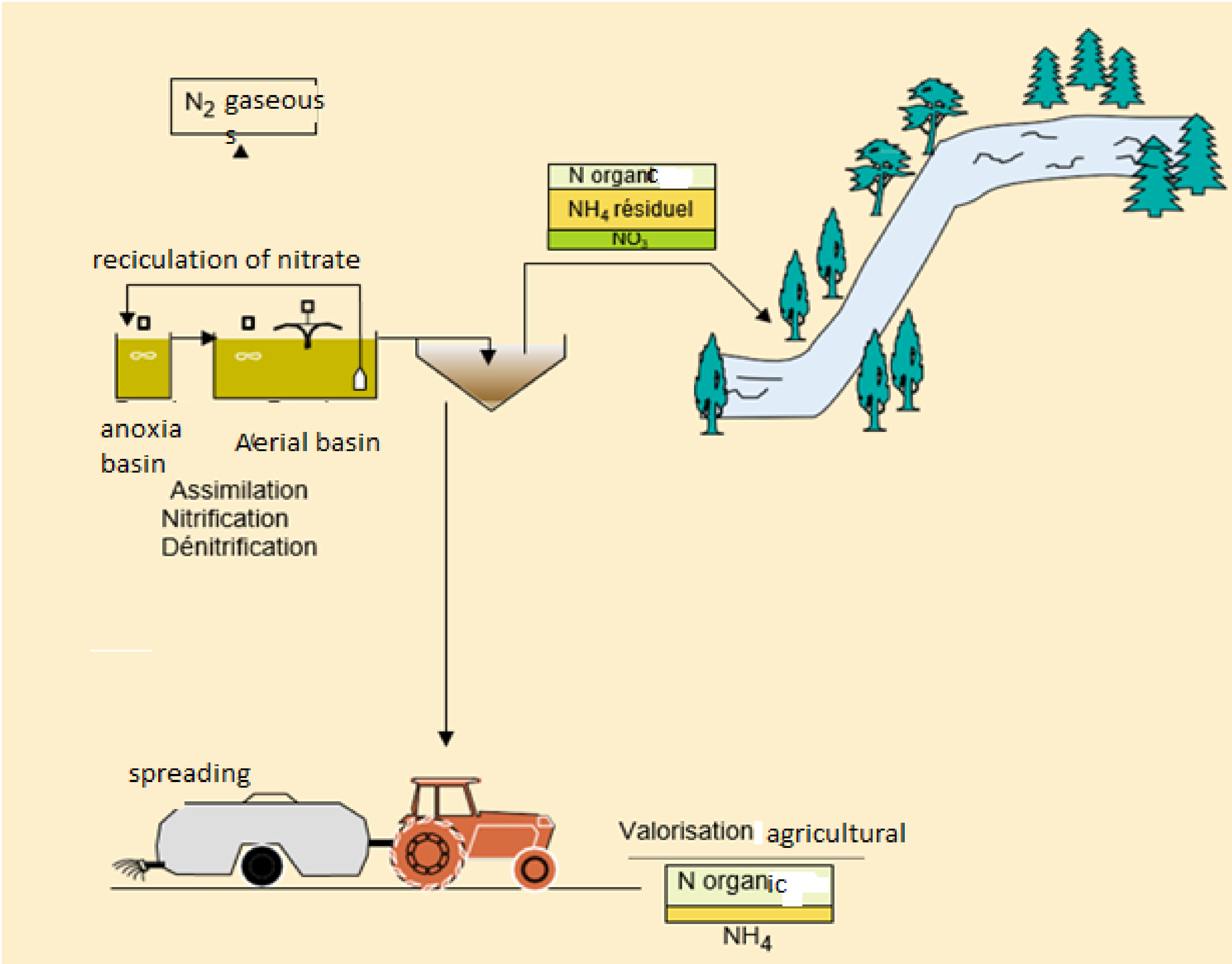
Most biodegradable organics can be used as e- donor. It will take 2.86 mg of COD to eliminate 1 mg/L of NO_3 –N.

Denitrification pathway:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow \tfrac{1}{2}\,N_2O \longrightarrow \tfrac{1}{2}\,N_2$$

Major greenhouse gas





N2 gaseous
N organic
NH4 résiduel
NO3
reciculation of nitrate
anoxia basin
Aerial basin
Assimilation
Nitrification
Dénitrification
spreading
Valorisation agricultural
N organic
NH4

- These are anoxic reactions, DO is inhibitory to denitrification.
- It is a base producing reaction.
- It is used extensively in wastewater treatment. It is estimated that most of the bacteria in an aerobic activated sludge can perform this anoxic reaction
- Low initial NO_3-N conc. favors N_2O production and high nitrate concentration favors N_2 production

