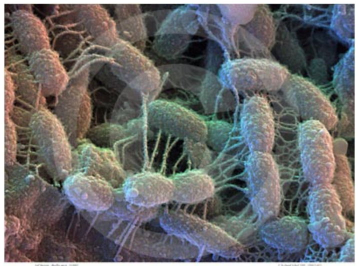
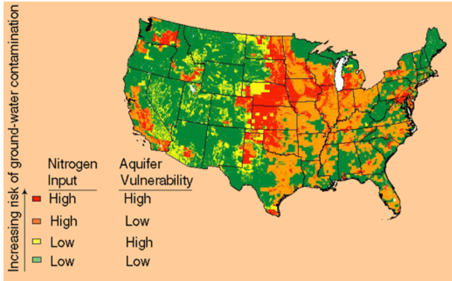
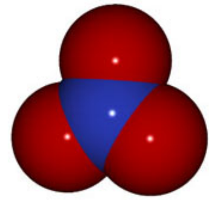
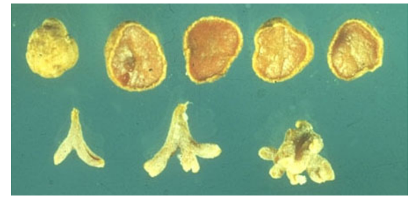
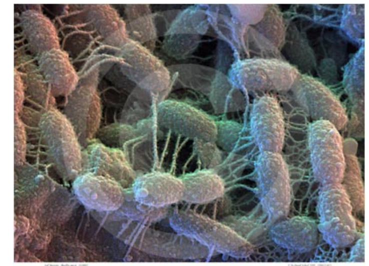


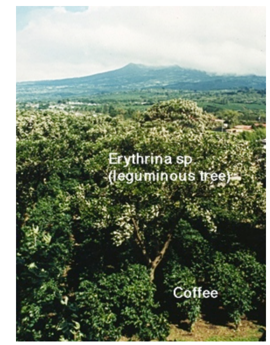


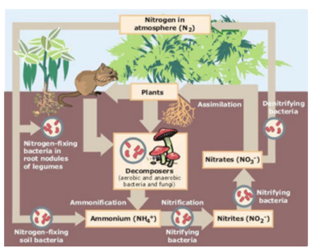




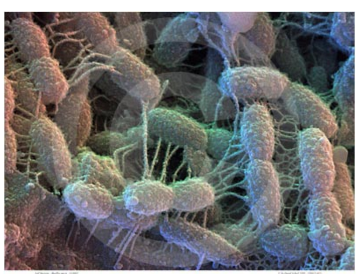


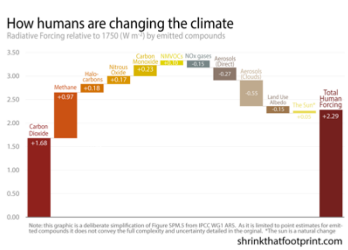
Microbial perspectives on the Nitrogen Cycle

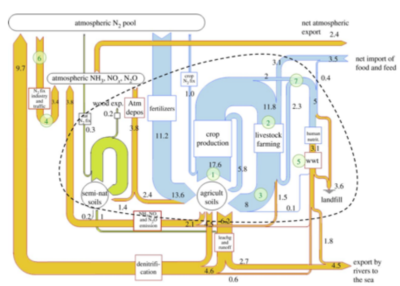


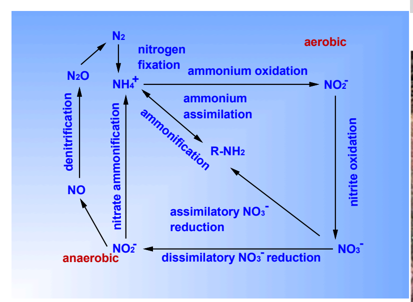


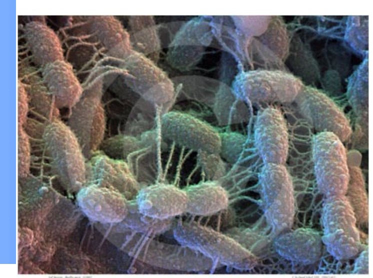
Much of material courtesy of **Raina Maier**, SWES



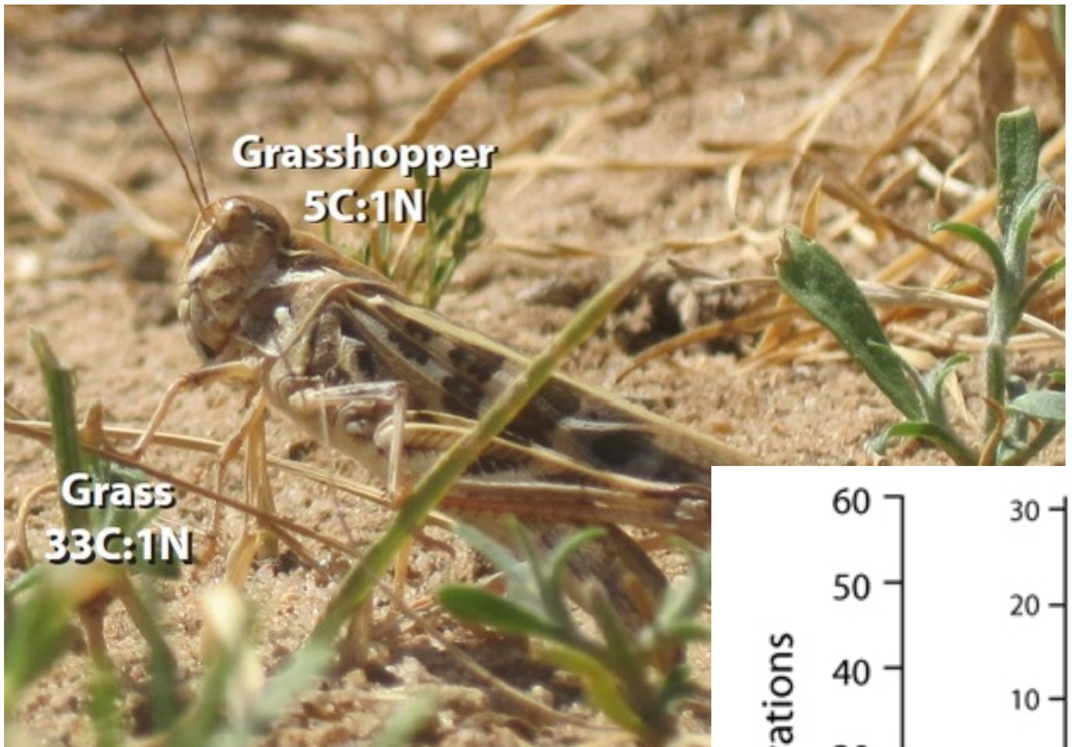


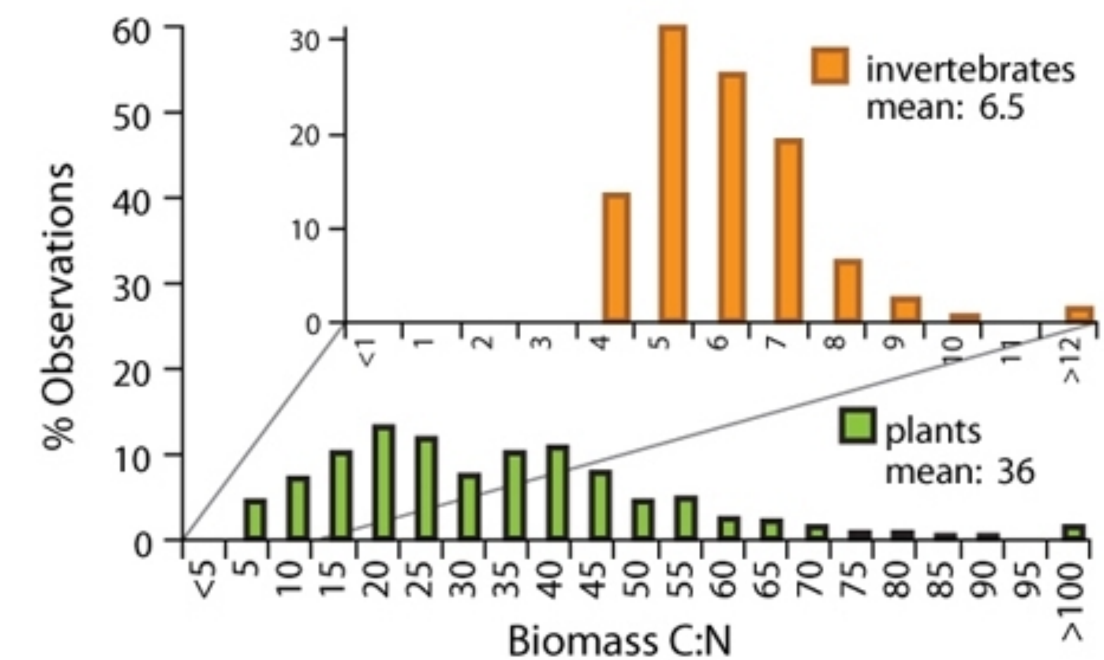






Why do organisms need N?
How much do they need?



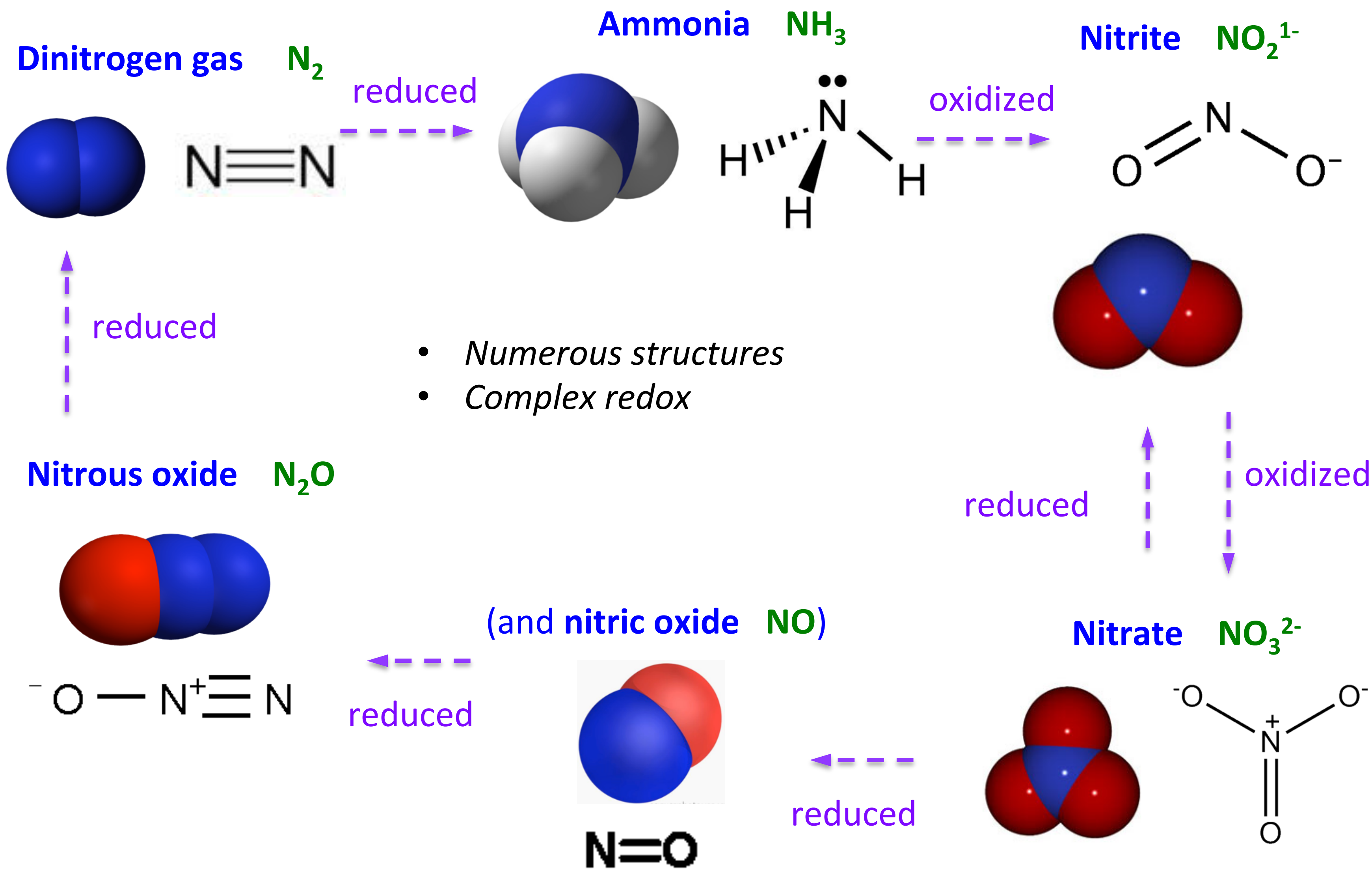


Key problem: 80%+ of global N is in atmopshere as N2

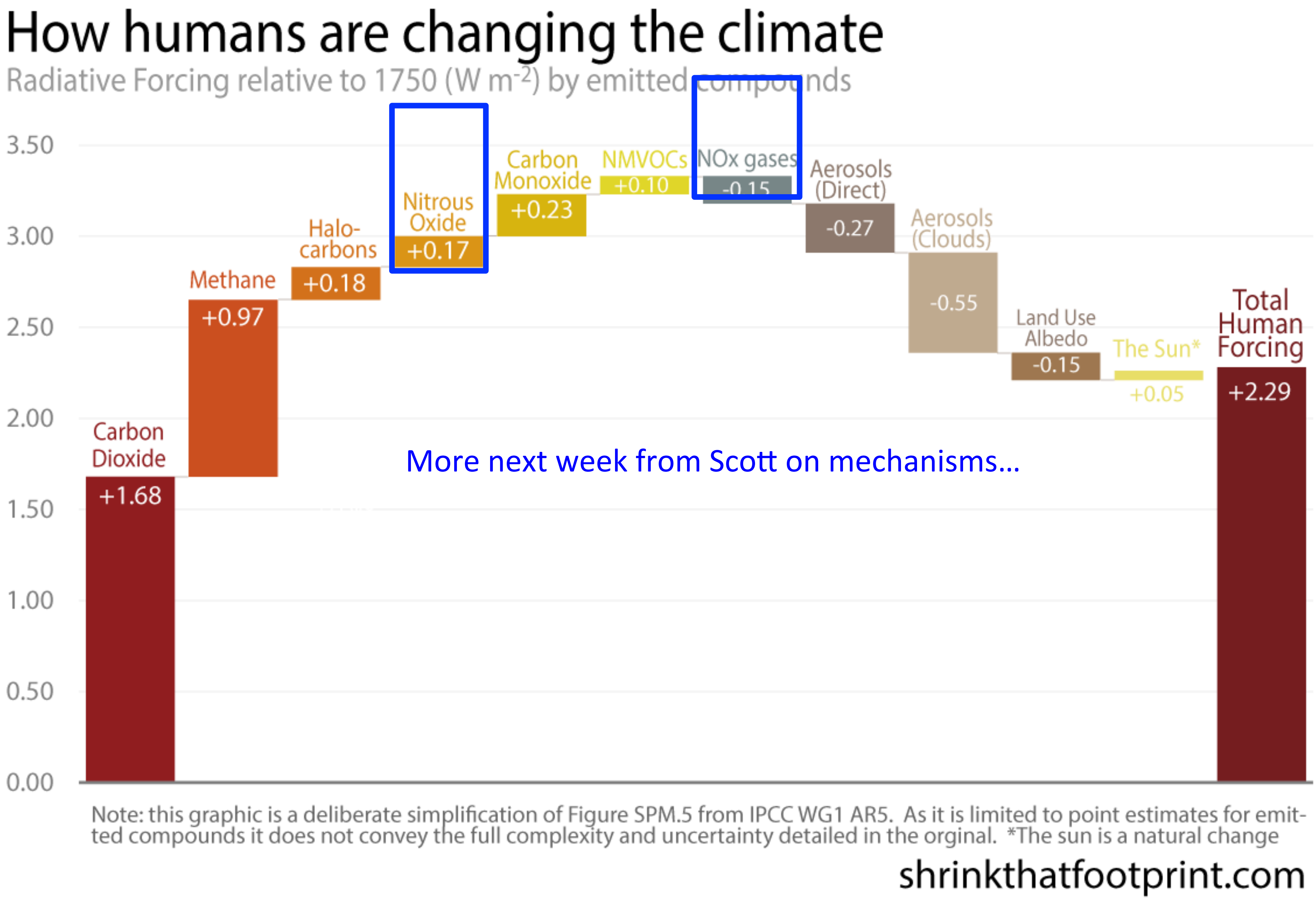
Only a few microbes can use N_2 as a cellular nitrogen source

