Nitrogen Fixation
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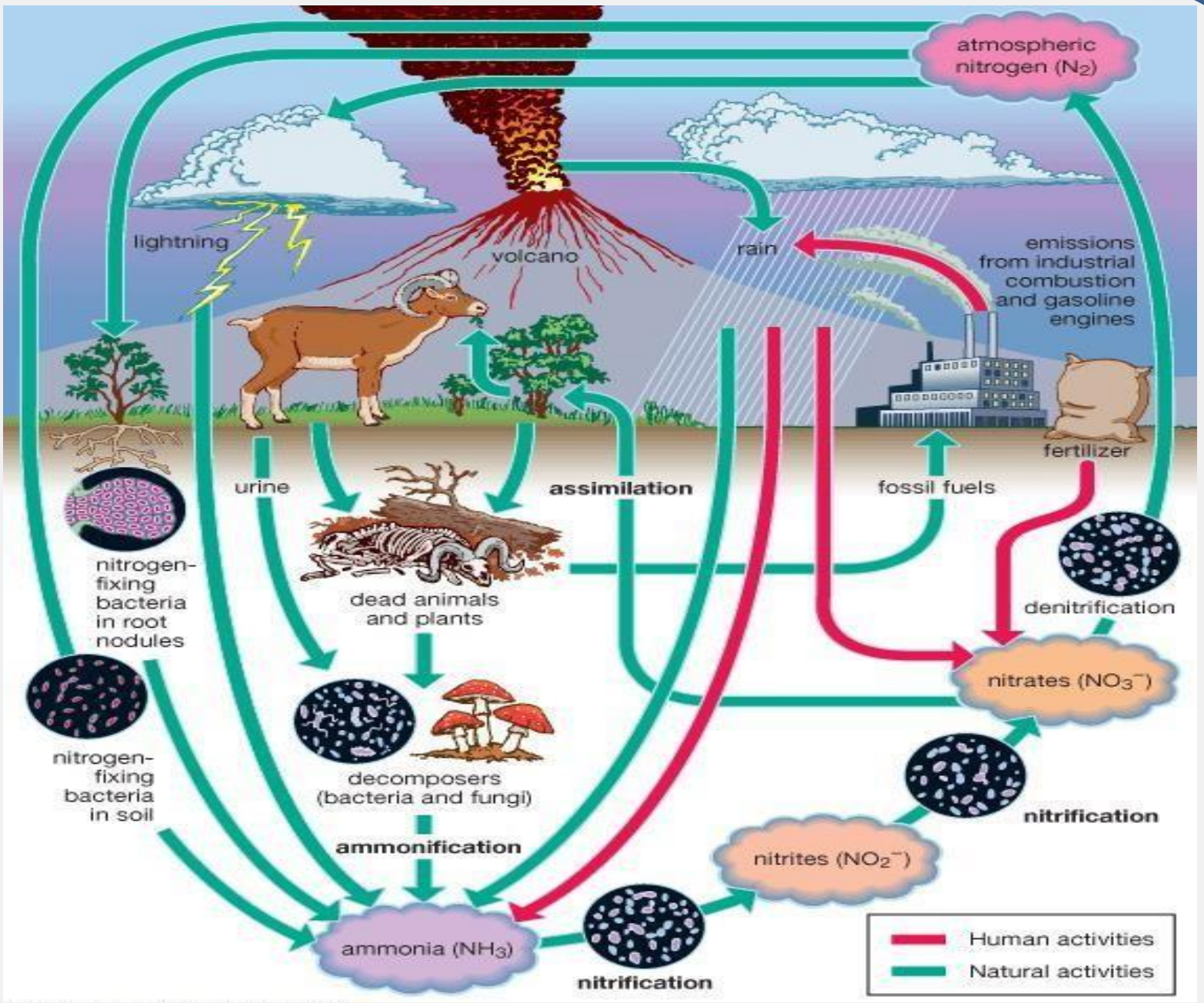
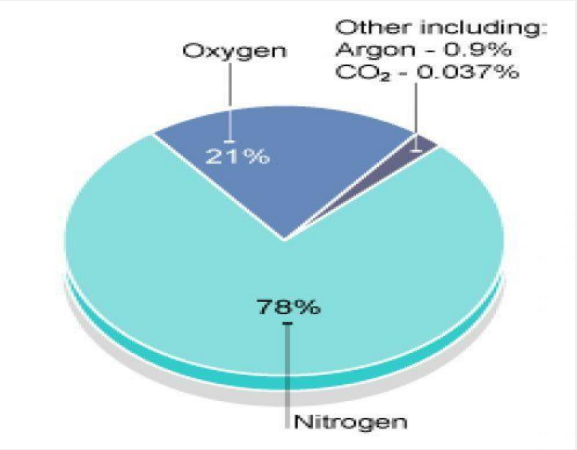

atmospheric nitrogen (N_2)
lightning
volcano
rain
emissions from industrial combustion and gasoline engines
fertilizer
urine
assimilation
fossil fuels
nitrogen-fixing bacteria in root nodules
dead animals and plants
denitrification
nitrates (NO_3^-)
nitrogen-fixing bacteria in soil
decomposers (bacteria and fungi)
nitrification
ammonification
nitrites (NO_2^-)
ammonia (NH_3)
nitrification
Human activities
Natural activities

NITROGEN

- Molecular nitrogen (N_2) is the major component (approximately 80%) of the earth's atmosphere.
- The element nitrogen is an essential part of many of the chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms.