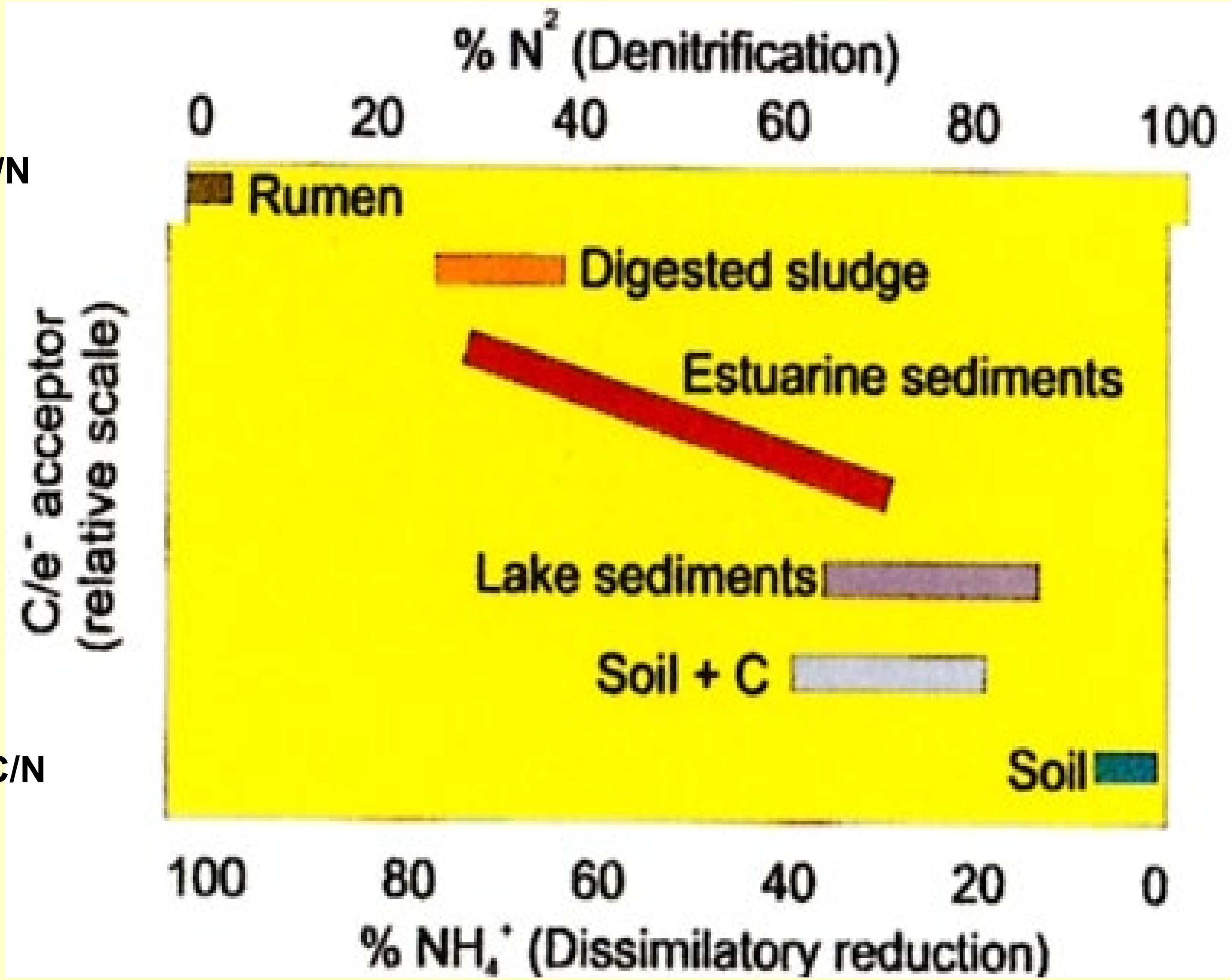
In addition to use organics as e^- donor, reduced sulfur compounds can be used as e- for this reaction

$$S^0 + 1.2NO_3^- + 0.4H_2O \rightarrow SO_4^{2-} + 0.6N_2 + 0.8H^+$$
$$\Delta G^{0'} = -547.6\ \text{kJ/reaction},$$

$$S_2O_3^{2-} + 1.6NO_3^- + 0.2H_2O \rightarrow SO_4^{2-} + 0.8N_2 + 0.4H^+$$
$$\Delta G^{0'} = -765.7\ \text{kJ/reaction}.$$

Competition between DNRA and denitrification in natural environments

HighC/N



LowC/N

6. Anaerobic NH_3 Oxidation (discovered in 1995, vandeGraaf,Appl.Env. Microbiol.61:1246)

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

Anammox process (autotrophic, NH_2OH and N_2H_4 as intermediates). These autotrophic bacteria form a separate and distinguish group in the microbial world. N_2O is not produced in this reaction. However, during its growth, Nitrite is oxidized to nitrate which compensates the reduction of CO_2 to cellular organic matter.

Overall reaction:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

Here is a website that provides information on this process:

http://www.anammox.com/index.html

It is a very slow growing culture

Various nitrogen species are involved in bacterial metabolism in four major categories:

- As nitrogen nutrient in biological cell synthesis:
  - Nitrogen gas – nitrogen fixation, convert N_2 to NH_3 and then uptake into proteins
  - Ammonia – Ammonia assimilation, uptake ammonia into proteins
  - Nitrate – Nitrate assimilation, reduce nitrate to ammonia and then uptake into proteins
- As energy source (electron donors) for aerobic autotrophic bacteria and O_2 is used as electron acceptor:
  - NH_3 oxidized to NO_2^- and then NO_3^-, to obtain chemical energy for the growth of these aerobic nitrifiers
- As electron acceptor in anoxic heterotrophic degradation of organics and sulfur:
- Others – Anammox process where NH_3 is served as electron donor and nitrite (NO_2^-) as electron acceptor.

Video