Nitrogen Reservoir	Metric tons nitrogen	Actively cycled
Atmosphere		
N_2	3.9×10^{15}	No
Ocean		
Biomass	5.2×10^{8}	Yes
Soluble salts (NO_3, NO_2^-, NH_4^+)	6.9×10^{11}	Yes
Dissolved and particulate organics	3.0×10^{11}	Yes
Dissolved N_2	2.0×10^{13}	No
Land		
Biota	2.5×10^{10}	Yes
Organic matter	1.1×10^{11}	Slow
Earth's crust	7.7×10^{14}	No

The key inorganic molecules in the N cycle are:



Some of these molecules impact climate:



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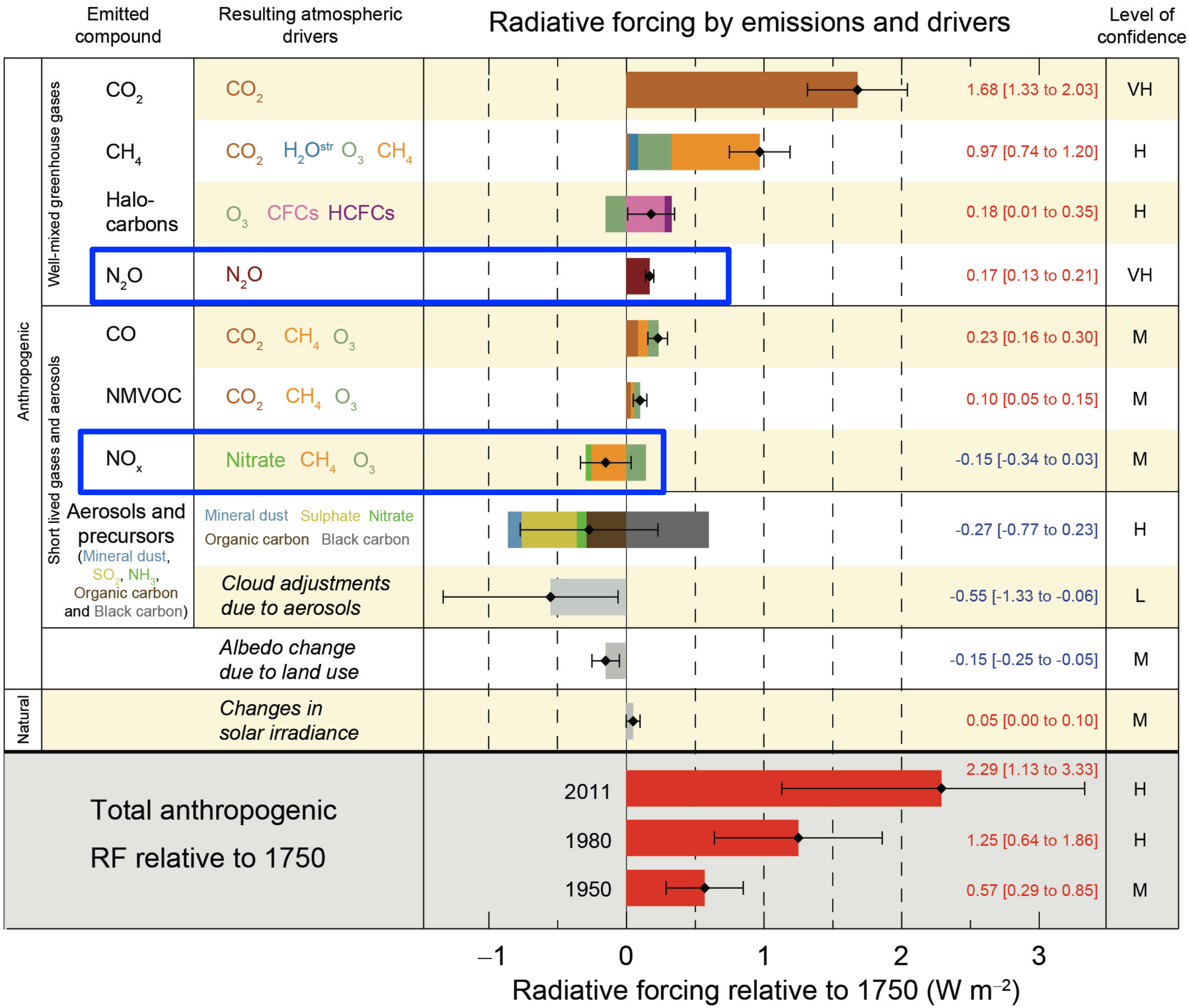


http://www.climatechange2013.org/images/figures/WGI_AR5_FigSPM-5.jpg

Ammonia is produced by:

1. *nitrogen fixation*
2. *ammonification*

Ammonia can be removed by:

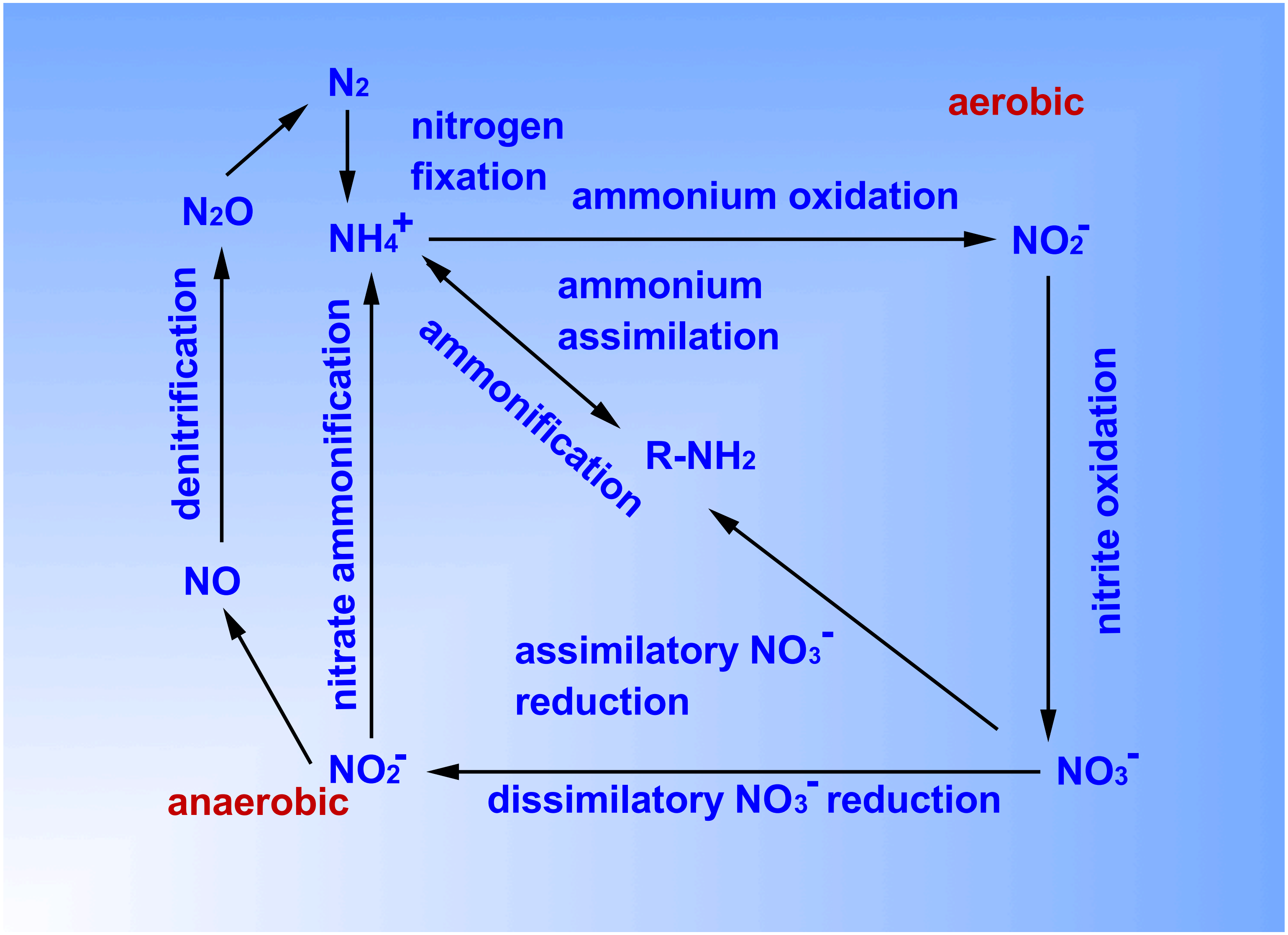
1. *assimilation* into organic matter
2. aerobic oxidation to nitrate through *nitrification*
3. anaerobic oxidation (and combination with nitrite) back to N_2 through *an*aerobic *amm*onia *ox*idation, aka *anammox*

Nitrate can be removed by:

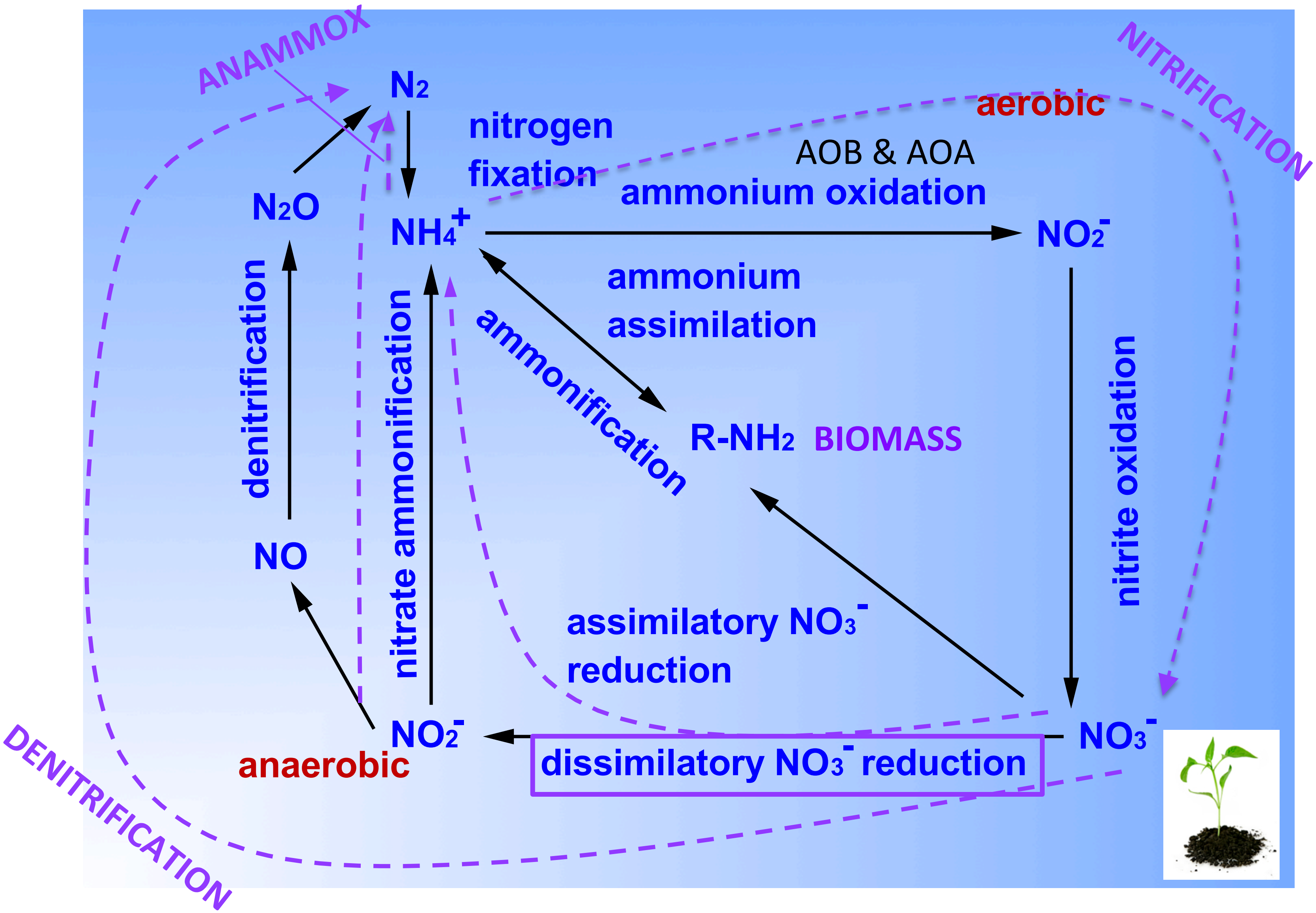
1. *assimilation* into organic matter
2. reduction to gaseous nitrogen products (NO, N_2O, N_2) through *denitrification*
3. reduction to NH_3 via *dissimilatory nitrate reduction to ammonia*

- Denitrification is the primary mechanism by which N_2 is produced biologically
- Denitrification and anammox result in losses of nitrogen from the biosphere

The Nitrogen Cycle



The Nitrogen Cycle



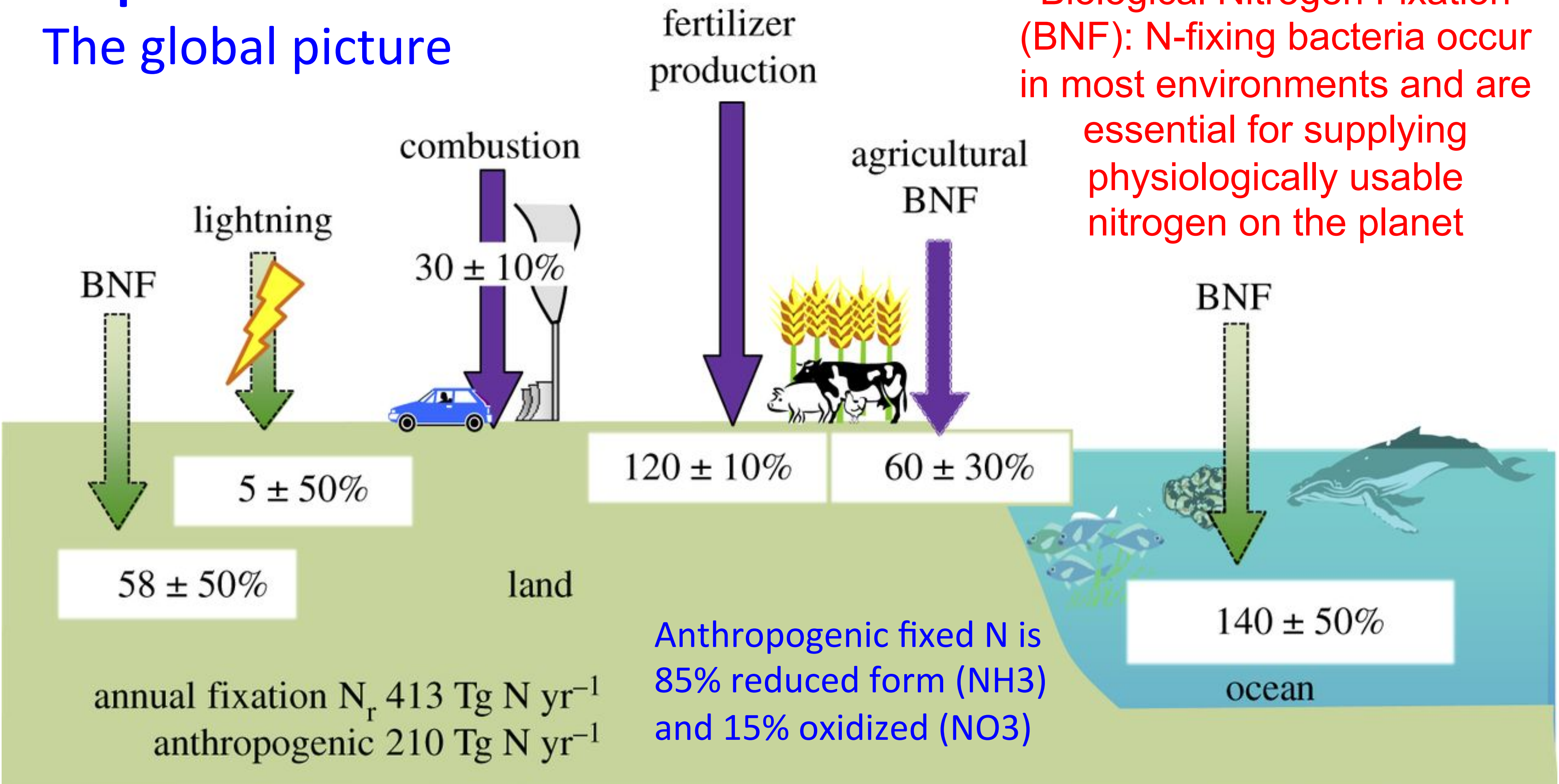
Key Processes and Prokaryotes in the Nitrogen Cycle	
Processes	**Example organisms**
Nitrification ($NH_4^+ \rightarrow NO_3^-$)	
$NH_4^+ \rightarrow NO_2^-$	*Nitrosomonas*
$NO_2^- \rightarrow NO_3^-$	*Nitrobacter*
Denitrification ($NO_3^- \rightarrow N_2$)	*Bacillus, Paracoccus, Pseudomonas*
N_2 Fixation ($N_2 + 8H \rightarrow NH_3 + H_2$)	
Free-living	
Aerobic	*Azotobacter* Cyanobacteria
Anaerobic	*Clostridium*, purple and green bacteria
Symbiotic	*Rhizobium* *Bradyrhizobium* *Frankia*
Ammonification (organic-N $\rightarrow NH_4^+$)	Many organisms can do this
Anammox ($NO_2^- + NH_3 \rightarrow 2N_2$)	*Brocadia*

