Nitrogen Cycle

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Introduction

- Nitrogen is an important element required by plants and animals as it a constituent of the cells.
- It is present in cells as organic molecules – proteins. The living organisms are incapable of taking in nascent Nitrogen.
- It is taken in combined form mainly nitrates.
- Gaseous nitrogen present in large quantity in the atmosphere of the earth is fixed by the microorganisms.
- Sometimes it becomes a limiting factor, as it is absorbed by the producers (green plants) of the ecosystem in inorganic combined form and later on passed to the consumers through food chain and food web.
- Inorganic combined form of nitrogen is ammonium or nitrate while the organic state of the elemental nitrogen in nature is humus, proteins, nucleic acid etc.

- Main source of nitrogen is atmosphere.
- Nitrogen is found in geochemical deposits – rocks having salts of ammonia, nitrite, nitrates gradually get converted to soil by weathering.
- These salts present in soil are water soluble and thus form soil solution. As a result they become available to the living organisms (macro and micro).
- Organic matter of living organisms gets cycled by food chain i.e. producers – consumers while the dead part gets degraded and decomposed by the process of humification.
- Humification liberates the organic compounds and converte them into simpler forms like fertilizers, which can be added to the plants specially the crops to provide them with nitrogen as ammonia or nitrate.
- Volcanic activities also provide nitrogen to the atmosphere.

In the nitrogen cycle two important steps occur which are as follows:

