MIC 319
FUNDAMENTALS OF AGRICULTURAL MICROBIOLOGY

CHAPTER 7
NUTRIENT CYCLE

BY

[redacted]

OBJECTIVE

- Illustrate the basic concept of nutrient cycle
- Clarify the steps in nitrogen cycle

CARBON CYCLE

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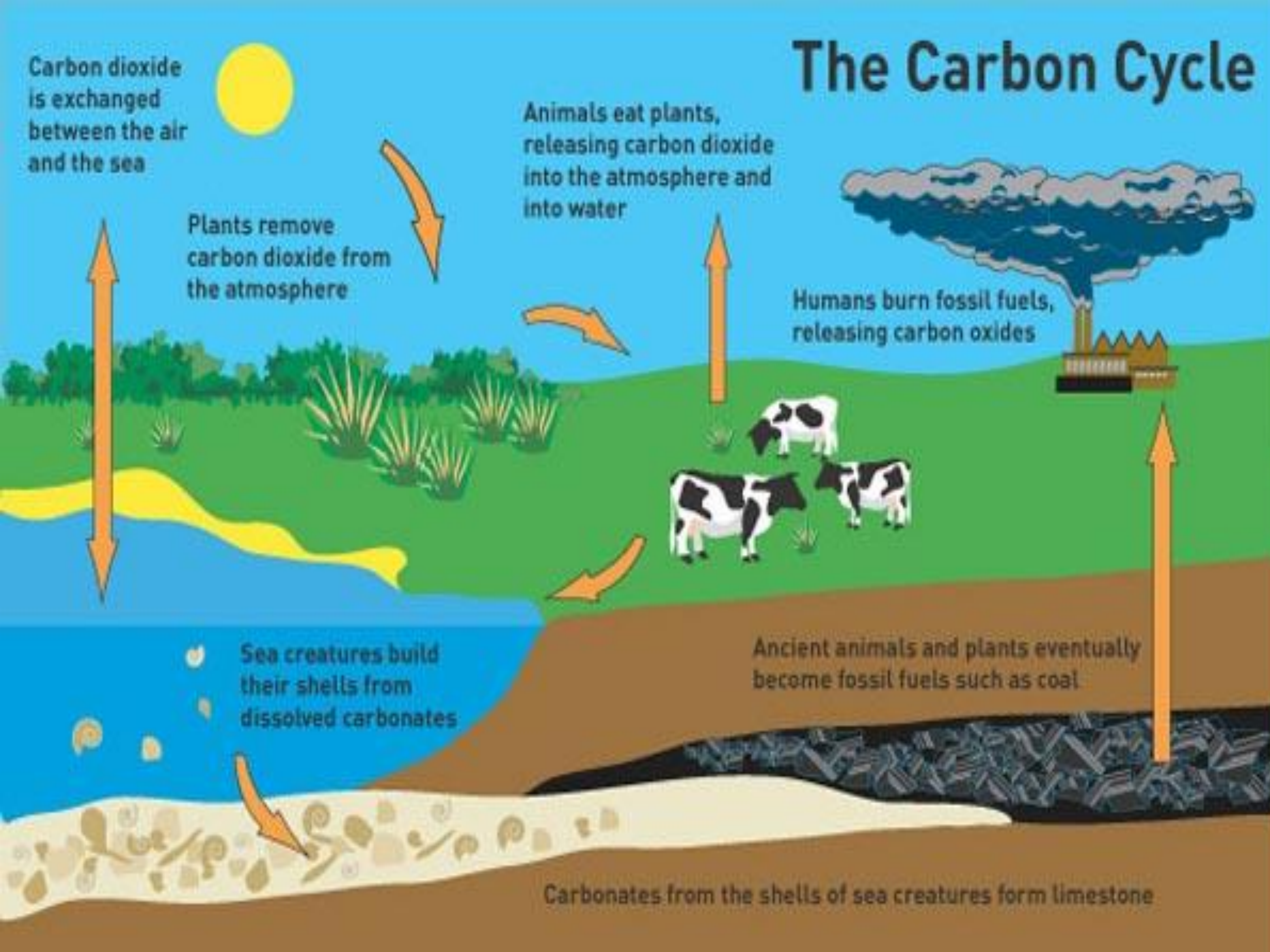
- Carbon >> is an element fundamental to all life.
- Nature has devised a way to recycle this elements, which is called the carbon cycle.
- Plants take in carbon as CO_2 through the process of photosynthesis and convert it into sugars, starches and other materials necessary for plant's survival.
- From the plants, carbon is passed up the food chain to all the other organisms.