Brock 2008

Step 1: N Fixation

The global picture

Biological Nitrogen Fixation (BNF): N-fixing bacteria occur in most environments and are essential for supplying physiologically usable nitrogen on the planet



Anthropogenic fixed N is 85% reduced form (NH3) and 15% oxidized (NO3)

Figure 1. Fowler et al 2013 PhilTransB
Global nitrogen fixation, natural and anthropogenic in both oxidized and reduced forms through combustion, biological fixation, lightning and fertilizer and industrial production through the Haber–Bosch process for 2010. The arrows indicate a transfer from the atmospheric N_2 reservoir to terrestrial and marine ecosystems, regardless of the subsequent fate of the N_r. Green arrows represent natural sources, purple arrows represent anthropogenic sources.

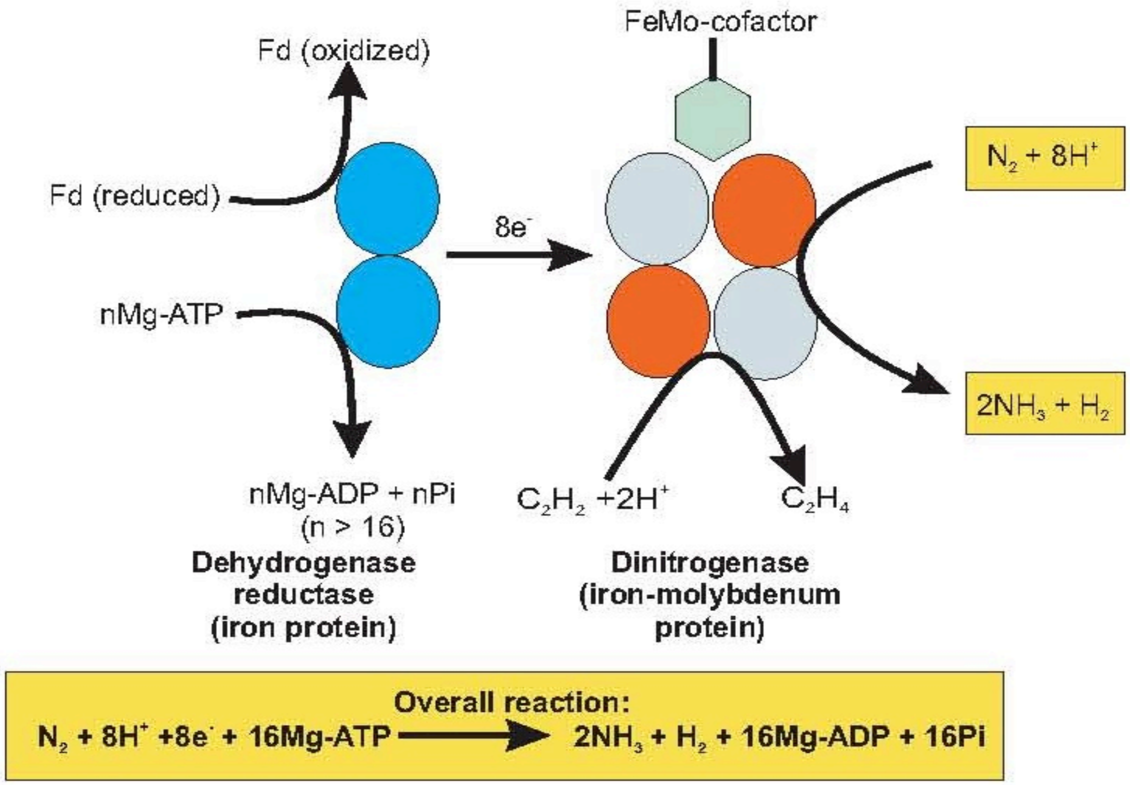
Fowler et al 2013: "There remain important limitations in understanding, including why, with such a widespread capability in ecosystems to fix atmospheric N2, organisms do not fix more N, when the benefits would provide advantages over competitor organisms that lack a nitrogen-fixing capability.

For many ecosystems, the availability of N in soils clearly down-regulates BNF, so perhaps the widespread application of Nr on farmland and deposition to semi-natural land has decreased non-agricultural BNF"

~83% of fertilizer N applied to crops and feedstocks is lost into the soils, water or atmosphere rather than ultimately consumed by humans…

N-fixation reaction is $N_2 + 3H_2 \rightarrow 2(NH_3)$

Reduction reaction done by enzyme NITROGENASE, 2-protein complex



- Nitrogenase is rapidly and irreversibly inactivated by oxygen, requiring various protective mechanisms eg. leghemoglobin

- In actively N_2-fixing microbes, 10-40% of protein is nitrogenase

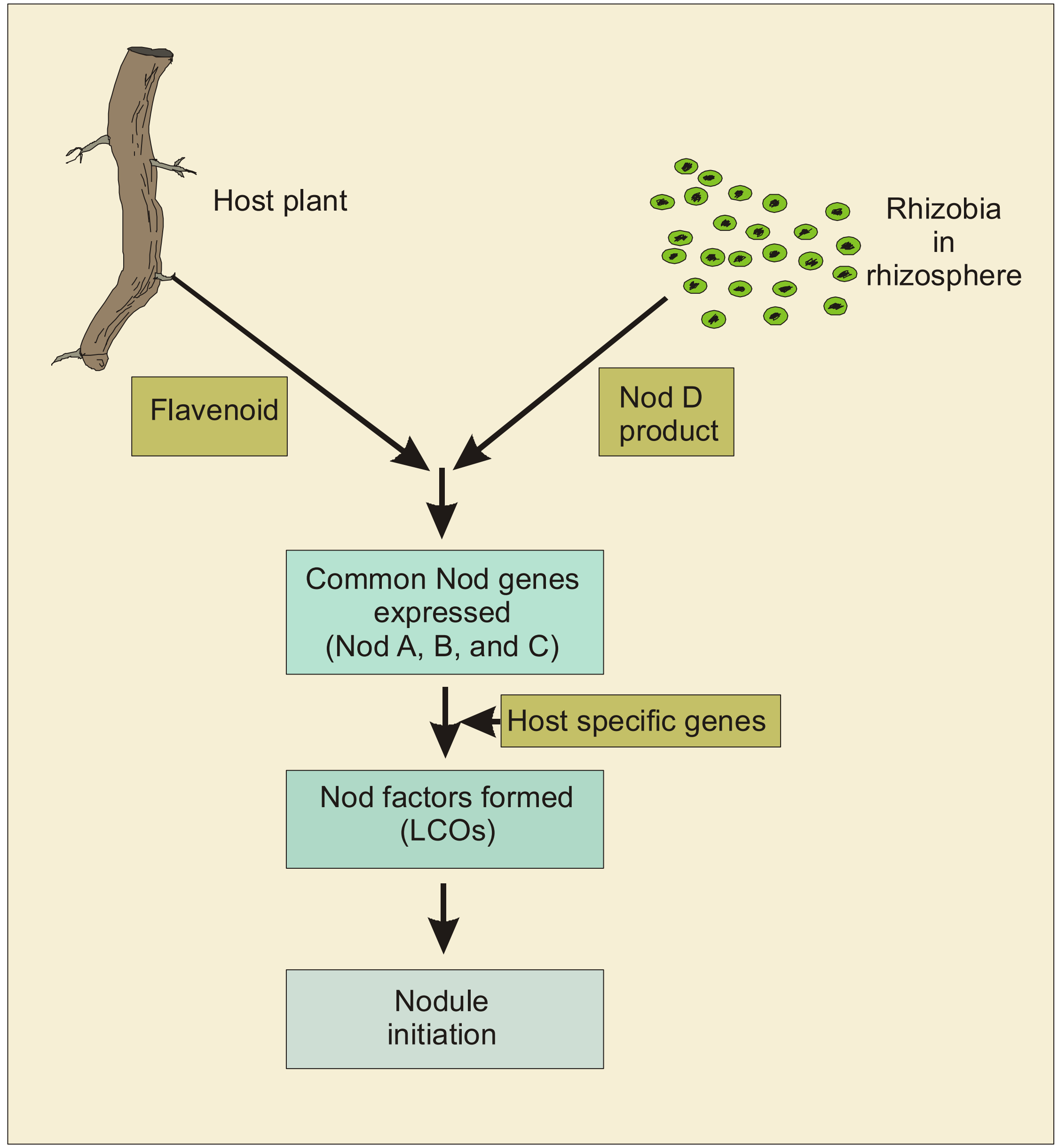
- Energy intensive; fueled by energy from fermentation, respiration or photosynthesis to provide reducing power and ATP

- Nitrogen fixation is carried out by free-living and symbiotic bacteria

- Rates of fixation depend on lifestyle (symbiotic or solo)

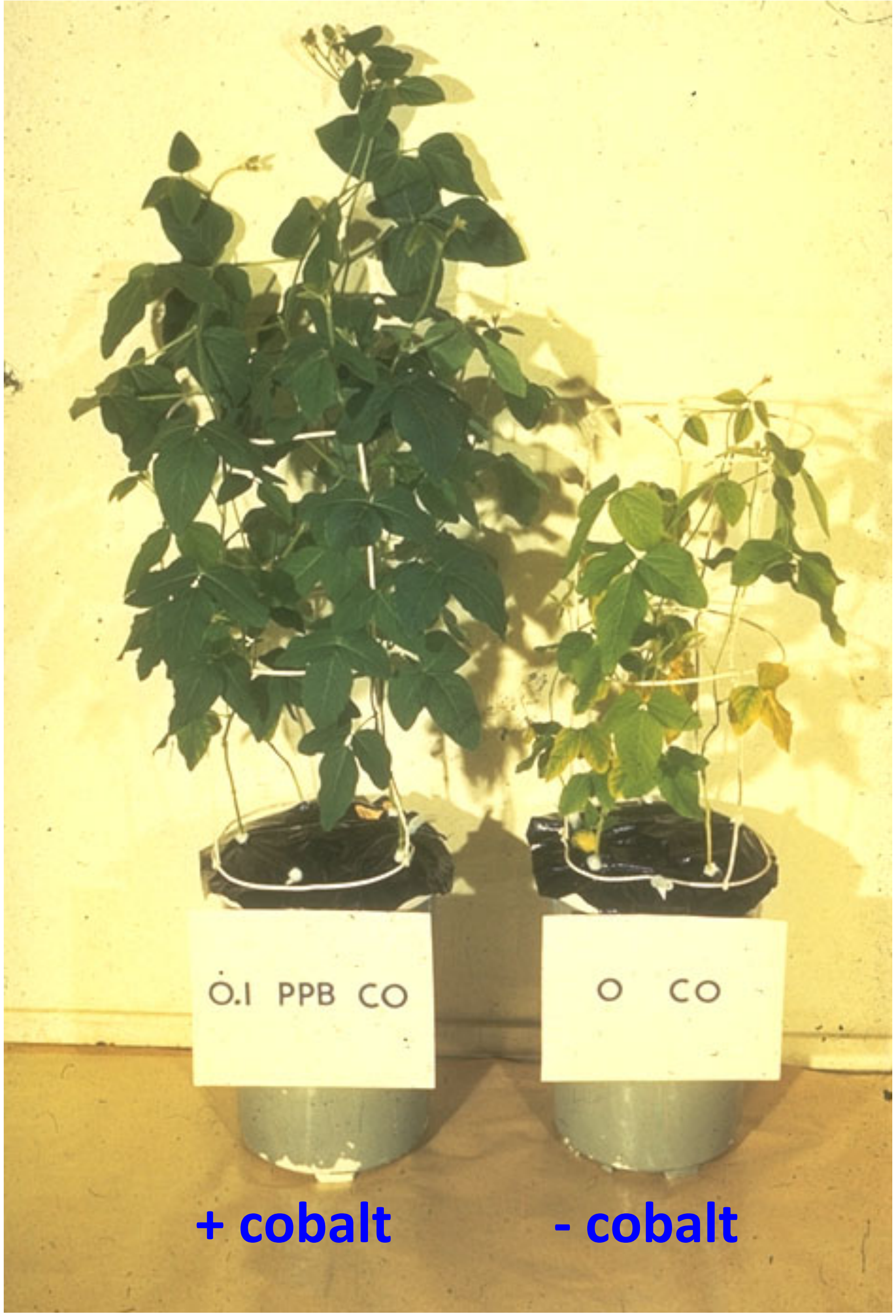
Rhizobia and legume crops' "symbiotic association"

There are specific recognition signals produced by both the plant (flavenoids) and the microbe (Nod factors). Once recognition occurs, *Rhizobium* infects the plant root. The microbe changes from its normal rod shape into large round bacteroid cells. that rapidly multiply forming nodules on the root that look like galls or tumors.