- However, N_2 cannot be used directly by biological systems to build the chemicals required for growth and reproduction. Before its incorporation into a living system, N_2 must first be combined with the element hydrogen. This process of reduction of N_2, commonly referred to as "nitrogen fixation"(N-fixation) may be accomplished chemically or biologically.

Sources of Nitrogen

- Atmospheric Nitrogen
  - 78% of atmosphere
  - Plants cannot utilize this form
  - Some Bacteria, Blue Green Algae, leguminous plants
- Nitrates, Nitrites and Ammonia
  - Nitrate is chief form
- Amino acids in the soil
  - Many soil organisms use this form
  - Higher plants can also taken by higher plants
- Organic Nitrogenous compounds in insects
  - Insectivorous plants

Nitrogen Fixation: Need

Nitrogen is an essential component of DNA, RNA, and proteins—the building blocks of life.

Although the majority of the air we breathe is *nitrogen*, most living organisms are *unable to use nitrogen* as it exists in the *atmosphere*!

What is Nitrogen Fixation?

"*Nitrogen Fixation*" is the process that converts the atmospheric nitrogen to useful/simpler compounds of nitrogen.

The conversion of N_2 to reactive forms of N is nitrogen fixation

- ***Nitrogen fixation done naturally by microbes***
- ***Humans do N fixation chemically = fertilizer production***

Nitrogen gets "*fixed*" when it is combined with oxygen or hydrogen.

Nitrogen fixation, any natural or industrial process that causes free nitrogen (N_2), which is a relatively inert gas plentiful in air, to combine chemically with other elements to form more-reactive nitrogen compounds such as ammonia, nitrates, or nitrites.

- Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and ultraviolet rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil microorganisms
- More than 90 percent of all nitrogen fixation is effected by them.
- Two kinds of nitrogen-fixing microorganism are recognized:

- Free-living (non-symbiotic) bacteria, including the cyanobacteria or (blue-green algae) Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia and Clostridium and mutualistic symbiotic bacteria such as Rhizobium, associated with leguminous plants, and various Azospirillum species, associated with cereal grasses.

- The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate the formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development.

- Within the nodules, the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g., alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

- Nitrogenous materials have long been used in agriculture as fertilizers, and in the course of the 19th century the importance of fixed nitrogen to growing plants was increasingly understood. Accordingly, ammonia released in making coke from coal was recovered and utilized as a fertilizer, as were deposits of sodium nitrate (saltpetre) from Chile.
- Wherever intensive agriculture was practiced, there arose a demand for nitrogen compounds to supplement the natural supply in the soil. At the same time, the increasing quantity of Chile saltpetre used to make gunpowder led to a worldwide search for natural deposits of this nitrogen compound.