CARBON CYCLE

- Both animals and plants release waste CO_2. This is due to the process called cell respiration where the cells of an organisms breakdown sugars to produce energy for the functions they are required to perform.
- This equation of cell respiration is as follows:

Cellular Respiration

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + \text{Energy}$$


The Carbon Cycle
Carbon dioxide is exchanged between the air and the sea
Plants remove carbon dioxide from the atmosphere
Animals eat plants, releasing carbon dioxide into the atmosphere and into water
Humans burn fossil fuels, releasing carbon oxides
Sea creatures build their shells from dissolved carbonates
Ancient animals and plants eventually become fossil fuels such as coal
Carbonates from the shells of sea creatures form limestone

CARBON CYCLE

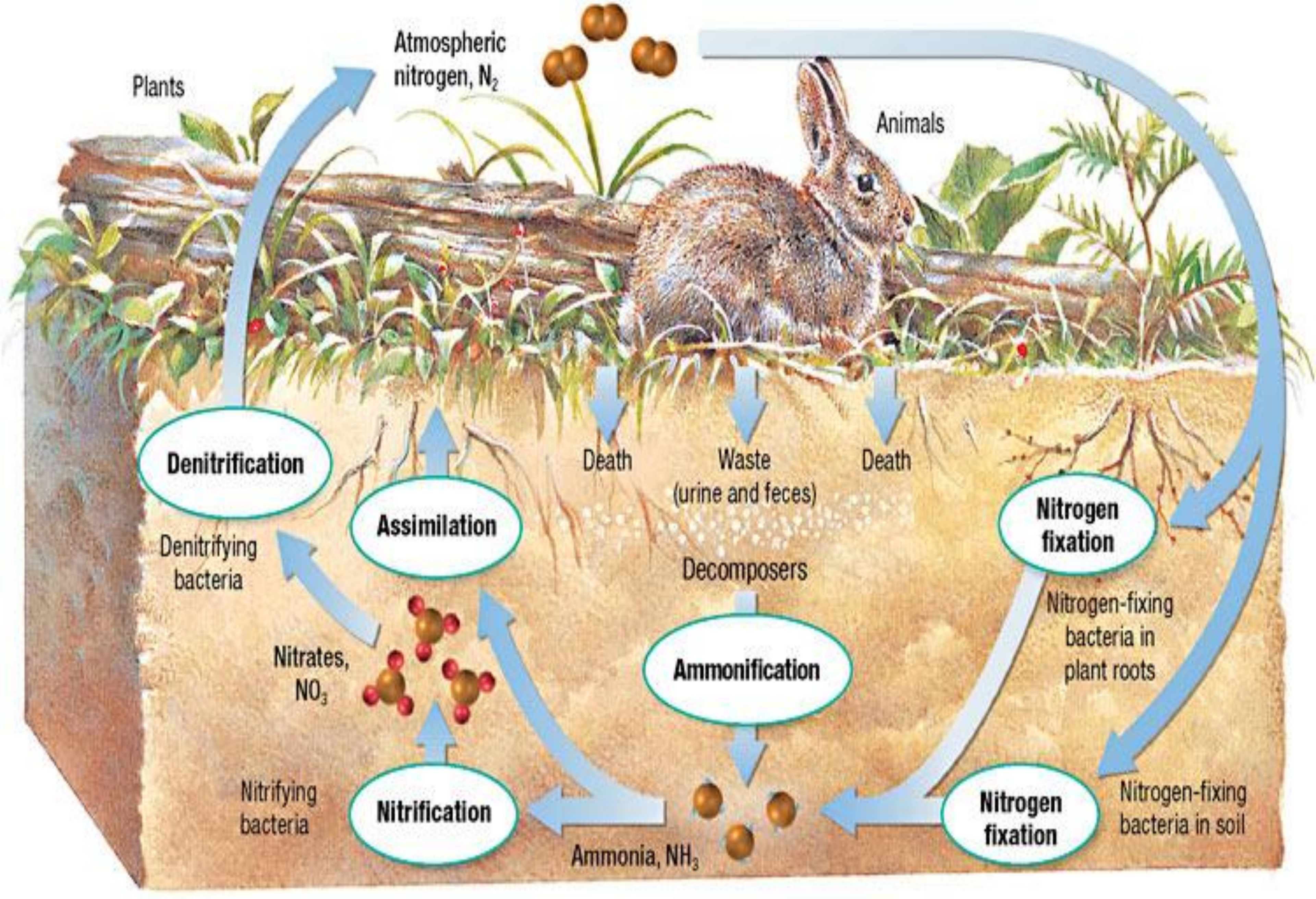
- CO_2 is returned to the atmosphere when plants and animals die and decompose. The decomposers release CO_2 back into the atmosphere again by other plant during photosynthesis.
- In this way the cycle of CO_2 being absorbed from the atmosphere and being released again is repeated over and over.
- In the carbon cycle, the amount of carbon in the environment always remains the same.