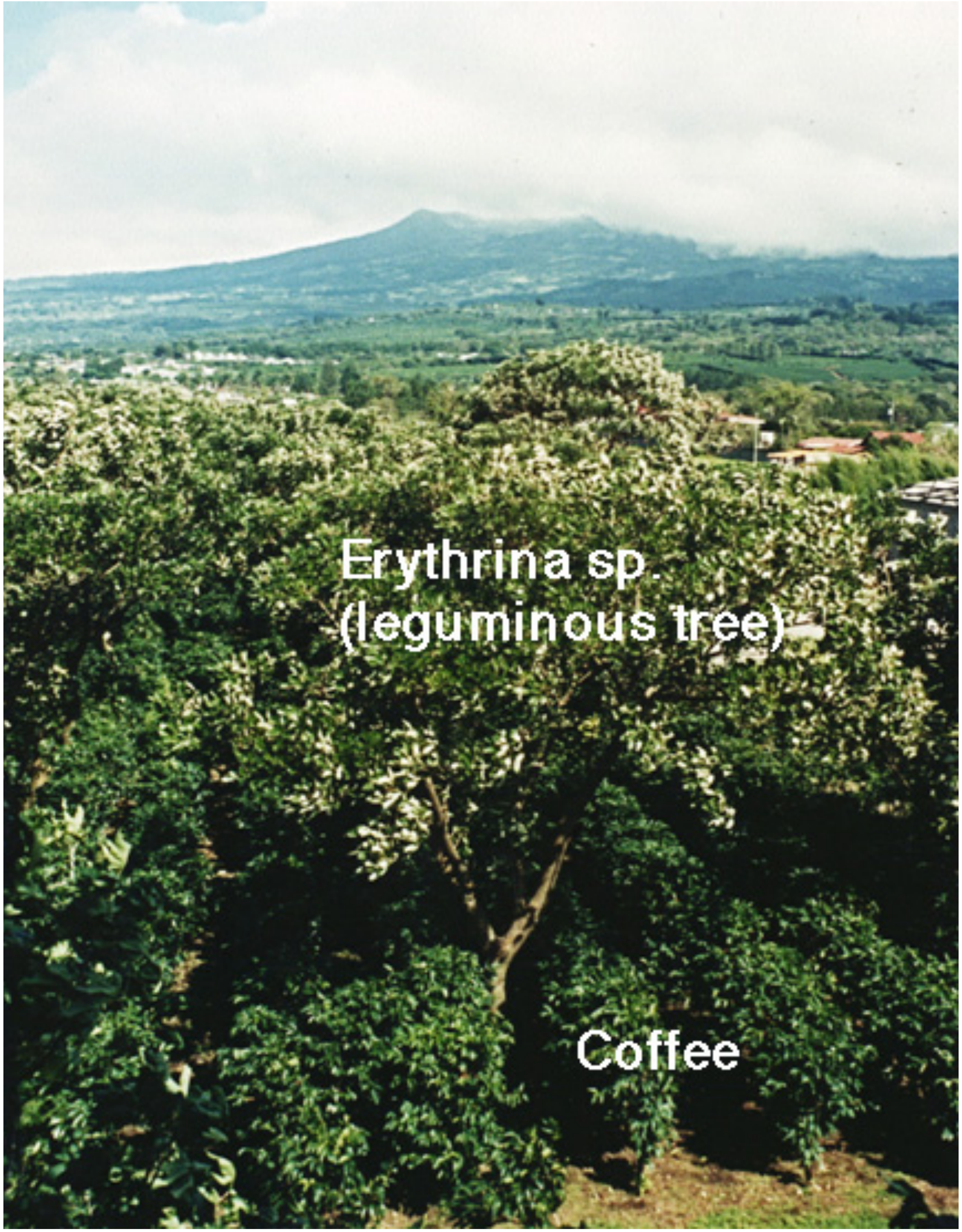
A few legumes (such as *Sesbania rostrata*) have stem nodules as well as root nodules. Stem nodules are capable of photosynthesis as well as nitrogen fixation.

Soybean grown in the presence (left) or absence of cobalt, an element essential for N fixation due to requirements of the microbial symbiont (*Bradyrhizobium japonicum* in this case).



Other nitrogen fixers:
There are 21 genera of non-legumes that fix N. These plants are important contributors of N in ecosystems where fixed N is scarce. This figure shows young plants of red alder (*Alnus rubra*). Alder is a N-fixing plant that forms a symbiotic association with an actinomycete of the genus *Frankia*. The plants on the left were inoculated with *Frankia*. The plants on the right were not inoculated and are displaying signs of extreme N deficiency.

The aquatic fern *Azolla* is the only fern that can fix nitrogen. It does so through a symbiotic association with a cyanobacterium (*Anabaena azollae*). *Azolla* is found worldwide and is sometimes used as a source of nitrogen for agriculture. The plants shown here are each about 2 cm across. The pale yellow plant has been deprived of cobalt (essential for the cyanobacterial symbiont) and thus is showing typical signs of N deficiency.



Interplanting of *Erythrina*, a leguminous tree, in a coffee plantation in the central highlands of Costa Rica. The tree provides shade as well as beneficial N for the coffee crop.

Douglas-fir foliage from an alder interplanting zone (right) compared to foliage from an area immediately outside the interplanting zone

Examples of free-living N-fixers:

Azotobacter	- aerobic
Beijerinckia	- aerobic, likes acidic soils
Azospirillum	- facultative
Clostridia	- anaerobic

Free-living bacteria must also protect nitrogenase from O_2

complex is membrane associated

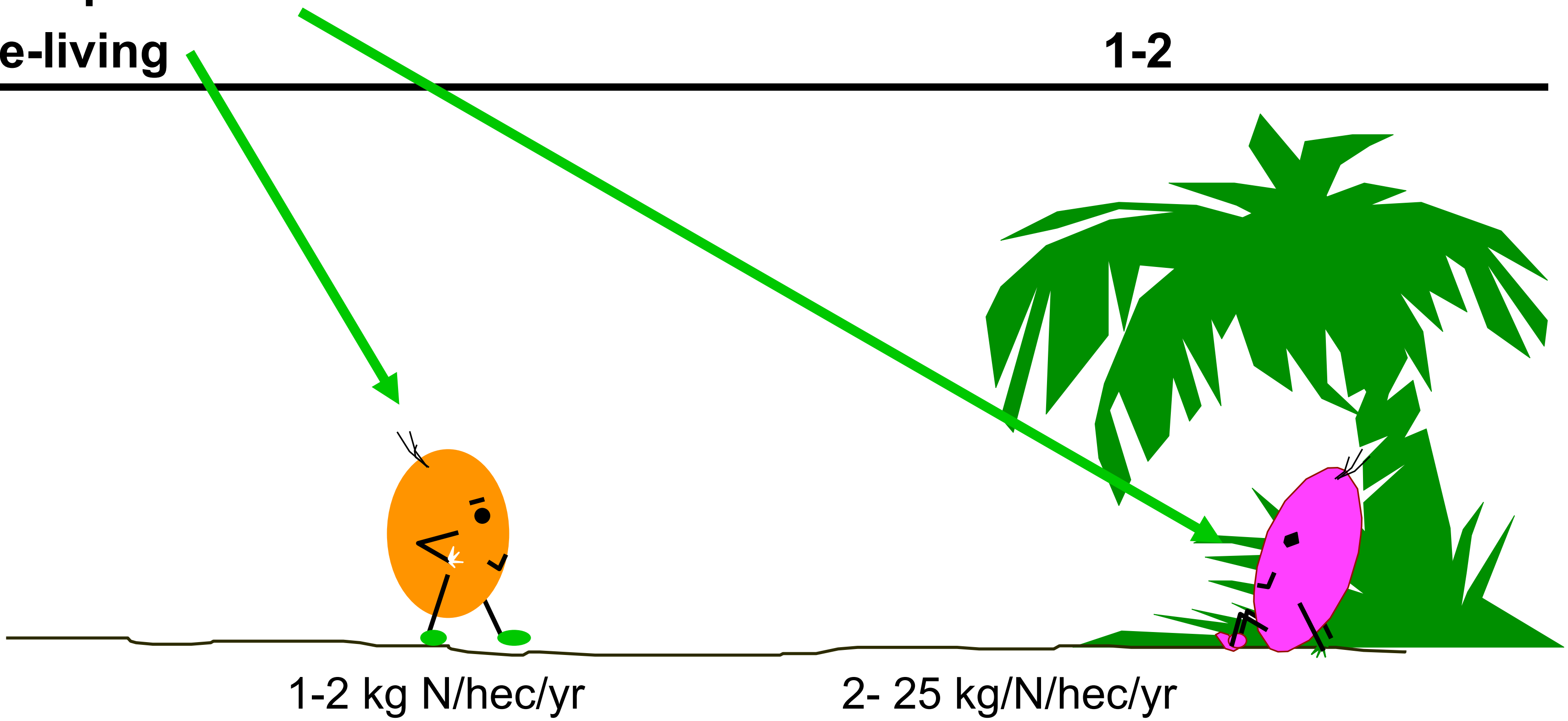
slime production

high levels of respiration

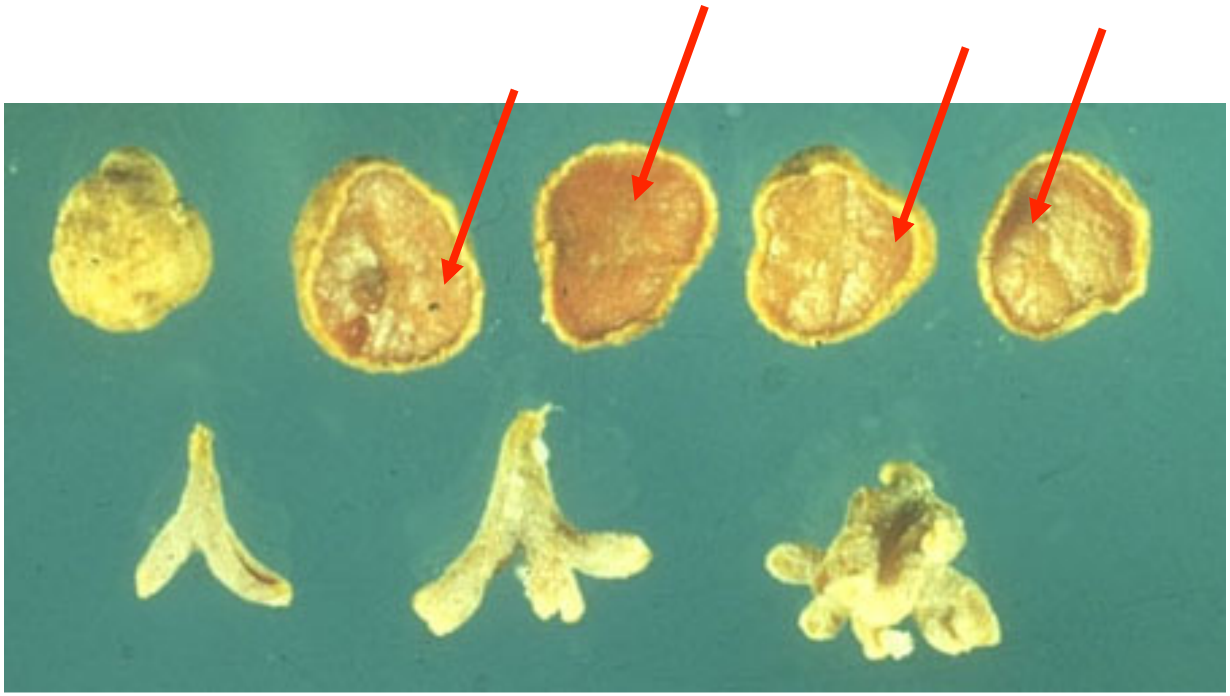
conformation change in nitrogenase when O_2 is present

Rates of Nitrogen Fixation

N_2 fixing system	Nitrogen fixation (kg N/hectare/yr)
Rhizobium-legume	200-300
Anabaena-Azolla	100-120
Cyanobacteria-moss	30-40
Rhizosphere assoc.	2-25
Free-living	1-2



Protecting nitrogenase from O2….



Soybean (top) and alfalfa nodules. The soybean nodules on the right have been hand-sectioned to show the typical pink appearance of the nodule interior which is due to leghemoglobin, an important oxygen-binding protein present in nodules.

Crude extract from soybean nodules showing the red appearance due to leghemoglobin. Nodules were ground in buffer, filtered through cheesecloth, and centrifuged to remove cell debris. The sample shown is the supernatant without any further purification. Leghemoglobin is about 50% of the total soluble protein.

Summary for nitrogen fixation:

- energy intensive
- end-product is ammonia
- inhibited by ammonia
- occurs in aerobic and anaerobic environments
- nitrogenase is O_2 sensitive

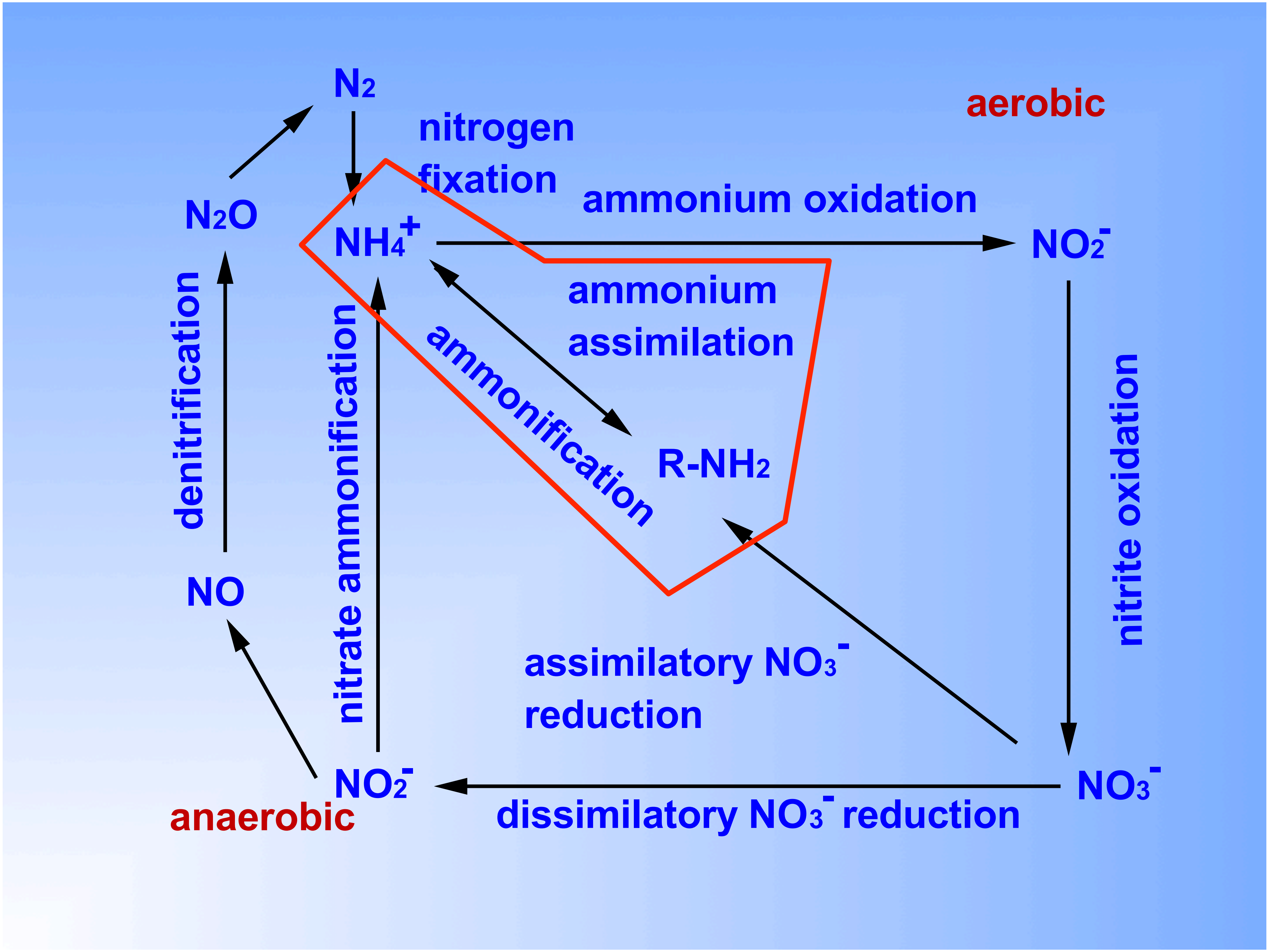
Fate of ammonium (NH_4^+) produced during nitrogen fixation

- plant uptake
- microbial uptake

} biological uptake

- adsorption to colloids (adds to CEC)
- fixation within clay minerals
- incorporation into humus
- volatilization
- nitrification

Side note: In the enviornment there is an equilibrium between ammonia and ammonium that is driven by pH. Ammonium formation is favored at acid or near-neutral pH. Thus, it is generally ammonium that is assimilated by cells into biomass.