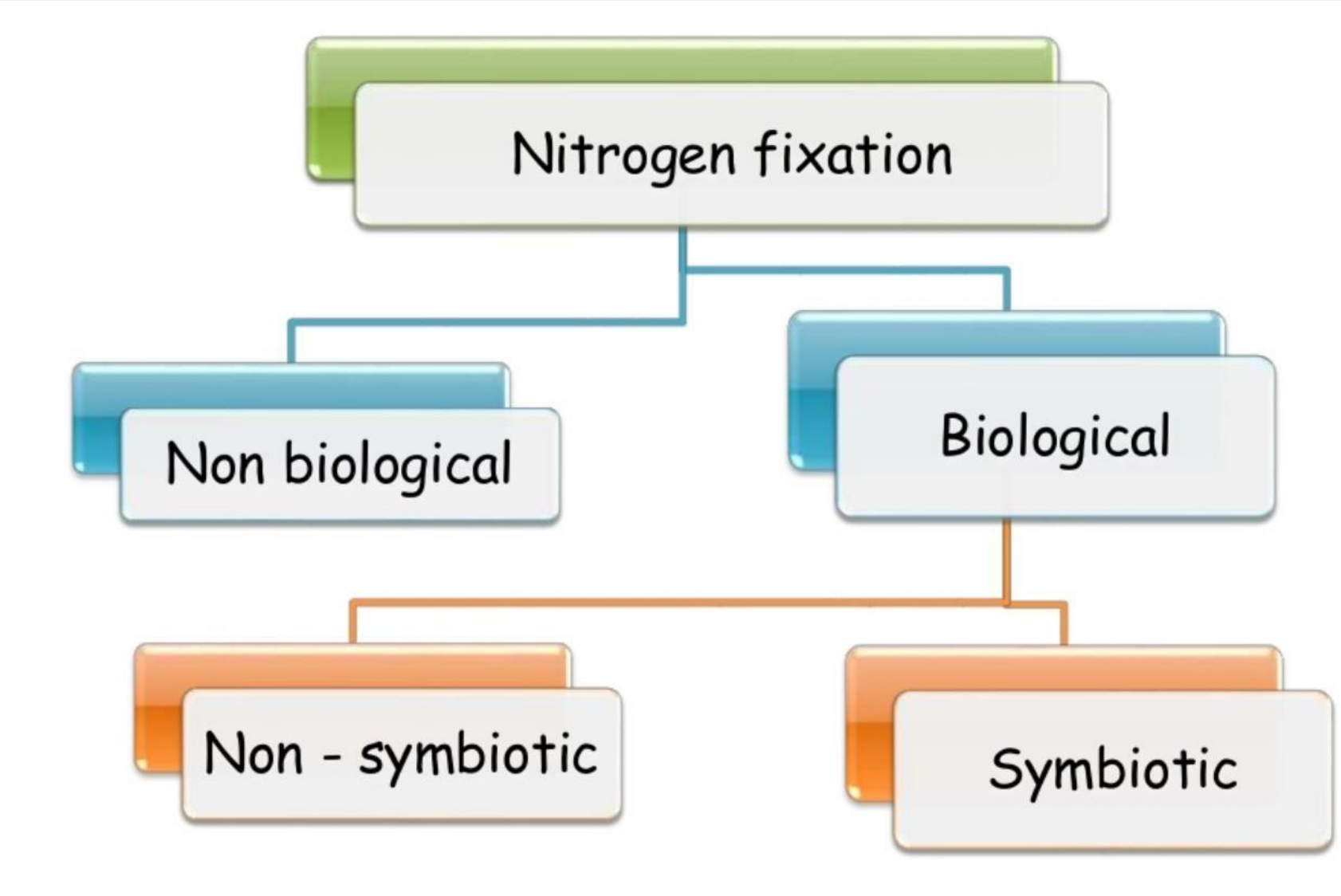
The Mechanism of Nitrogen Fixation

- The general chemical reaction for the fixation of nitrogen is identical for both the chemical and the biological processes.

$$N_2+3H_2+Energy = 2NH_3$$

- The triple bond of N must be broken and three atoms of hydrogen must be added to each of the nitrogen atoms. Living organisms use energy derived from the oxidation ("burning") of carbohydrates to reduce molecular nitrogen (N_2) to ammonia (NH_3). The chemical process of nitrogen fixation involves "burning" of fossil fuels to obtain the electrons, hydrogen atoms and energy needed to reduce molecular nitrogen.

Types of Nitrogen Fixation



Non-Biological Nitrogen Fixation

- The micro-organisms do not take place
- Found in rainy season during lightning

1. $N_2 + O_2 \xrightarrow{\text{lightning}} 2\,NO$
   (Nitric oxide)
2. $2NO + O_2 \xrightarrow{\text{oxidation}} 2NO_2$
   (Nitrogen per oxide)
3. $2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$
4. $4NO_2 + 2H_2O + O_2 \longrightarrow 4HNO_3$
   (Nitric acid)
5. $CaO + 2HNO_3 \longrightarrow Ca(NO_3)_2 + H_2O$
   (Calcium nitrate)
6. $HNO_3 + NH_3 \longrightarrow NH_4NO_3$
   (Ammonium nitrate)
7. $HNO_2 + NH_3 \longrightarrow NH_4NO_2$
   (Ammonium nitrite)

Biological Nitrogen Fixation

- Fixation of atmospheric Nitrogen into nitrogenous salts with the help of micro-organisms
- Two types
  - Symbiotic
  - Non-symbiotic

Non-Symbiotic

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Bacteria: special type (nitrogen fixing bacteria) types -
  - Free living aerobic : *Azotobacter, Beijerenckia*
  - Free living anaerobic : *Clostridium*
  - Free living photosynthetic : *Chlorobium, Rhodopseudomonas*
  - Free living chemosynthetic :*Desulfovibro, Thiobacillus*

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Symbiotic

- Fixation of free nitrogen by micro-organisms in soil living symbiotically inside the plants
- 'Symbiosis' – coined by DeBary
- Three categories
  - Nodule formation in leguminous plants
  - Nodule formation in non-leguminous plants
  - Non nodulation

Nitrogen Fixation: Processes

Processes

In-vivo

Within a living organism

Biological Fixation

In-vitro

In an artificial environment outside a living organism

Atmospheric Fixation

Industrial Fixation

Biological Fixation(BNF)

(where MOST nitrogen fixing is completed)

There are two types of "Nitrogen Fixing Bacteria"

Free Living Bacteria
("fixes" 30% of N_2)

Symbiotic Relationship Bacteria
("fixes" 70% of N_2)

In Vitro Nitrogen Fixation

- "*in vitro*" is a Latin word which means "within the glass".
- A procedure performed ***in vitro*** is performed not in a living organism but in a controlled environment, such as in a test tube or Petri dish.
- Because the test conditions may not correspond to the conditions inside of the organism, this may lead to results that do not correspond to the situation that arises in a living organism. Consequently, such experimental results performed *in vitro*, are in contradiction with *in vivo*.
- *in vitro* experiments are performed to model *in vivo* experiments.

Importance of in vitro nitrogen fixation

- It provides a model for in vivo nitrogenase systems and to employ molybdenum.