NITROGEN CYCLE

NITROGEN CYCLE

- Nitrogen (N) >> is an essential nutrient used in relatively large amounts by all living things.
- The difficulty from a plant's point of view is that the N_2 in the atmosphere is very non-reactive and is not plant-available,
- Plants obtain all the O_2 and C they need from the air but they get no N.
- The conversion of N_2 to N compound and from N compound back to N_2 is called the Nitrogen Cycle.

NITROGEN CYCLE



NITROGEN CYCLE

- Nitrogen cycle involves several processes:

1) Nitrogen fixation
2) Ammonification
3) Nitrification
4) Denitrification

1) NITROGEN FIXATION

1) NITROGEN FIXATION

- This is the first step in the N cycle.
- Defined as the reduction of atmospheric N_2 to ammonia.
- Can only be done biologically by a small and highly specialized group of microorganisms in the presence of the enzyme nitrogenase which catalyzes the reduction of nitrogen gas (in atm) to ammonia.

$$N_2 + 8\,H^+ + 8\,e^- \rightarrow 2\,NH_3 + H_2$$

- The ammonia is now combined with organic acids to form amino acids and proteins.

1) NITROGEN FIXATION

- Nitrogen can be fixed from the atmosphere by:

a) Non Biological fixation (Fixation on N from lightning)

b) Biological fixation

a) Non Biological Fixation

- Nitrogen may be fixed by the electrical discharge of lightning in the atmosphere.

Lightning + N_2 + O_2 -------→ 2 NO

- The nitrous oxide formed combines with oxygen to form NO_2

2 NO + O_2 ------------------→ 2 NO_2

- NO2 readily dissolves in water to produce nitroc and nitrous acids

2 NO_2 +H_2O -------------→ HNO_3 + HNO_2

- These acids readily release the hydrogen, forming nitrate and nitrite ions.
- The nitrate can be readily utilized by plants and microorganisms.

HNO_3-------------→ H+ + NO_3^- (nitrate ions)

HNO_2-------------→H+ + NO_2^- (nitrite ions)

a) Biological fixation

- Biological fixation may be symbiotic or non symbiotic

>> **Symbiotic N fixation** (Symbiotic N Fixers) refers to microorganism fixing N while growing in association with a host plant.

- Both the plant and microorganism benefits from this relationship.
- The most widely known example of such a symbiotic association is between *Rhizobium* bacteria and plants such as soybean, peanut and alfafa.