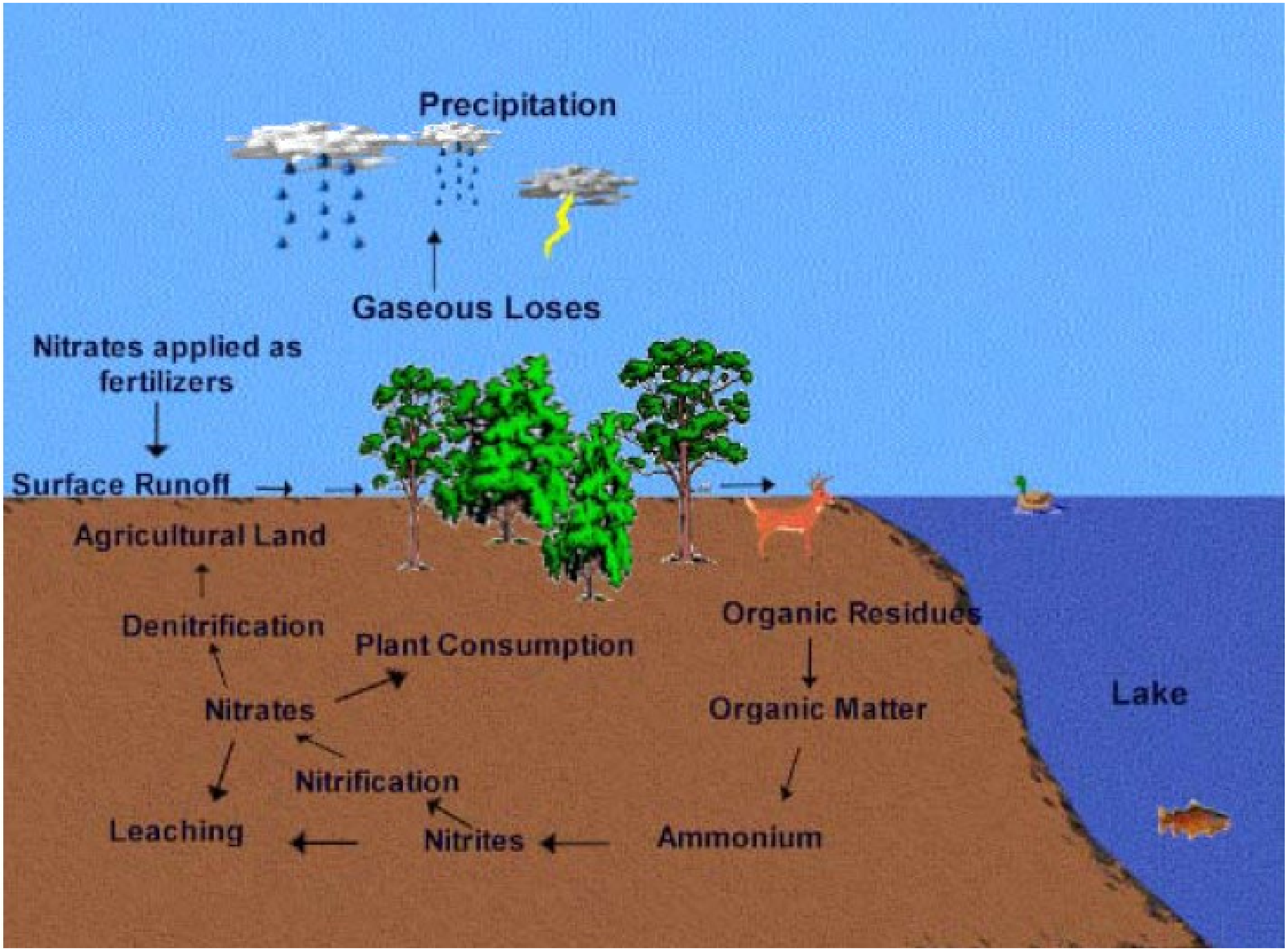
1. **Mineralisaton –**

- In this process the microbes convert unavailable organic form of nitrogen to simpler assimilable form of nitrogen.
- This involves processes of both ammonification and nitrification.

2. **Immobilisation –**

- In this process the available form of Nitrogen i.e. ammonia and nitrate are utilized by the living organisms, basically plants and microbes, who convert it into bound organic form. Thus the nitrogen gets immobilized.
- In summary the nitrogen cycle involves ammonification, nitrification and denitrification.

Nitrogen Cycle



Ammonification

- Nitrogen in the form of ammonia is utilizable by the living organisms.
- The bound organic nitrogen present in organic matter as proteins, amines, amides, urea etc are converted to ammonia by the microbial population.
- The degree of decomposition varies with the type of microbes acting upon the dead remains.
- Protein is degraded by the proteolytic enzymes which are extracellular in nature.

$$\text{Protein} \longrightarrow \text{Polypetide} \longrightarrow \text{Aminoacids}$$

- The micro-organisms helping in the operation of ammonification are:
- Among bacteria – *Pseudomonas, Bacillus, Clostridium, Serratia* etc.
- Among Fungi - *Alternaria, Aspergillus, Penicillium, Mucor* etc.
- The end products of aerobic decomposition of protein are carbon dioxide, ammonia, water, sulphates while anaerobic decomposition (putrefaction), liberates hydrogen sulphide, mercaptans etc.
- Bacteria are found to liberate more ammonia compared to fungi.

$$\text{Urea} + \text{water} \longrightarrow \text{Ammonia} + \text{Carbon dioxide}$$

- Microbes involved production of Ammonia from Urea are – *Bacillus, Proteus, Micrococcus, Sarcina etc.*

$$RCH_2NH_2 + O_2 + H_2O \longrightarrow RCHO + NH_2 + H_2O_2$$

- *Amines are acted upon* by *Pseudomonas, Protoaminobacter and the enzyme involved – aminooxidases.*

Amide $\xrightarrow{\textit{Amidase}}$ *Ammonia*

- *Amides are* converted to ammonia by the enzyme amidase.