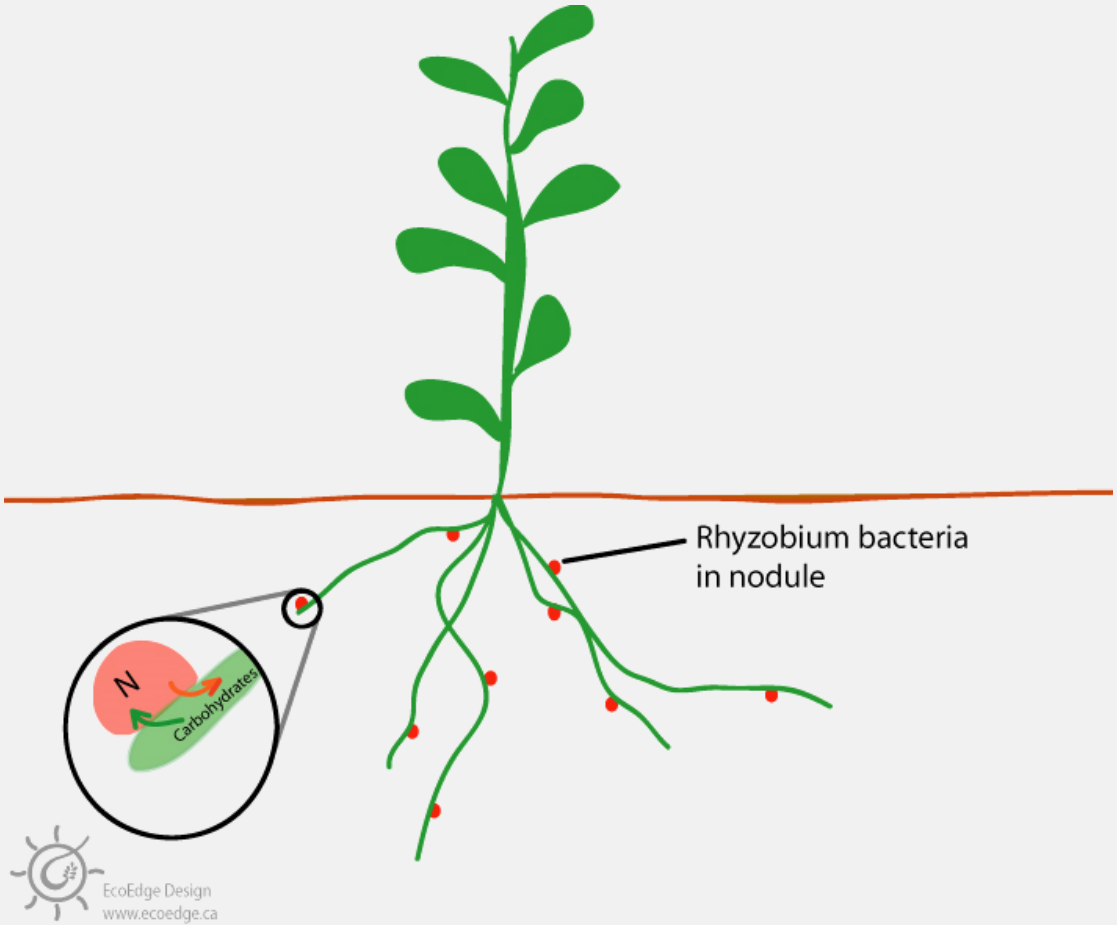
- It provides in sight into the development of useful catalyst for the industrial fixation of nitrogen.

Limitations of in vitro nitrogen fixation

- It is not a continuous process, i.e., after fixing one nitrogen molecule to two molecules of NH_3, reaction stops.

- Reaction stops when the metal is completely oxidized as there is no more electron supply but in *in vivo*, the reaction goes on because there is an enzyme nitrogenase which assists the continuous supply of oxygen and hence the electrons.

In vivo Nitrogen fixation



In vivo Nitrogen fixation

- In vivo processes are the processes which occur or made to occur within a living organism.

- Certain symbiotic or free living bacteria are able to fix nitrogen into useful compounds.

- Symbiotic bacteria fixes dinitrogen in association with plants e.g. the bacterium Rhizobium which is associated with the nodules on the roots of leguminous plants.

- Asymbiotic bacteria are free living bacteria which fixes atmospheric nitrogen e.g. Azotobacter.

- In biological nitrogen fixation two moles of ammonia are produced from one mole of nitrogen gas, using 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

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

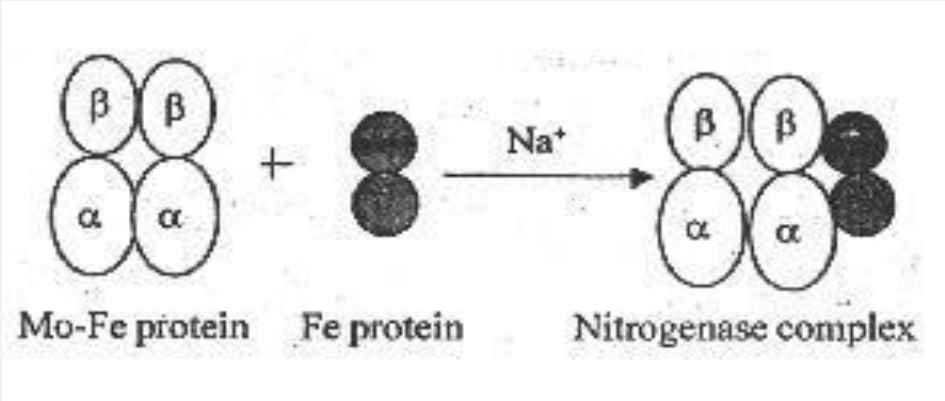
- These bacteria contain the enzyme called nitrogenase which is responsible for nitrogen fixation