N2
aerobic
nitrogen
fixation
ammonium oxidation
N2O
NH4+
NO2-
ammonium
assimilation
denitrification
nitrate ammonification
ammonification
R-NH2
nitrite oxidation
NO
assimilatory NO3-
reduction
NO2-
NO3-
anaerobic
dissimilatory NO3- reduction

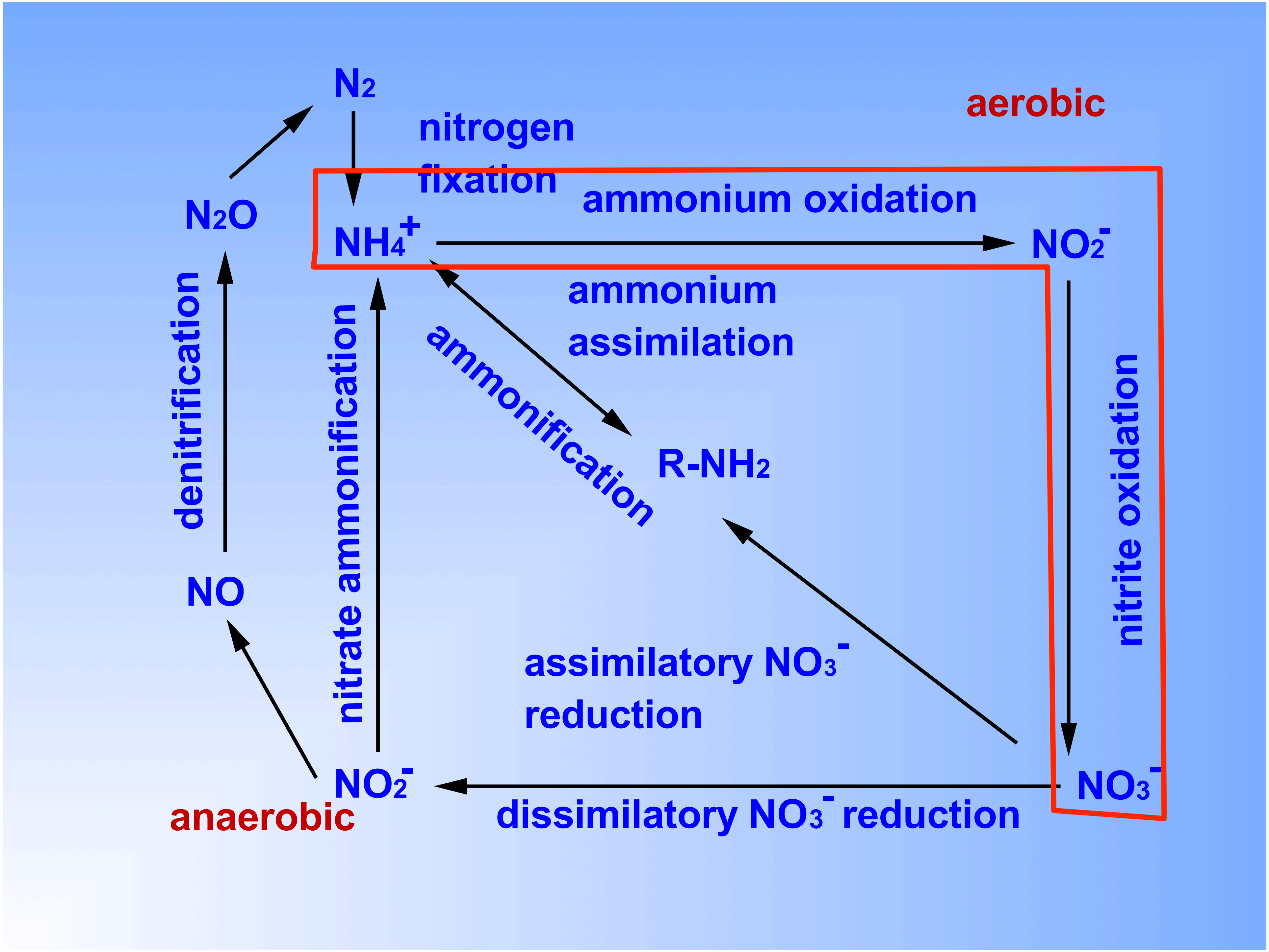
Ammonium assimilation and ammonification

NH_4^+ is assimilated by cells into:

- proteins
- cell wall constituents
- nucleic acids

Release of assimilated NH_4^+ is called ammonification aka **mineralization**. This process can occur intracellularly or extracellularly

- proteases
- chitinases
- nucleases
- ureases



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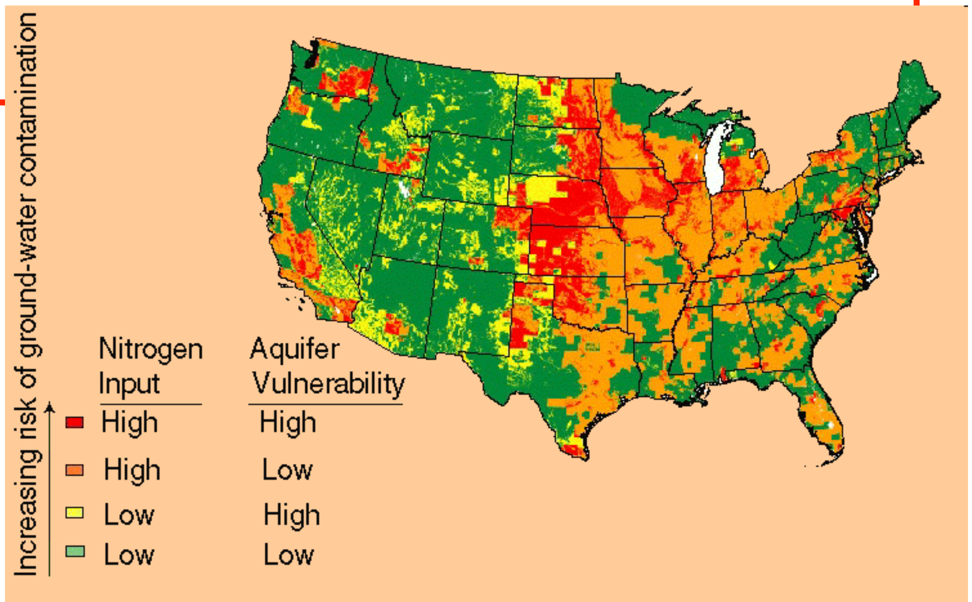
assimilatory NO3-
reduction
NO2-
NO3-
anaerobic
dissimilatory NO3- reduction

Nitrification

Chemoautotrophic aerobic process

NH_4^+ —*Nitrosomonas*→ NO_2^- —*Nitrobacter*→ NO_3^-

Nitrification is important in areas that are high in ammonia (septic tanks, landfills, feedlots, dairy operations, overfertilization of crops). The nitrate formed is highly mobile (does not sorb to soil). As a result, nitrate contamination of groundwater is common. Nitrate contamination can result in methemoglobenemia (blue baby syndrome) and it has been suggested (not proven) that high nitrate consumption may be linked to stomach cancer.

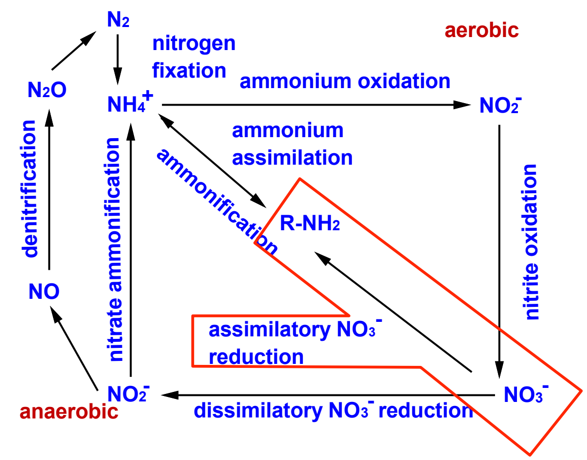


- Nitrification is an chemoautotrophic, aerobic process
- Nitrification is sensitive to a variety of chemical inhibitors and is inhibited at low pH. (There are a variety of nitrification inhibitors on the market)
- Nitrification in managed systems can result in nitrate leaching and groundwater contamination

What is the fate of NO_3^- following nitrification?

- plant uptake
- microbial uptake

} biological uptake (**assimilatory nitrate reduction**)

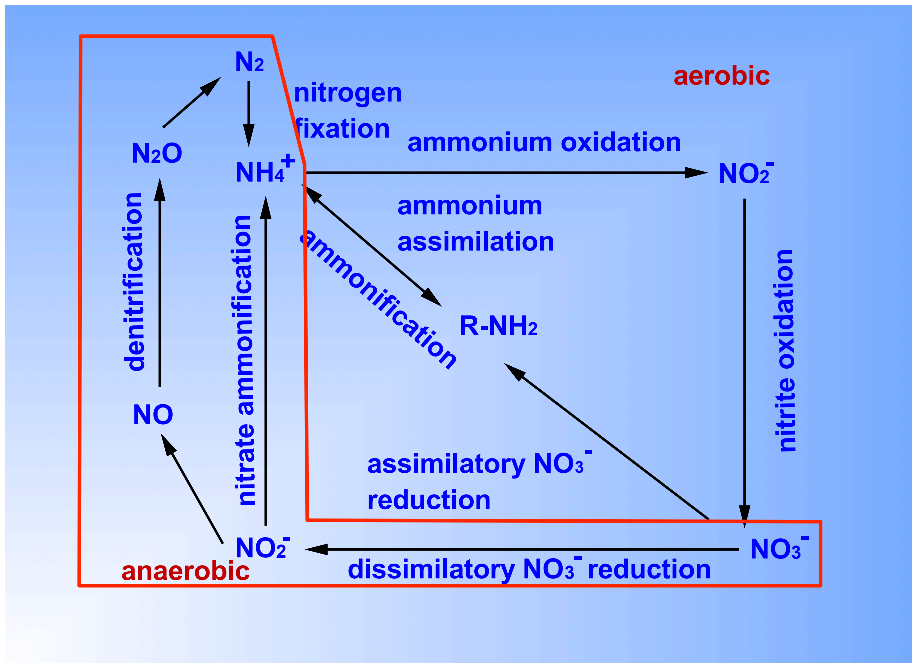


- many plants prefer nitrate which is reduced in the plant to NH3 prior to use. However, nitrogen in fertilizer is usually added as ammonia or urea.
- microorganisms prefer ammonia since uptake of nitrate requires a reduction step
- assimilatory nitrate reduction is inhibited by ammonium
- Oxygen does not inhibit this process
- nitrate is more mobile than ammonium leading to leaching loss

What is the fate of NO_3^- following nitrification?

- accumulation (disturbed vs. managed)
- fixation within clay minerals
- leaching (groundwater contamination)

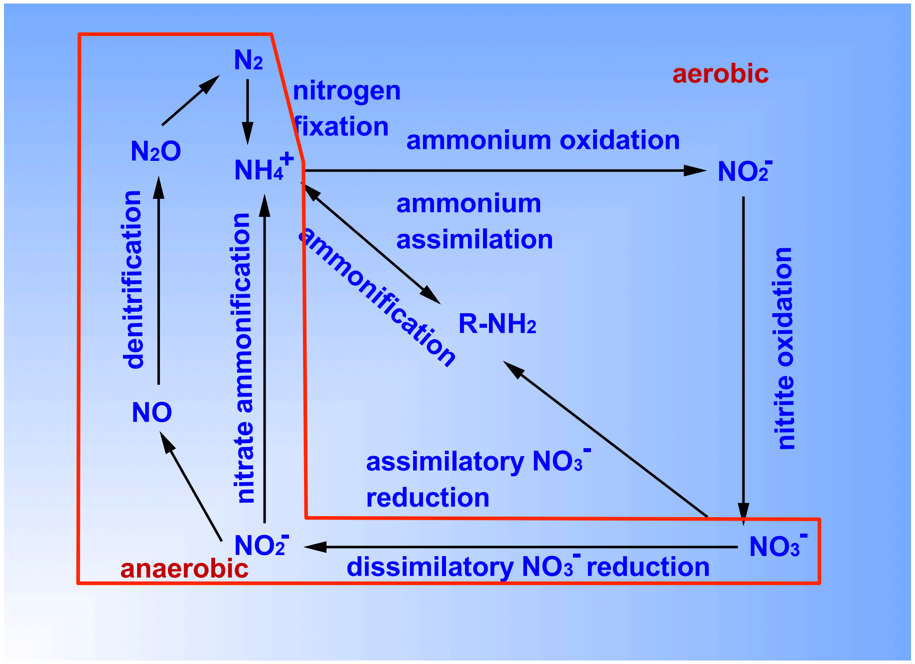
- dissimilatory nitrate reduction
  - nitrate ammonification
  - denitrification



Dissimilatory nitrate reduction

dissimilatory reduction of nitrate to ammonia (DNRA)

- Anaerobic respiration using nitrate as a TEA – less energy produced
- inhibited by oxygen
- not inhibited by ammonium
- Fermentative bacteria predominate
- found in a limited number of carbon rich, TEA-poor environments



stagnant water
sewage plants
some sediments

denitrification

- Anaerobic respiration using nitrate as a TEA – more energy produced
- inhibited by oxygen
- not inhibited by ammonium
- many heterotrophic bacteria are denitrifiers
- produces a mix of N_2 and N_2O

Denitrification

- returns fixed N to atmosphere: $NO_3 \rightarrow NO \rightarrow N_2O \rightarrow N_2$

- get formation of NO, N_2O

NO, N_2O deplete the ozone layer

Reaction of N_2O with ozone

O_2 + UV light $\rightarrow$ O + O

O + O_2 $\rightarrow$ O_3 (ozone generation)

N_2O + UV light $\rightarrow$ N_2 + O^*

N_2O + O^* $\rightarrow$ 2NO (nitric oxide)

NO + O_3 $\rightarrow$ NO_2 + O_2 (ozone depletion)

NO_2 + O^* $\rightarrow$ NO + O_2

Denitrification requires a set of 4 enzymes:

$NO_3 \rightarrow NO_2$ (nitrate reductase)

$NO_2 \rightarrow NO$ (nitrite reductase)

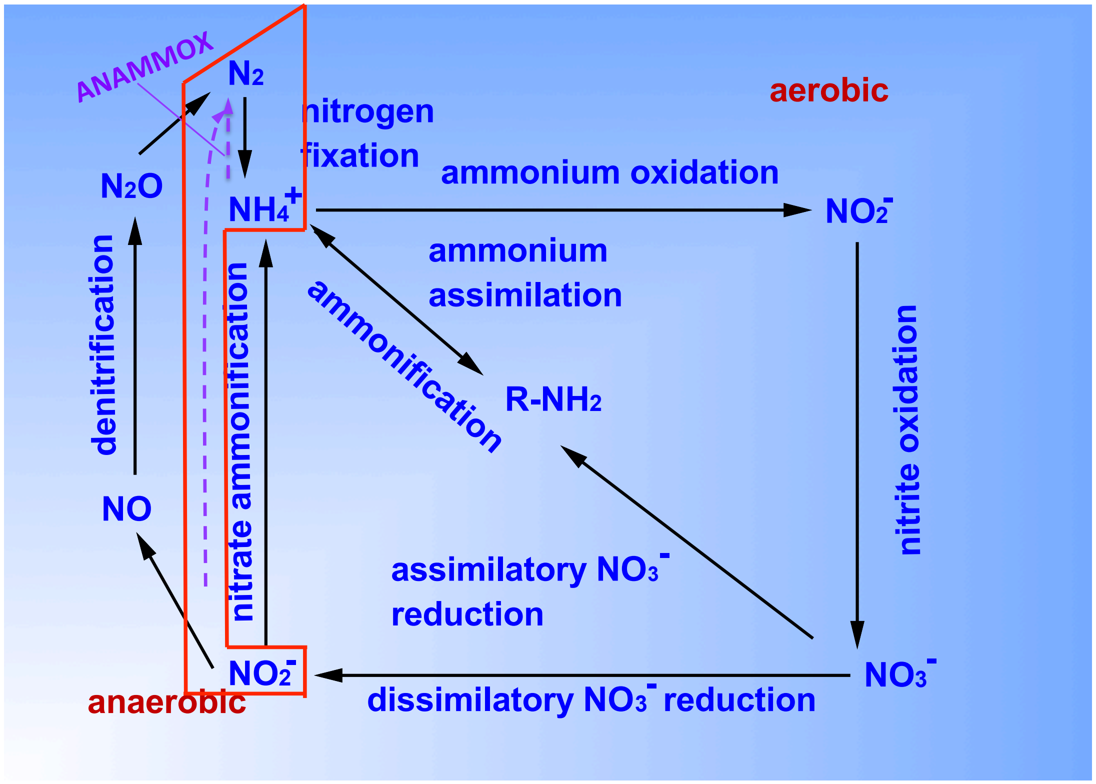
$NO \rightarrow N_2O$ (nitric oxide reductase)