- Ammonification is a predominant phenomenon in aerobic soils with more organic residues.
- In acidic soils the process is carried out by fungi, therefore less ammonia production.
- In soils having carbohydrate, ammonia production is low as the microbes have a preference to carbohydrates compared to nitrogenous compounds.
- Besides the microbial population, animals are also capable of metabolizing nitrogenous compounds e.g. production of uric acid.
- Nitrogenous compounds are source of carbon, nitrogen and energy for the organisms.
- Ammonia is a volatile compound and is positively charged. Hence it either gets dispersed or may bound to the negatively charged clay particles in soil.
- Thus ammonia may get readily oxidized leading to Nitrification which is the next step of the Nitrogen cycle

- The **Nitrification process is oxidation of:**

  Ammonia $\longrightarrow$ Nitrite $\longrightarrow$ Nitrate

- It is of two types:

  1. **Heterotrophic Nitrification**

  ▪ This is carried out by heterotrophic bacteria and fungi occurring usually in acidic soils e.g. *Pseudomonas, Corynebacterium, Aspergillus, Nocardia etc.*

  ▪ This process does not make significant conversion of Ammonia to Nitrite to Nitrate.

  2. **Chemoautotrophic Nitrification**

  ▪ This is a bacterial process occurring in neutral to alkaline type of soils.

  The process of nitrification completes in the following two steps -

  ✓ **Step 1**: Ammonia is oxidized to nitrite mediated by *Nitrosomonas.*

  ✓ **Step 2**: Nitrite is further oxidized to nitrate mediated by *Nitrobacter.*

- Since the above two steps are closely coupled, therefore nitrite does not accumulate.
- Both the steps are aerobic and release energy.
- The microbes carrying out the process are chemoautotrophs. Other bacterial species capable of converting ammonia to nitrite are
- *Nitrosospira, Nitrosococcus, Nitrosolobus, Nitrosovibrio* while *Nitrospira, Nitrospina, Nitrococcus converts*

$$\textit{nitrite} \longrightarrow \text{nitrate}$$

- In aquatic system nitrification is carried by *Nitrosococcus, Nitrosolobus, Nitrobacter, Nitrospira, Nitrospina, Nitrococcus.*

- Organisms with prefix Nitroso are Ammonia oxidisers. While with prefix Nitro are Nitrite oxidisers.
- Nitrosomonas and Nitrobacter are common in nature and are found to occur in close association.
- As conversion of nitrite to nitrate is a fast process, thus the level of nitrite does not rise. Hence it is beneficial for the plants since nitrite is phytotoxic.