Composition of Nitrogenase

•composed of two proteins

•Fe protein also called reductase

•Fe-Mo protein

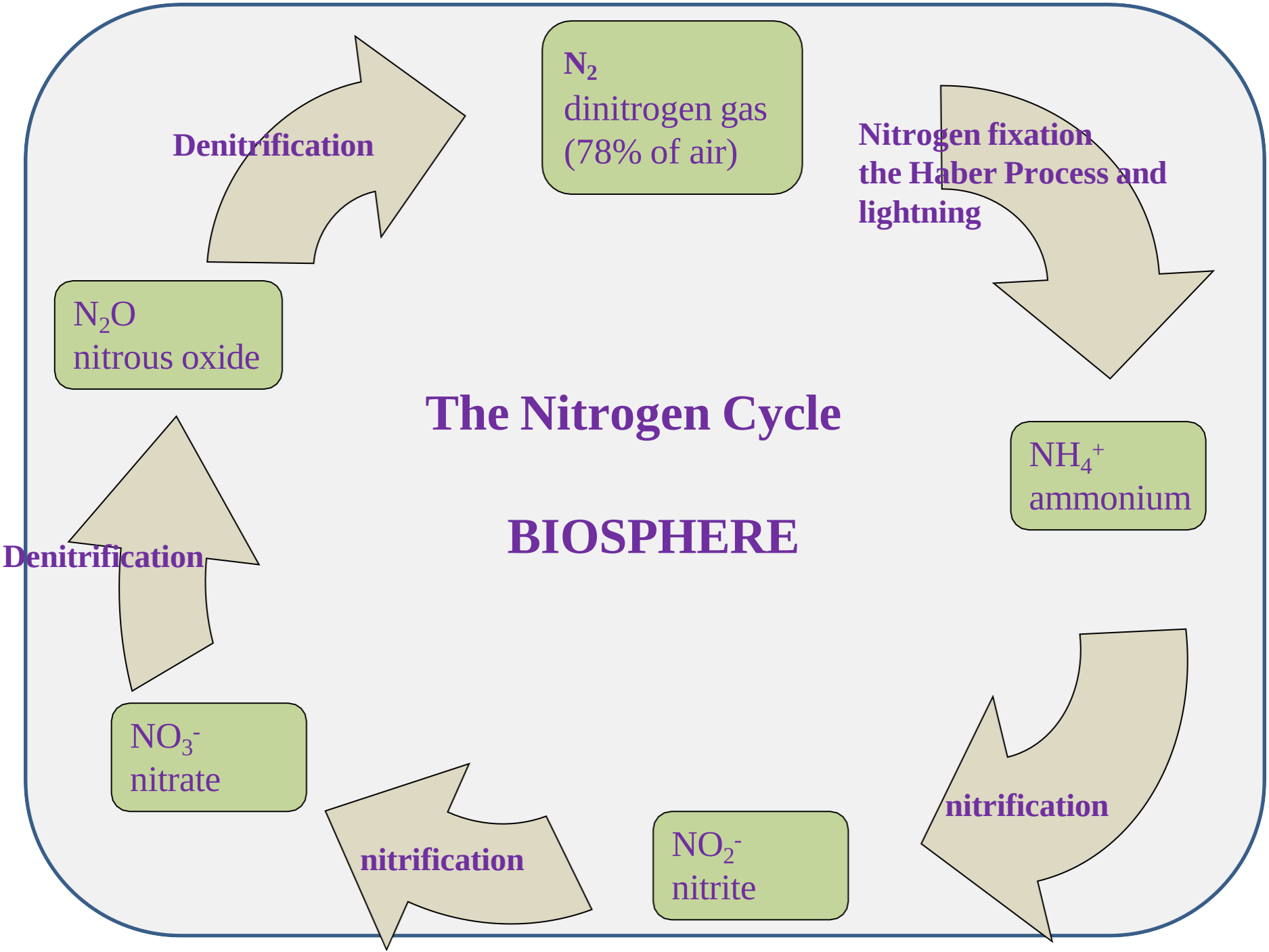


•Fe-Mo protein contains Mo-Fe as cofactor and protein P cluster.

•Mo-Fe cofactor is the active site of nitrogen fixation where dinitrogen is supposed to bind.

•These components are present in the ratio of one Mo-Fe protein to two Fe proteins .It is anaerobic in nature.

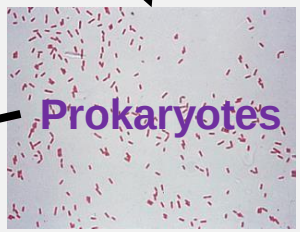
•Nitrogenase also catalyses reduction of acetylene to ethylene apart from nitrogen to ammonia.


N_2
dinitrogen gas
(78% of air)
Nitrogen fixation
the Haber Process and
lightning
NH_4^+
ammonium
NO_2^-
nitrite
nitrification
NO_3^-
nitrate
nitrification
N_2O
nitrous oxide
Denitrification
Denitrification
The Nitrogen Cycle
BIOSPHERE

The Nitrogen Cycle

N_2
dinitrogen gas
(78% of air)