$N_2O \rightarrow N_2$ (nitrous oxide reductase)

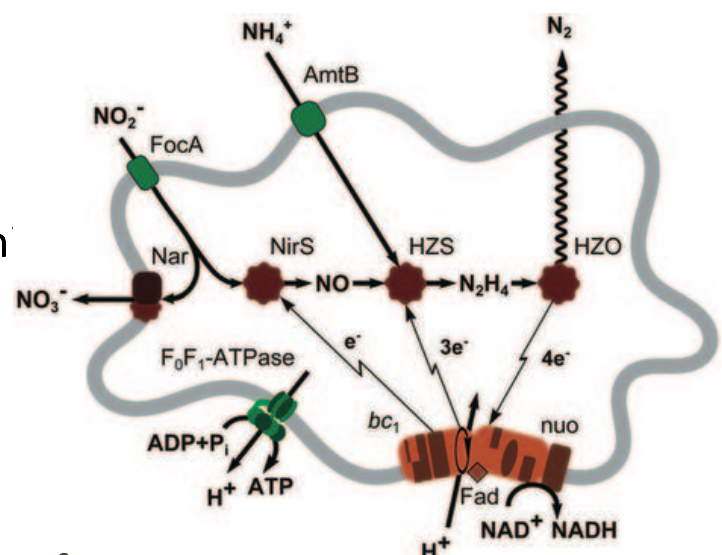
High [NO_3] favors N_2 production

Low [NO_3] favors N_2O production

Anammox $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2Ox$



- may be responsible for 30-50% of the dinitrogen gas produced in the oceans.
- thus major sink for **fixed** nitrogen, and so limits oceani primary productivity
- 5(+?) known anammox genera, all bacteria within phylum Planctomycetes: Brocadia, Kuenenia, Anammoxoglobus, Jettenia (all fresh water species), and Scalindua (marine species)



Inferred Scalindula transformations:

RECAP OF main Microbial N tranformations

Ammonia is produced by:

1. *nitrogen fixation*
2. *ammonification*

Ammonia can be removed by:

1. *assimilation* into organic matter
2. aerobic oxidation to nitrate through *nitrification*
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3. reduction to NH_3 via *dissimilatory nitrate reduction to ammonia*

- Denitrification is the primary mechanism by which N_2 is produced biologically
- Denitrification and anammox result in losses of nitrogen from the biosphere

Global inputs of reactive nitrogen

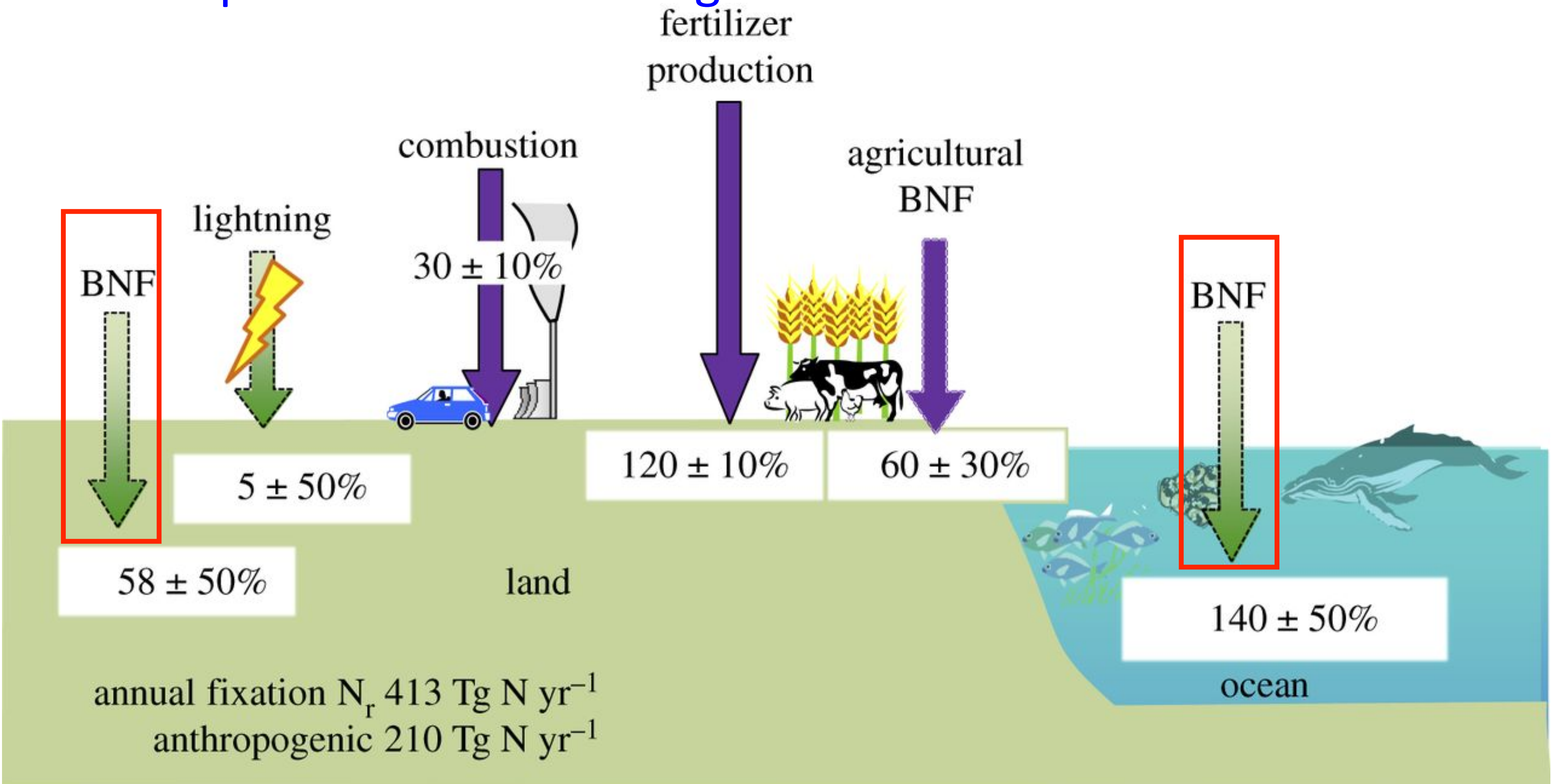


Figure 1. Fowler et al 2013 PhilTransB
Global nitrogen fixation, natural and anthropogenic in both oxidized and reduced forms through combustion, biological fixation, lightning and fertilizer and industrial production through the Haber–Bosch process for 2010. The arrows indicate a transfer from the atmospheric N_2 reservoir to terrestrial and marine ecosystems, regardless of the subsequent fate of the N_r. Green arrows represent natural sources, purple arrows represent anthropogenic sources.

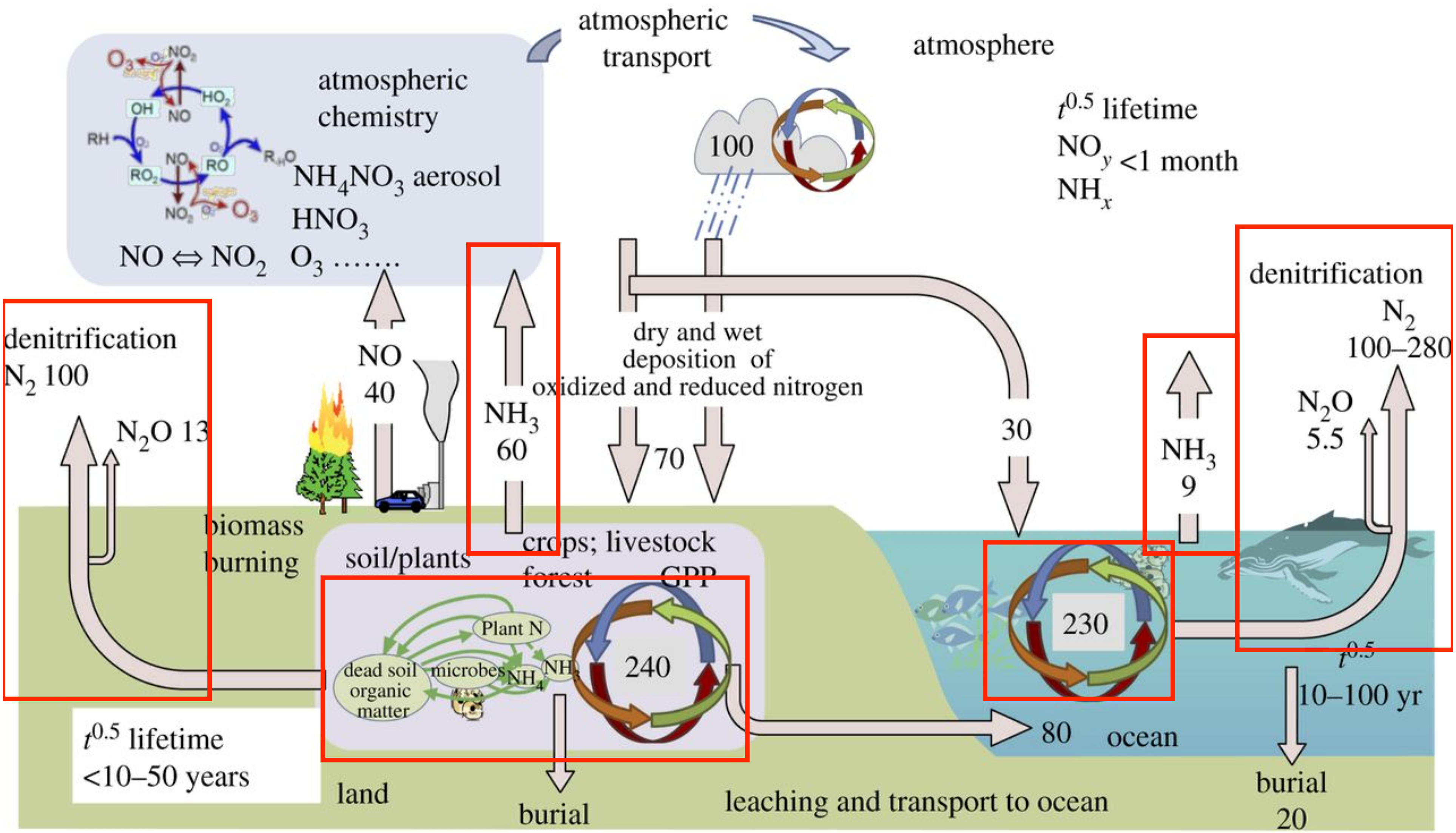
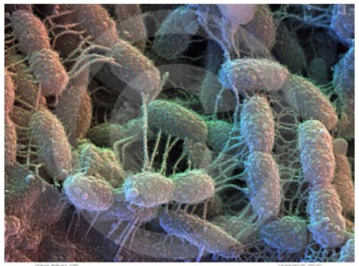
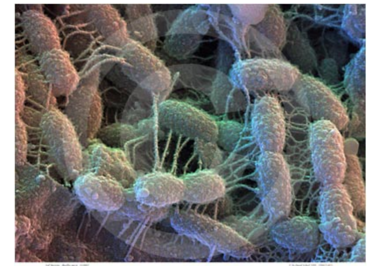


Figure 2. Fowler et al 2013 PhilTransB

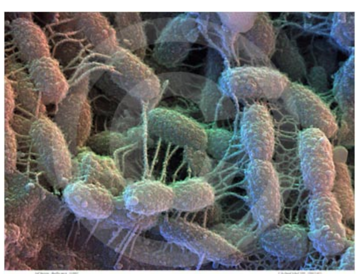
The processing and fluxes of reactive nitrogen in terrestrial and marine systems and in the atmosphere (Tg N yr^{-1}), showing the dominant forms of the N_r in the exchanges and the magnitude of the boundary fluxes, and approximate lifetimes, integrated over global scales.