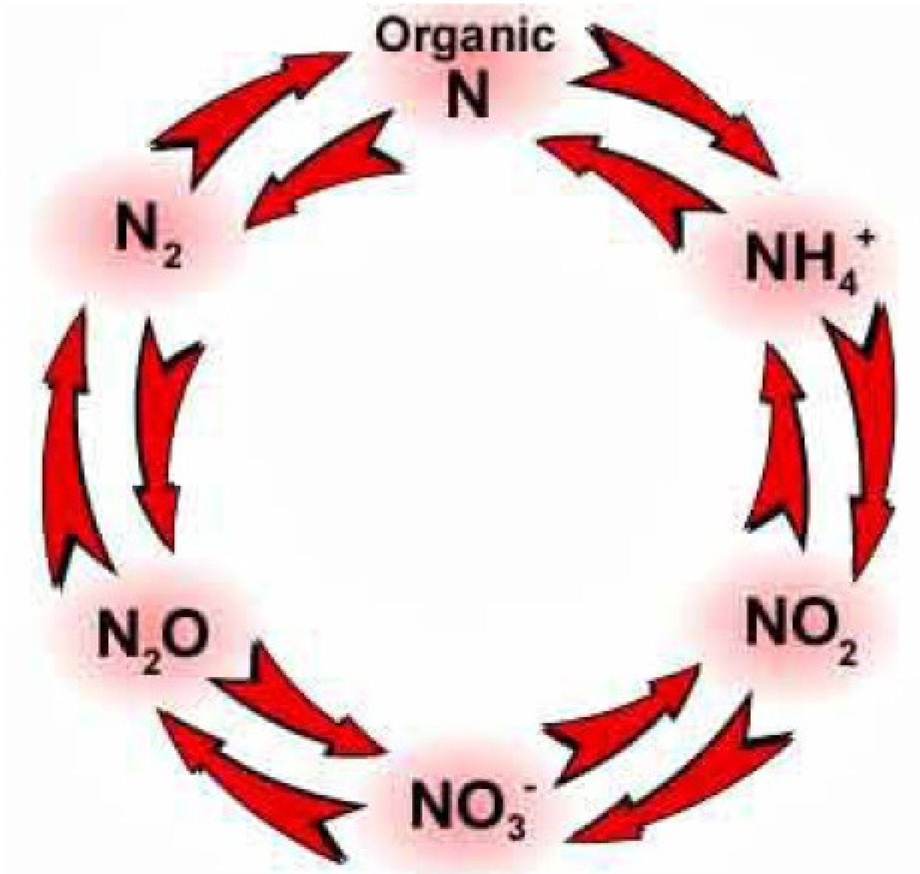


Fig: Interconversion of different nitrogenous compound present in nitrogen cycle

- The nitrification process is found to be pH sensitive.
- Oxygen is also important because the nitrifiers are obligate aerobes. Nitrification rate declines in moist soils.
- Nitrifiers are incapable of tolerating arid conditions therefore moisture is required.
- The organisms are mesophilic, so nitrate production is high at 30-35º C.
- Nitrifications is dependent on carbon : nitrogen ratio.
- It proceeds at a faster rate when organic matter has higher nitrogen content.

- Plants roots readily take up nitrate ions for organic compound assimilation.
- Since nitrite and nitrate ions are negatively charged they do not bound to the clay particles which are also negatively charged.
- As a result they at times leach down into the groundwater through soil column. Leached down nitrite and nitrate cause pollution problems.
- The level of nitrate should not exceed 10ppm in drinking water.
- The accumulation of nitrate in tissues (water, vegetables, fruits) causes infant methaemoglobinemia (blue baby syndrome) and animal methaemoglobinemia.
- Nitrite which is phytotoxic on reacting with secondary amines in environment or in foods, forms – N- nitrosamines which are carcinogenic in nature.

- **Nitrate Reduction:** In this process nitrate is reduced to nitrite and then to ammonia.
- The nitrate reduction process could be assimilatory or dissimilatory.

1. **Assimilatory :** This occurs in aerobic conditions and is carried out by heterogenous microbial population comprising of bacteria, fungi and algae.

This process is inhibited by excess of ammonia. Here oxygen is the electron acceptor.

2. **Dissimilatory :** This occurs under anaerobic conditions also known as nitrate respiration. The process is carried out by facultative anaerobic bacteria e.g. *Bacillus, Achromobacter, Flavobacterium, Nocardia, Spirillum.*

Here nitrate ion acts as electron acceptor. This is not inhibited by excess of ammonia.

The process is important in stagnant waters, water logged soils, sediments and sewage plants.

Denitrification

- It is the process whereby fixed Nitrogen i.e. nitrite and nitrate are converted back to molecular Nitrogen.
- The sequence of events occurring is this process are –

Nitratre ⟶ Nitrite ⟶ Nitric oxide ⟶ Nitrous oxide ⟶ Nitrogen (molecular)

- Thus in this process Nitrogen is released back in the atmosphere, thus becoming unavailable to the organisms.
- Bacterial denitrifiers are heterotrophs. The process is anaerobic and organisms are facultative anaerobes.
- The organisms are *Pseudomonas, Bacillus, Alcaligenes, Paracoccus, Micrococcus.*

- *Thiobacillus denitrificans* is a chemoautotrophs which reduces nitrate $\longrightarrow$ molecular Nitrogen.
- Denitrification depends on number and efficiency of the denitrifying organisms present and environmental parameters, viz, soil temperature, moisture content, oxygen status, organic matter, pH of the soil and nitrate concentration.
- Denitrification differs from dissimilatory nitrate reduction (DNR) process that Nitrate Nitrogen gas in denitrification while nitrate is reduced to ammonia in DNR process.