Biological nitrogen fixation



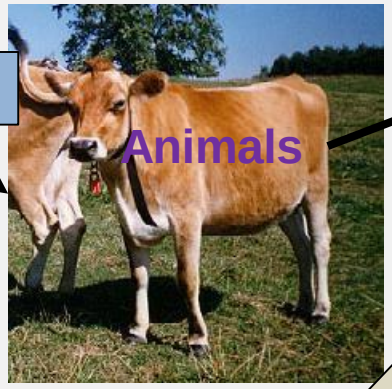
assimilation

NH_4^+
ammonium

nitrification

N_2O
nitrous oxide

consumption



uptake

NO_3^-
nitrate

NO_2^-
nitrite

Non-symbiotic nitrogen fixation

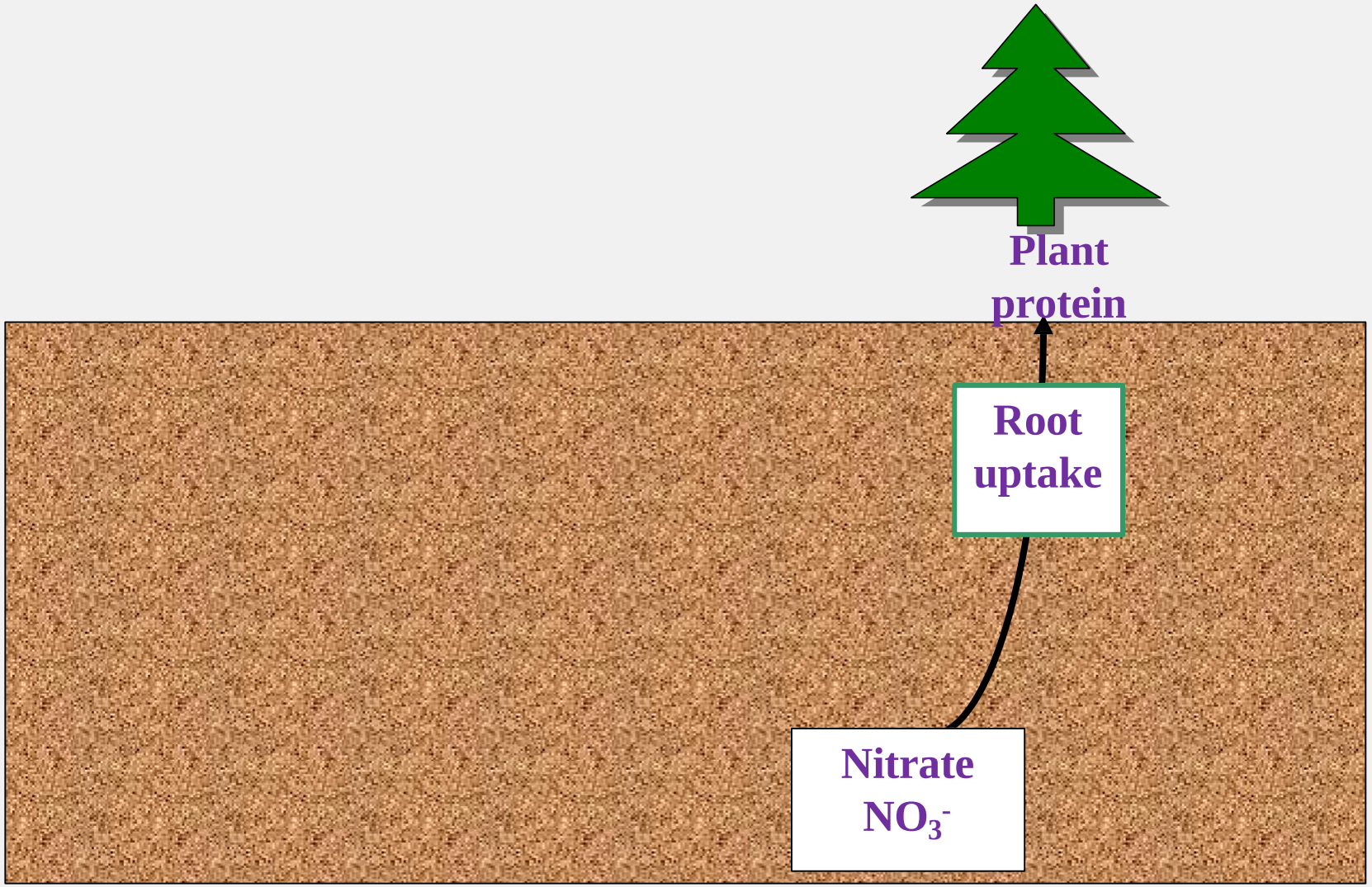
Aquatic:

Cyanobacteria
Anabaena
Nostoc

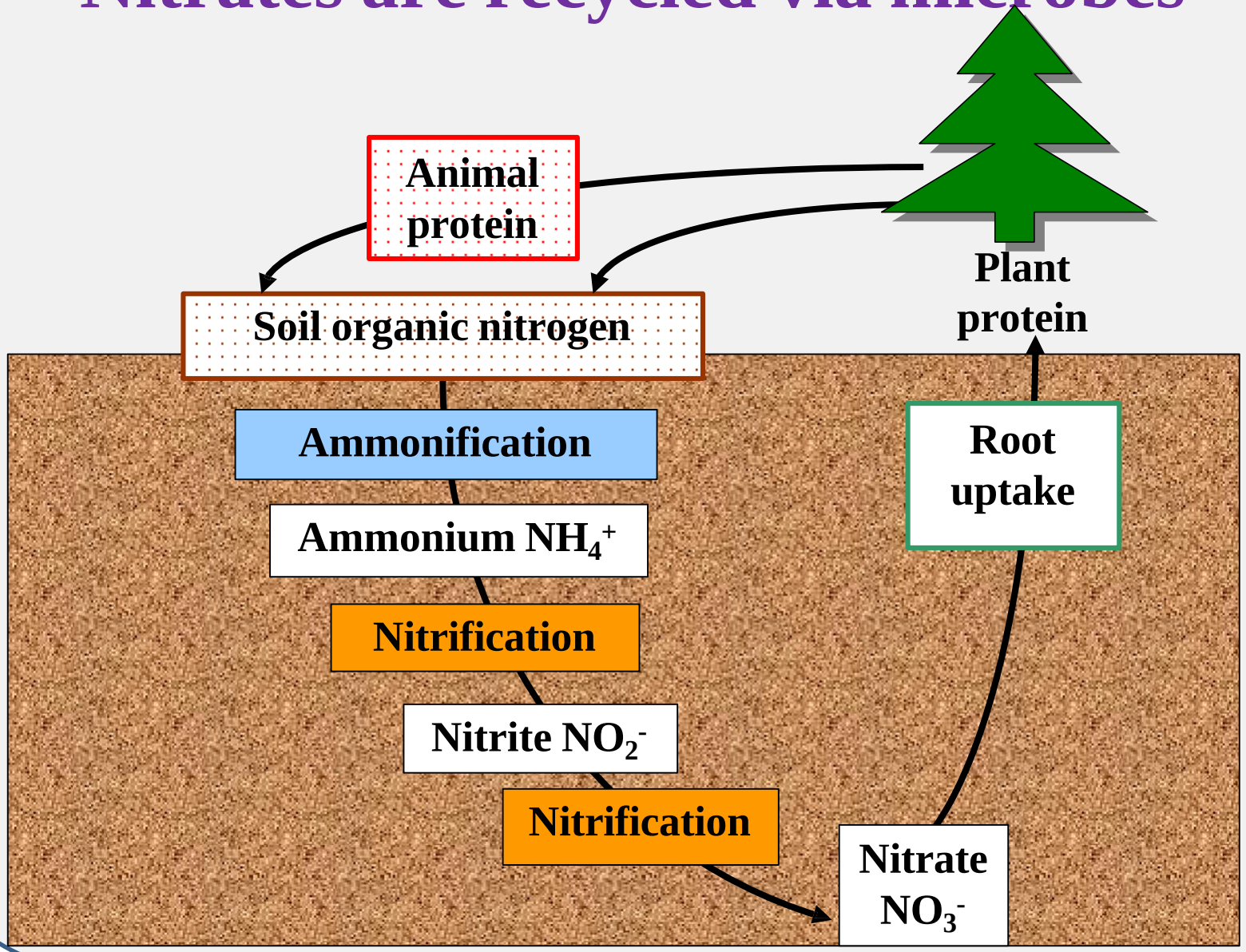
Terrestrial and rhizosphere-associated:

Azospirillum
Azotobacter
Acetobacter
Klebsiella
Clostridium

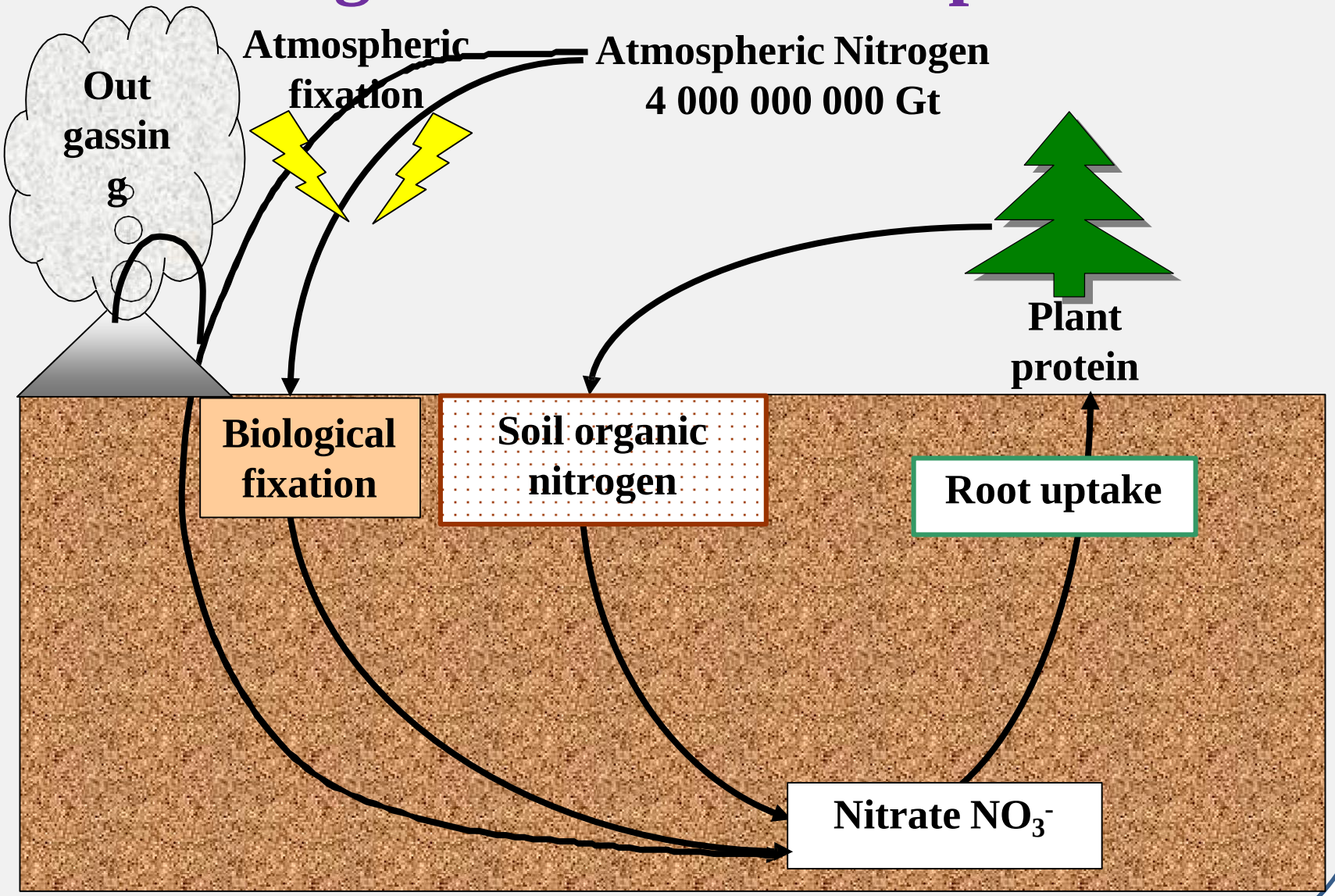
Nitrates are essential for plant growth



Nitrates are recycled via microbes



Nitrogen from the atmosphere



Atmospheric Pollution

- This also happens inside the internal combustion engines of cars
- The exhaust emissions of cars contribute a lot to atmospheric pollution in the form of NO_x
- These compounds form **photochemical smogs**
- They are **green house gases**
- They dissolve in rain to contribute to **acid rain** in the form of nitric acid
- The rain falling on soil and running into rivers
- They contribute to the **eutrophication** of water bodies