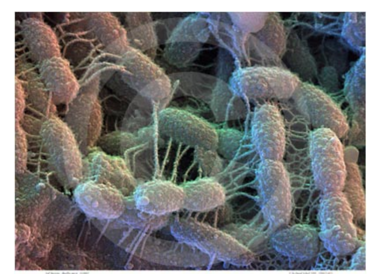




EXTRA

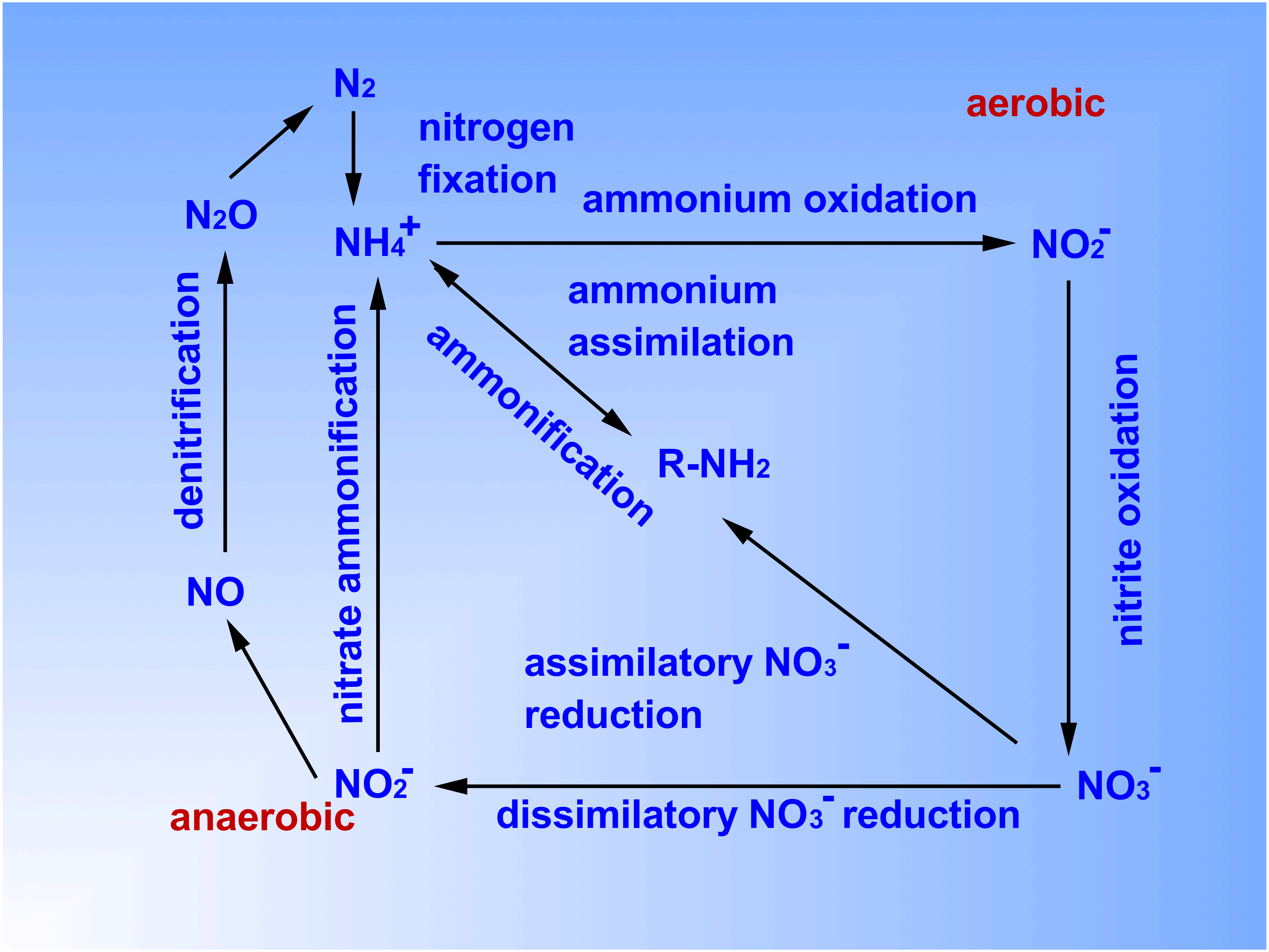
SLIDES

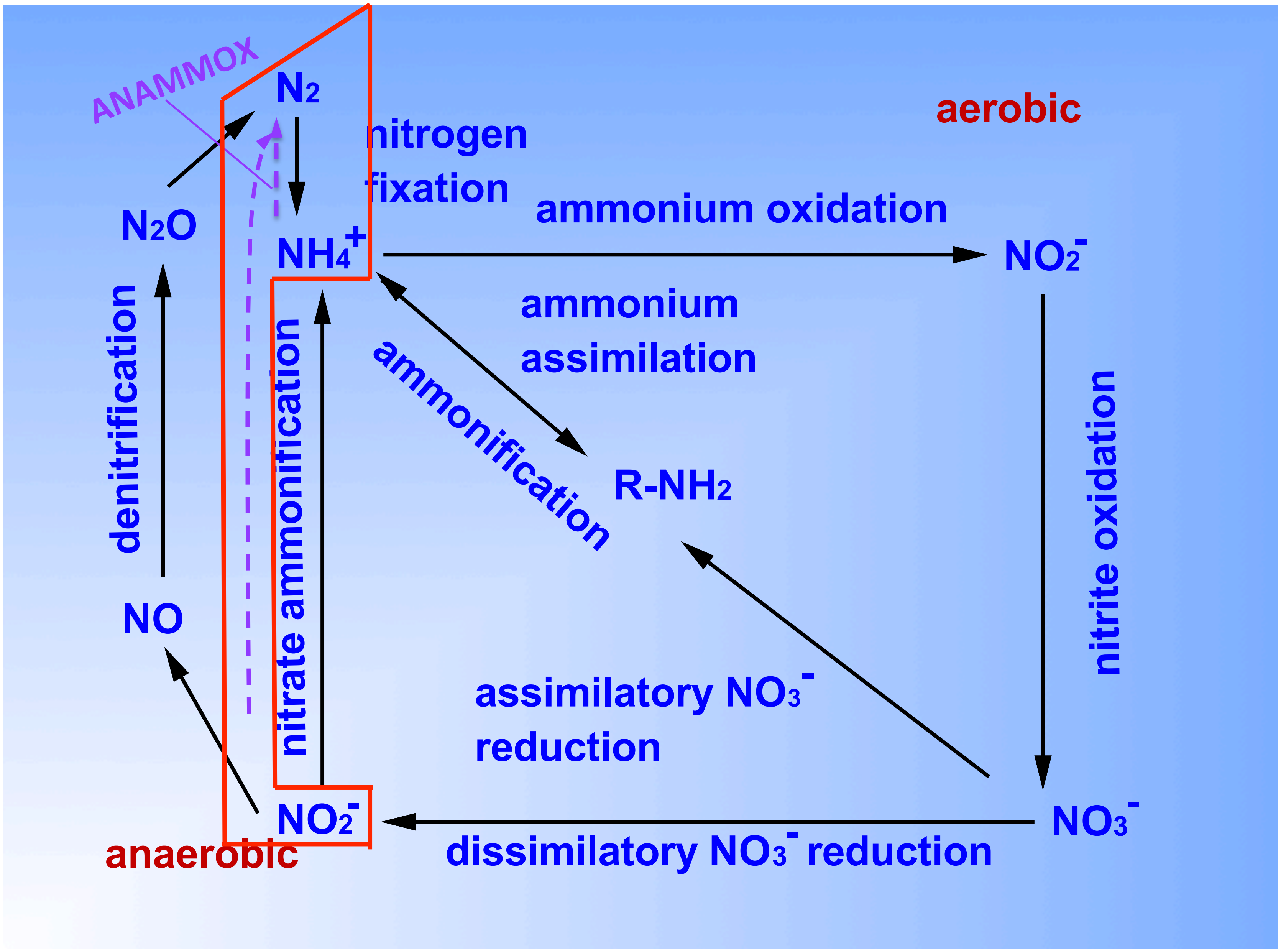




The Azolla event

- 49 million years ago
- Blooms of Azolla in the Artic Ocean
- Died and sank to ocean floor, incorporated into sediment.
- Resulted in CO_2 drawdown which is speculated to have transformed earth from “greehouse” 13 C to “icehouse” -9 C (in Artic).


N2
nitrogen
fixation
aerobic
N2O
NH4+
ammonium oxidation
NO2-
ammonium
assimilation
denitrification
nitrate ammonification
ammonification
R-NH2
nitrite oxidation
NO
assimilatory NO3-
reduction
NO2-
NO3-
anaerobic
dissimilatory NO3- reduction



ANAMMOX
N2
nitrogen fixation
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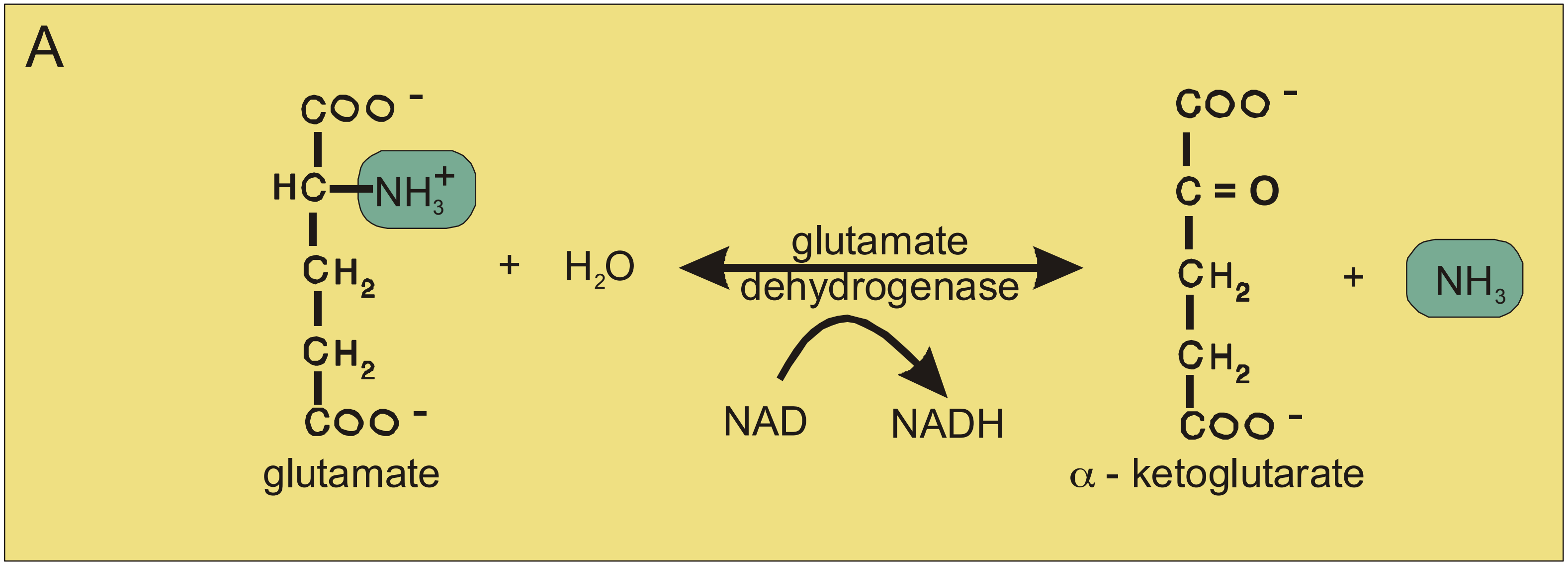
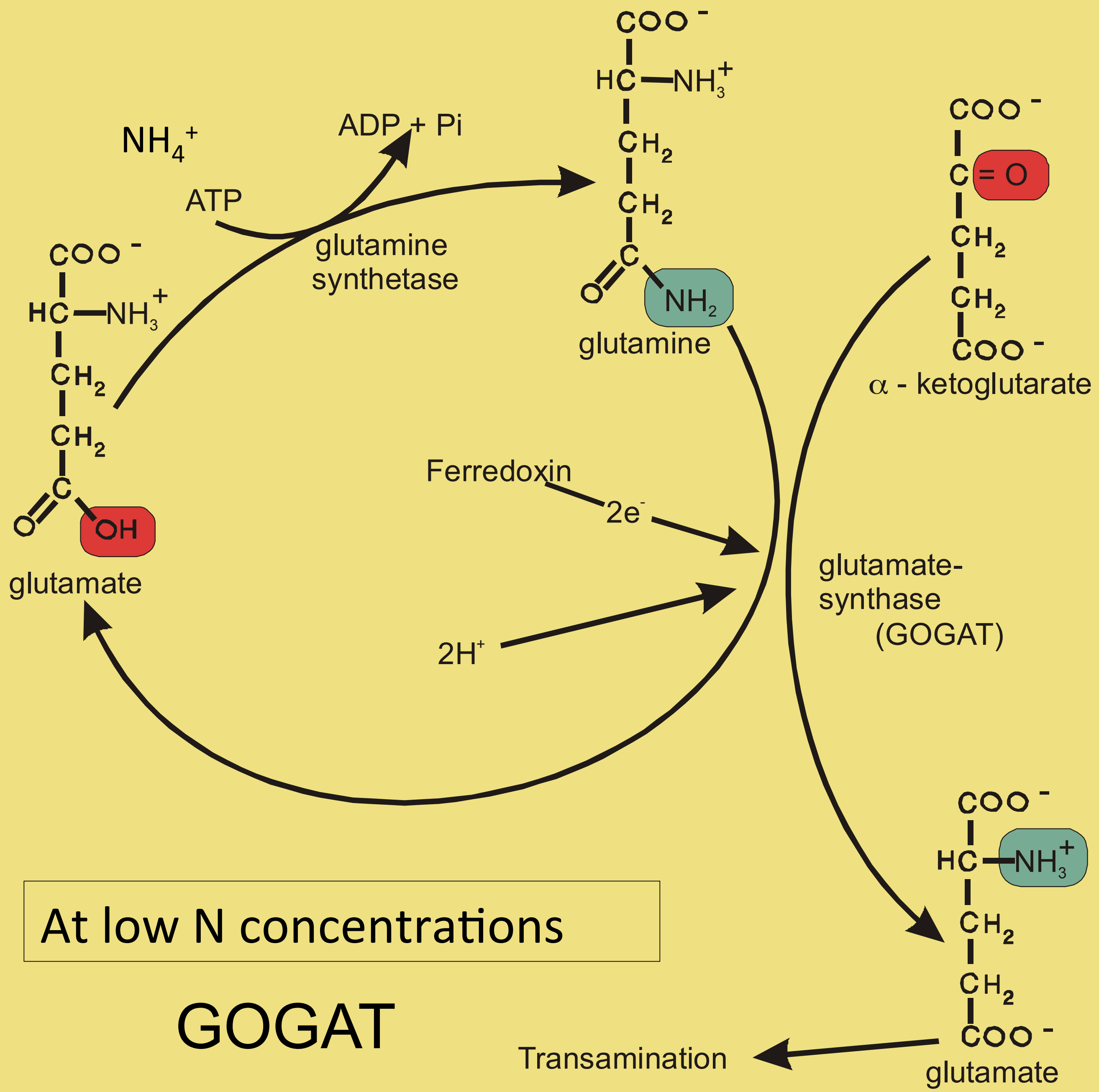
N2O measurement http://youtu.be/fAHkpOJ4cwA
AU N2O agricultural monitoring
http://youtu.be/ADkCHQKr6xk