a) Biological fixation

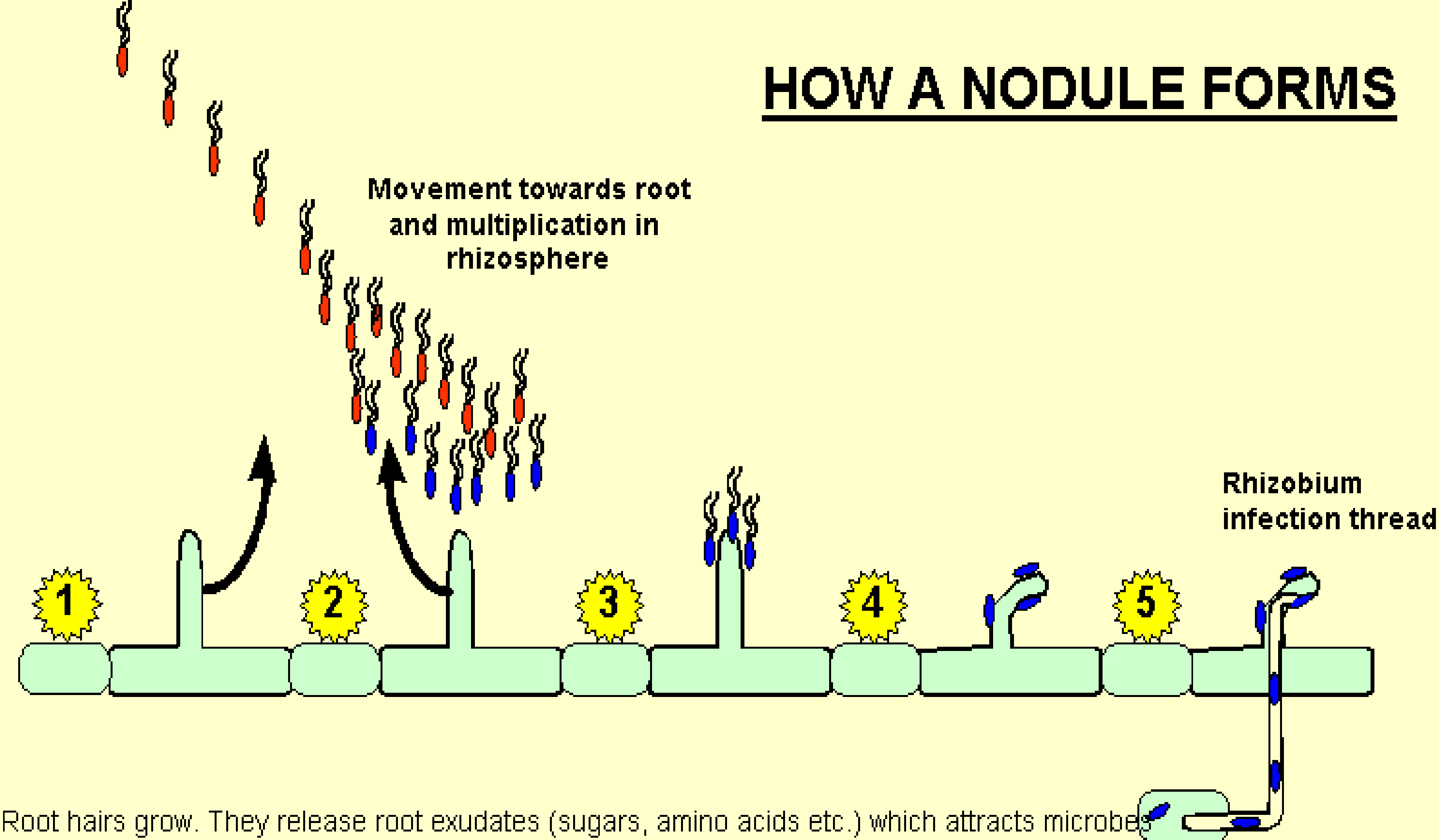
- This bacteria **infect** the plant's root and form **nodules**
- The bacteria within this nodule fix N_2 and make it available to the plant (70% of all N_2 fixed in world)
- Symbiotic nitrogen fixers are associated with plants and provide the plant with nitrogen in exchange for the plants carbon (energy source) and a protected home.

a)Biological fixation

>>> **Non-symbiotic N fixation** (free living N fixers) is carried out by free living bacteria and blue-green algae in the soil.

- The amount of N fixed by these organisms is much less than the amount fixed symbiotically.
- Free living nitrogen fixers that generate ammonia for their own use (e.g bacteria living in soil but not associated with a root) include bacteria, *Azospirilium*, *Azotobacter* spp. And Clostridium spp. (30% of all N_2 fixed in world)

HOW A NODULE FORMS



1. Root hairs grow. They release root exudates (sugars, amino acids etc.) which attracts microbes towards the root (chemotaxis) setting up the microbial 'rhizosphere'
2. The root hairs release specific flavenoid exudates to specifically attract *Rhizobia* bacteria towards the root and to induce specific *nod* (nodulation) genes in the bacteria (I.e. red to blue)
3. The *Rhizobia* attach to the root hair surface(probably via lectin binding)
4. In reponse to a *Rhizobia* signal, the root hair curls entrapping the *Rhizobia*
5. The *Rhizobia* digests the cell wall and forms an infection thread (I.e. a tube) down into the root cortex where they then divide, the cortical cells expand and a root nodule is formed. The tube seals off to prevent other unwanted guests. The bacteria are extracellular at this stage. Once they reach the potential nodule site they invade the cells and set up hor

Factor affecting N Fixation

- When soil nitrogen (NO_3 or NH_4) levels are high, the formation of nodules is inhibited.
- also, anything that impacts the carbohydrates production will effect the amount of N fixed. In order for the nitrogen to be used by succeeding crops, the nodules and plant must be incorporated into the soil, or no nitrogen will be gained.
- Harvesting for animal feed reduces the chances for a net nitrogen gain, unless the manure is returned to the soil.