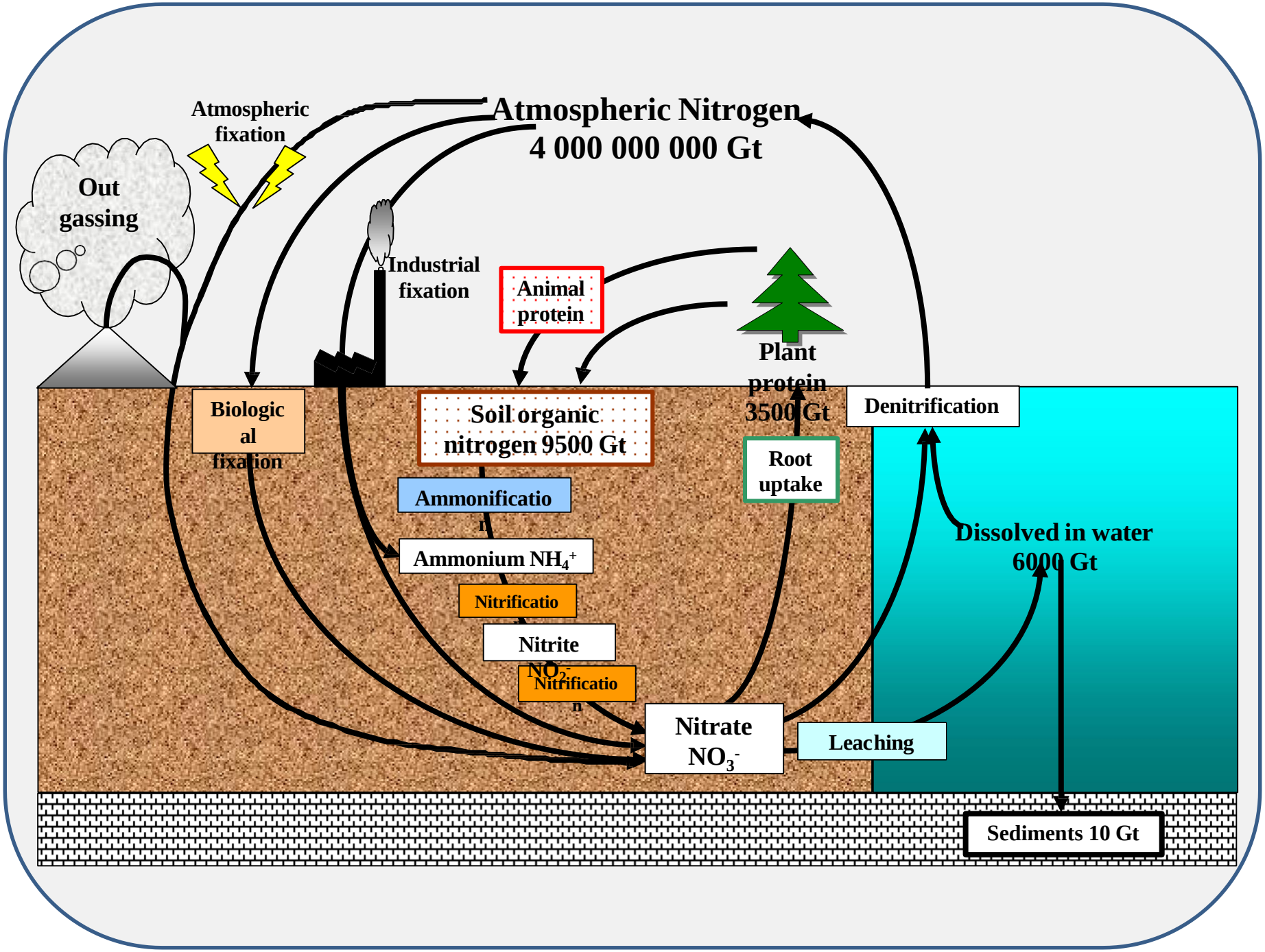
Return to the atmosphere: Denitrification

- Nitrates and nitrites can be used a source of oxygen for ***Pseudomonas*** bacteria
- Favourable conditions: Cold waterlogged (anaerobic) soils

$2NO_3$ - □ $3O_2$ + N_2□providing up to 2385kJ

$$2NO_2^- \rightarrow 2O_2 + N_2 \uparrow$$

- The liberated oxygen is used as an electron acceptor in the processes that oxidise organic molecules, such as glucose
- These microbes are, therefore, **heterotrophs**


Atmospheric Nitrogen
4 000 000 000 Gt
Atmospheric fixation
Out gassing
Industrial fixation
Animal protein
Plant protein 3500 Gt
Biologic al fixation
Soil organic nitrogen 9500 Gt
Root uptake
Denitrification
Ammonificatio n
Ammonium NH_4^+
Nitrificatio
Nitrite NO_2^-
Nitrificatio n
Nitrate NO_3^-
Leaching
Dissolved in water 6000 Gt
Sediments 10 Gt