Nitrogen Fixation

- **Nitrogen Fixation:** This occurs biologically.
- Atmospheric Nitrogen (molecular Nitrogen) is fixed biologically non-symbiotically or symbiotically.
- This is carried out with the help of the enzyme nitrogenase. Organisms involved in this phenomenon are basically prokaryotic microbes.
- They are either bacteria or cyanobacteria or Actinomycetes.
- Symbiotically fixed nitrogen is higher than fixed by free living nitrogen fixers though they are wide spread in soil.
- In aquatic habitats, cyanobacteria play a key role in nitrogen fixation while actinomycates show symbiotic association with non-legunminous plants and bacteria with legumes.

- Microbes which fix atmospheric nitrogen in soil or aquatic habitat or those inhabiting the rhizosphere are known as diazotrophs (azo means nitrogen i.e. they are molecular nitrogen fixer).
- Microbial population present in the rhizosphere may show enhanced nitrogen fixation since organic compounds are available to them from the root exudates.
- As a result the environment around the rhizosphere becomes conducive/congenial for growth of the microbial population.
- Among the free nitrogen fixers (asymbiotic) some are aerobes (*Azotobacter) while others are anaerobic (Clotridium) or microaerophilic (Azospirillum).*
- Cyanobacteria shows both symbiotic as well as non symbiotic association.

- Some filamentous cyanobacteria (BGA-Blue green algae) possess specialized cells known as heterocyst which are site for nitrogen fixation.
- Some filamentous non heterocystous cyanobacteria are capable of fixing nitrogen e.g. *Oscillatoria, Trichodesmium, Lyngbya, Microcoleus.*
- *Certain* cyanobacteria form symbiotic associations in which they occur as endophytes e.g. *Anabaena* or *Nostoc* in coralloid roots of *Cycas.*
- Cyanobacteria also form symbiotic association with the water fern *Azolla (Anabaena- Azolla). They are also reported to be associated with the thallus of Anthoceros (Bryophytes).*
- Their association in the paddy fields of the tropics is well documented.
- The heterocystous cyanobacteria like *Anabaena, Nostoc fix nitrogen* symbiotically as well as asymbiotially in nature.

- Nitrogen fixation is also found to be associated with grasses like *Paspalam notatum.*
- Symbiotic nitrogen fixation occurs in the nodules of leguminous plants. Best known example of this association is the (bacteria) *Rhizobium legume association.*
- *Rhizobium is known to* even form stem nodules where it is called as *Azorhizobium and the plants are Sesbania rostrata, and Aeshynomene.*
- *Rhizobium is also reported to be associated with a non* leguminous plant e.g. *Trema.*

- *Actinomycetes (Frankia) – are also known to form nodules in non-leguminous plants e.g. Casuarina, Alnus etc. This is known as actinorrhizal association.*
- Lichens can also be accounted for nitrogen fixation.
- Here nitrogen is fixed by the algal partner called as phycobiont.
- Mycorrhiza also help in nitrogen fixation as their spores are reported to harbour nonsymbiotic nitrogen fixing bacteria.
- Nitrogen fixers are reported from the phylloplane (leaf surface) also apart from being associated in the rhizosphere (root region).
- Some bacteria are reported to form leaf nodules which are capable of nitrogen fixation in the members belonging to family Rubiaceae (*Psychotria) and Myrsianaceae (Ardisia).*
- *These nodules are* reported to harbour the bacteria *Klebsiella.*

- The environment factors affecting biological nitrogen fixation (BNF) are temperature, water stress, water logging, salinity, pH, availability of nutrients like P, K, Mo, and Fe.
- Nitrogen fixation also depends on the organic content of the soil.