2) AMMONIFICATION

2) AMMONIFICATION

- **Second step** in N cycle.
- The biochemical process whereby ammoniacal nitrogen is released from nitrogen- containing organic compounds.
- Soil bacteria decompose organic nitrogen forms in soil to the ammonium form. This process is referred to as ammonification.
- The amounts of nitrogen released for plant uptake by this process is most directly related to the organic matter content.
- The initial breakdown of a urea fertilizer may also be termed as an ammonification process.

- In the plant, fixed nitrogen that is locked up in the protoplasm (organic nitrogen) of N_2 fixing microbes has to be released for other cells.
- This is done by the process of ammonification with the assistance of deaminating enzymes.
- <u>In the plant:</u>

alanine (an amino acid) + deaminating enzyme > ammonia = pyruvic acid

- <u>In the soil:</u>

RNH_2 (Organic N) = heterotropic (ammonifying) bacteria > NH_3 (ammonia) + R

> In soils NH_3 is rapidly converted to NH_4 when hydrogen ions are plentiful ($pH < 7.5$)

Fate of Ammonium

- Ammonium has several divergent pathways from this point forth.
- Plants and algae can assimilate ammonia and ammonium directly for the biosynthesis.
- The remaining bulk of decomposed by products is utilized by bacteria in a process called nitrification.
- Some are used by heterotroph for further assimilation of organic carbon. Some are fixed by clay particles and made unavailable or other uses.

3) NITRIFICATION

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- This is the **third step** in nitrogen cycle.
- Conversion of ammonium to nitrate (NO_3^-)
- Performed by several species of nitrifying bacteria that live in the soil

$$NH_4^+ \rightarrow NO_3^- \text{ (Nitrate)}$$

Organic N (protein amino acids

most microb

NH_4^+

AMMONIFICATION

O_2
Nitrosomonas
$4H^+$

NO_2^-

O_2
Nitrobacter

NO_3^-

NITRIFICATION

- The **nitrification** process is primarily accomplished by **two groups** of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonium or nitrate.

>>> In the first step of nitrification:

- ammonia-oxidizing bacteria oxidize ammonia to nitrite according to equation (1).

$$NH_3 + O_2 \text{ ----}\rightarrow NO_2^- + 3H + 2e^- \qquad (1)$$

- Nitrososomas is the most **frequently** identified genus associated with this step, although other genera, including Nitrosococcus and Nitrosospira.
- Some subgenera, Nitrosolobus and Nitrosovibrio, can also autotrophically oxidize ammonia. (Watson et al. 1981).

>>>> In the second step of the process:

- nitrite-oxidizing bacteria oxidize nitrite to nitrate according to equation (2)

$$NO_2^- + H_2O \dashrightarrow NO_3^- + 2H + 2e^- \quad (2)$$

- Nitrobacter is the most frequently identified genus associated with this second step, although other genera, including Nitrospina, Nitrococcus and Nitrospira can also autotrophically oxidize nitrite.

Environmental influences

- Physical and chemical factors affect the rate of ammonium oxidation.
- **Acidity** : acid environmental rate slower, due to effect on the responsible species. > enhanced by liming
- **Oxygen** : since it is oxidation process, oxygen is necessary, effect the microorganisms involved.
- **Water level**: waterlogging can create anaerobic condition.
- **Temperature**

Nitrate pollution

- Excessive nitrification may lead to undesirable conditions.

1) Eutrophication
2) Infant and animal methemoglobinemia
3) Formation of nitrosamines

4) DENITRIFICATION

Denitrification

- Fourth and last step of N cycle
- Involves conversion of NO_3 to N_2 gas in the presence of low oxygen levels.

$$C_6H_{12}O_6 + 4NO_3^- \longrightarrow 6CO_2 + 6H_2O + 2N_2 \text{ (gas)} + NO + NO_2$$

- Bacterial denitrification is the microbial reduction of NO_3 to NO_2 or N.
- E.g : *Pseudomonas* use NO_3 instead of O_2 as a terminal electron acceptor.

- **Denitrification** is accelerated under anaerobic (flooded or compacted) conditions and high nitrogen inputs.
- Denitrification results in environmental pollution (destroys ozone) and also contributes to global warming since nitrous oxides do have a minor effect as a greenhouse gas.
- Through nitrification and denitrification 10-20% of applied N is lost.