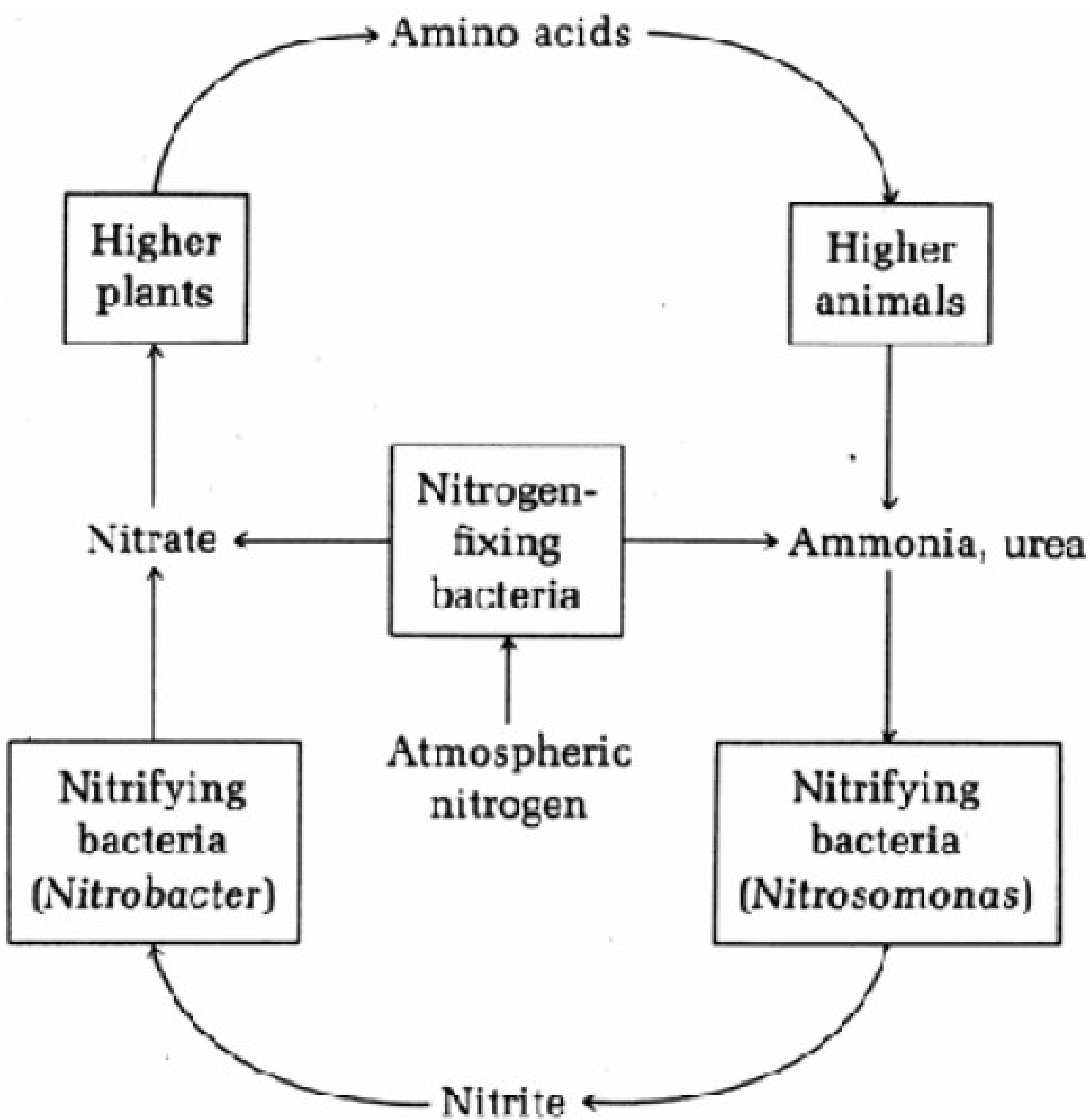


Fig: Represents biological nitrogen fixation and the organisms involved in the process