At high N concentrations



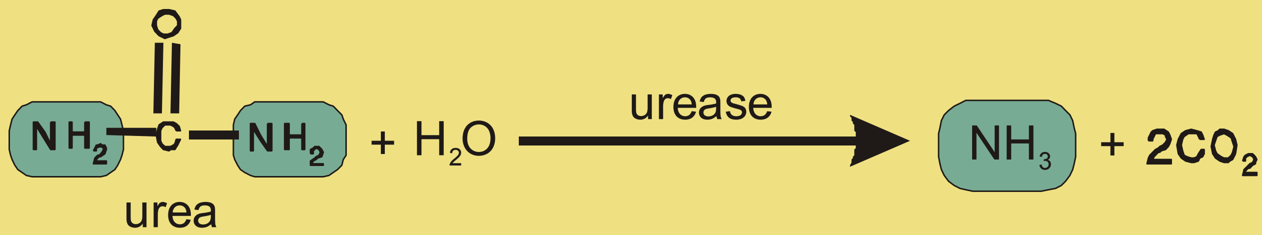
B



At low N concentrations

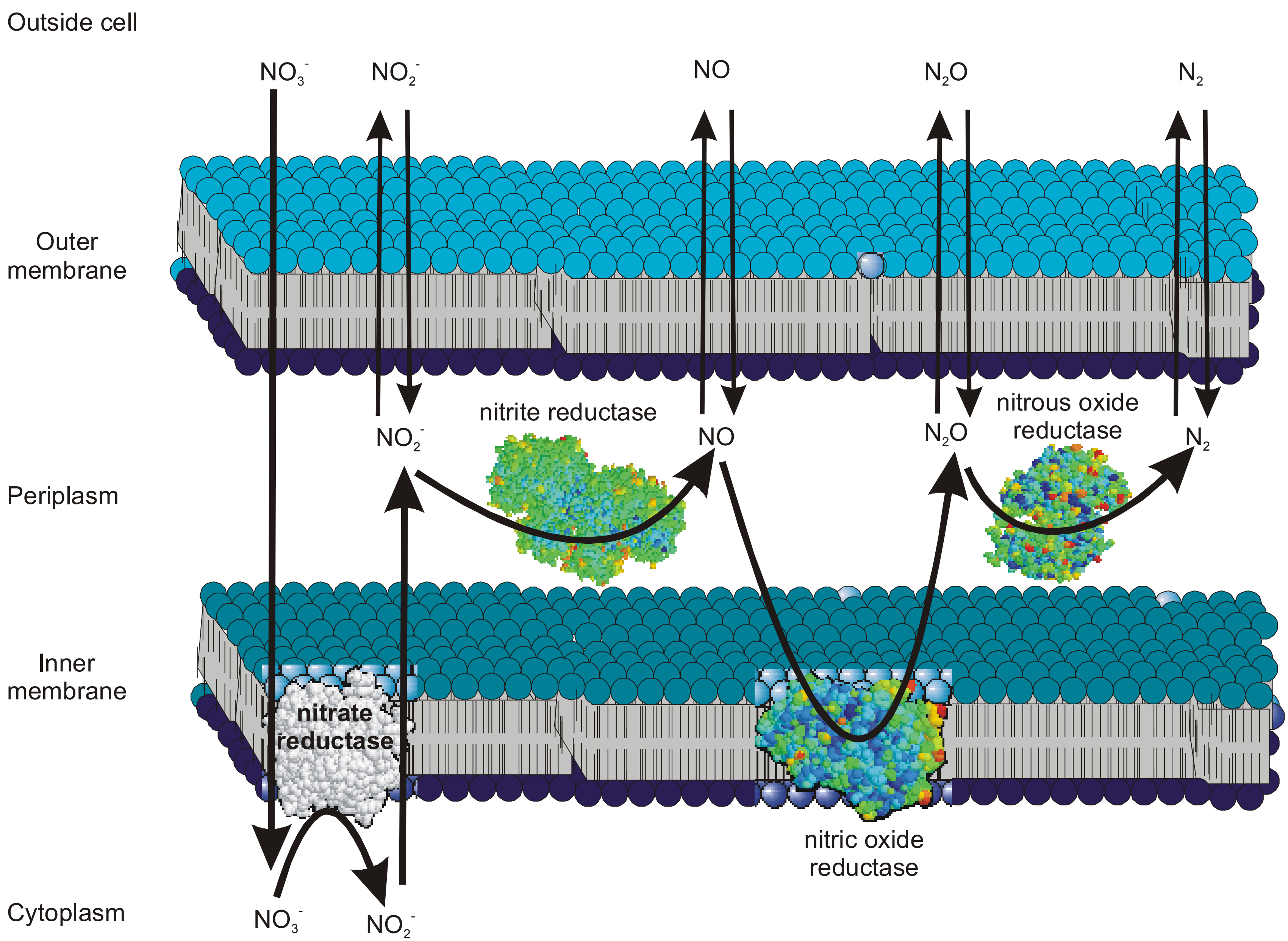
GOGAT

C

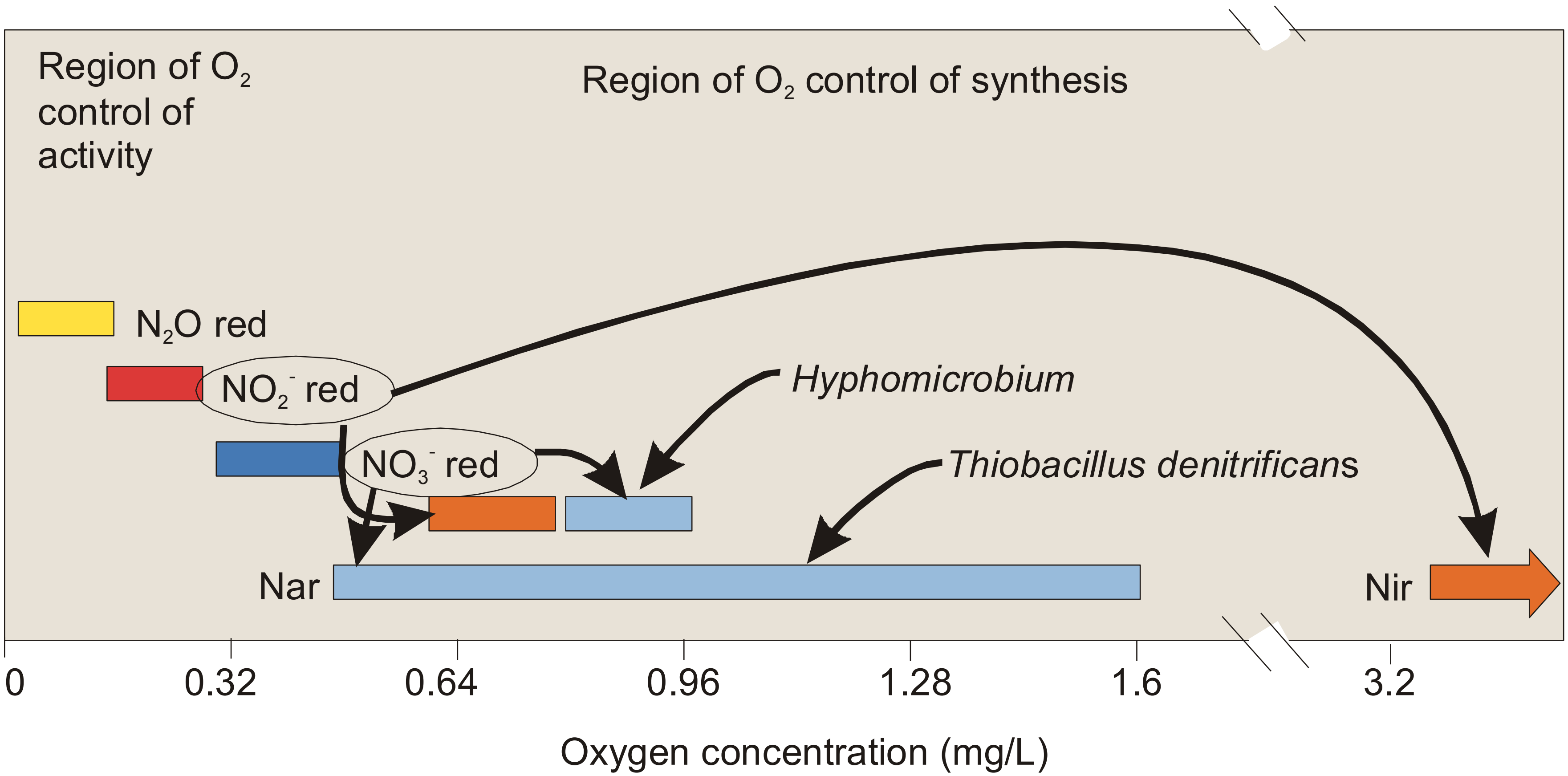


<u>Summary for ammonia assimilation and ammonification</u>

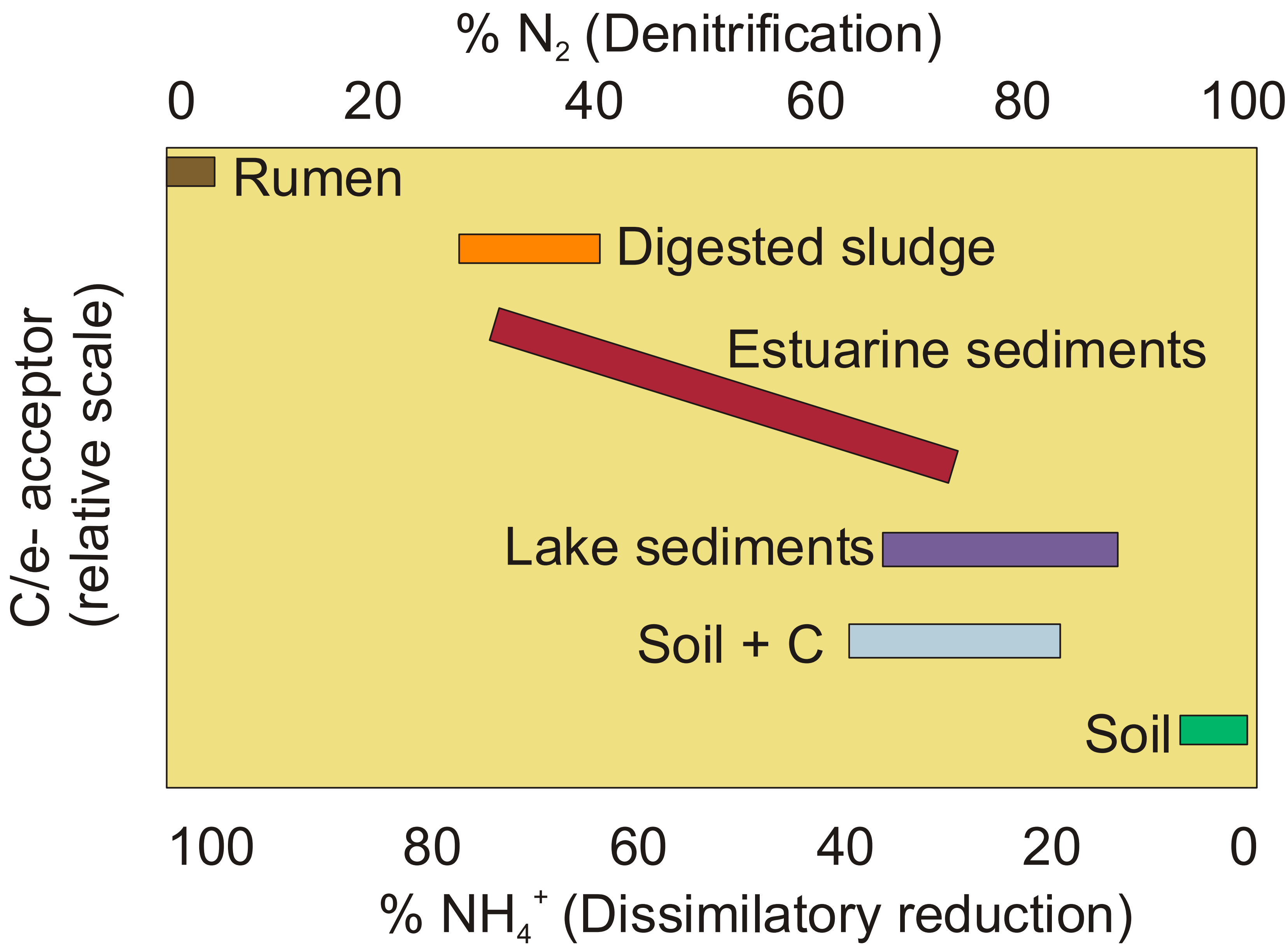
- Assimilation and ammonification cycles ammonia between its organic and inorganic forms
- Assimilation predominates at C:N ratios > 20
- Ammonification predominates at C:N ratios < 20



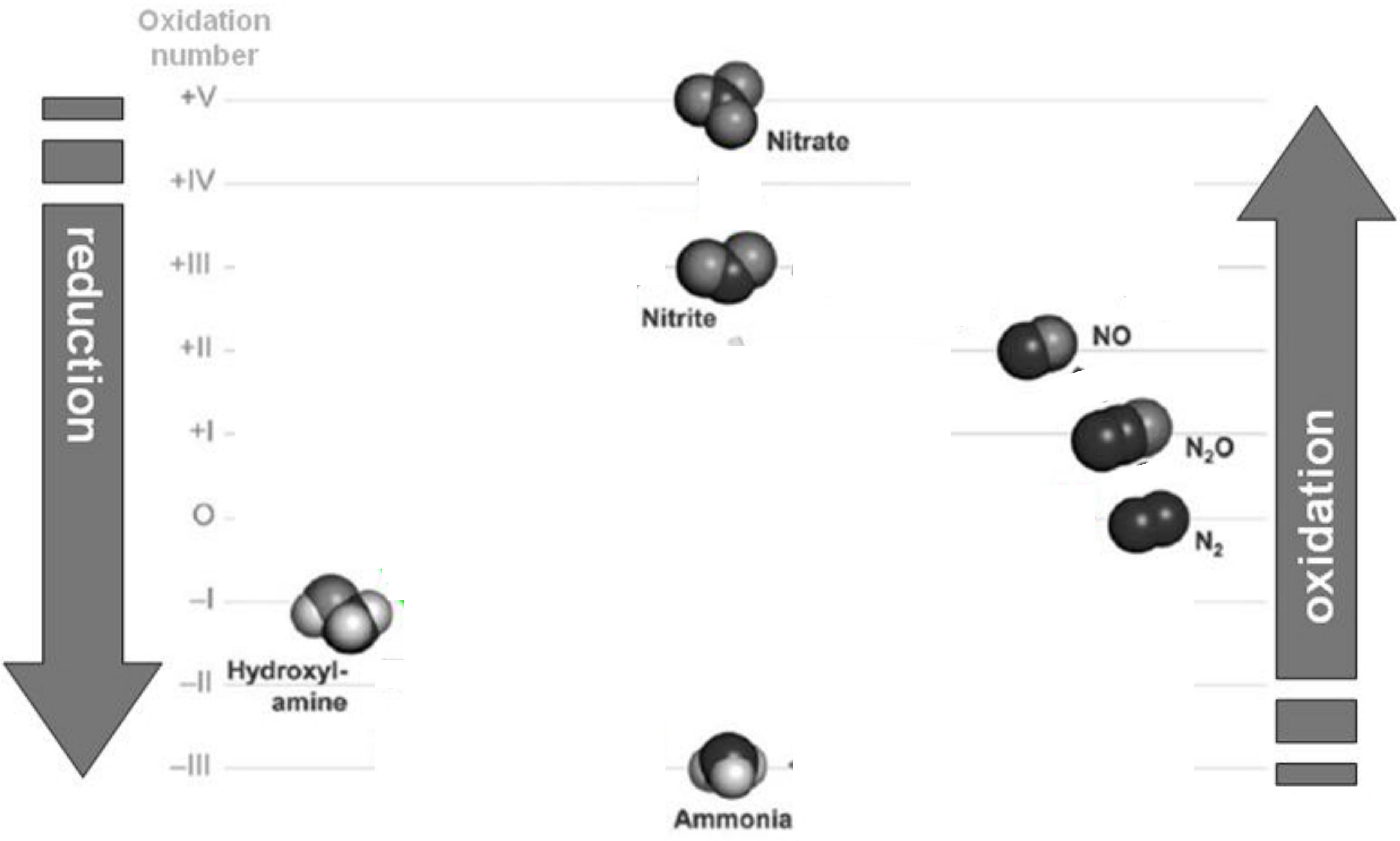
Outside cell
NO_3^-
NO_2^-
NO
N_2O
N_2
Outer membrane
nitrite reductase
NO_2^-
NO
N_2O
nitrous oxide reductase
N_2
Periplasm
Inner membrane
nitrate reductase
nitric oxide reductase
Cytoplasm
NO_3^-
NO_2^-



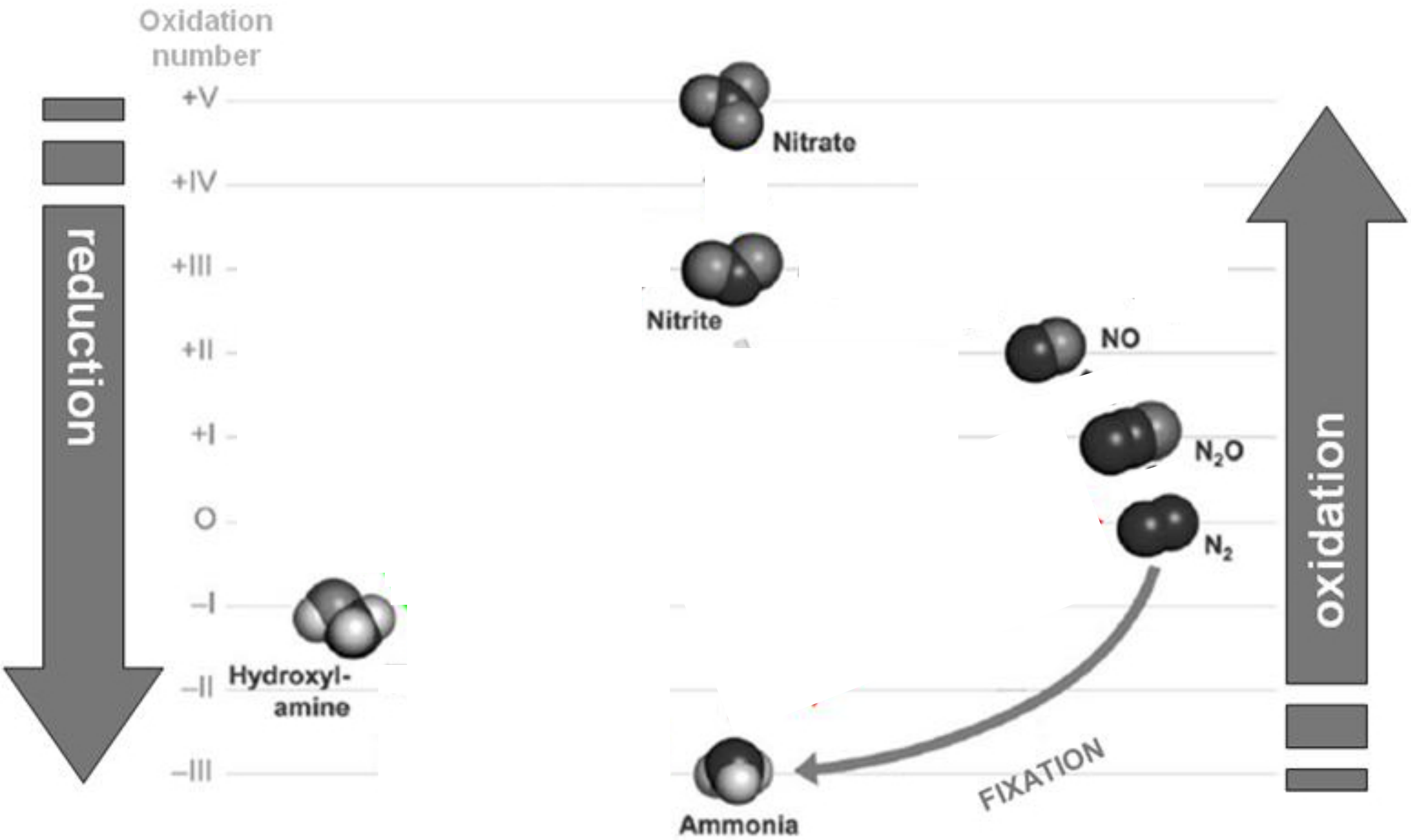
Region of O_2 control of activity
Region of O_2 control of synthesis
N_2O red
NO_2^- red
NO_3^- red
Hyphomicrobium
Thiobacillus denitrificans
Nar
Nir
0
0.32
0.64
0.96
1.28
1.6
3.2
Oxygen concentration (mg/L)



% N2 (Denitrification)
0
20
40
60
80
100
Rumen
Digested sludge
Estuarine sediments
Lake sediments
Soil + C
Soil
C/e- acceptor
(relative scale)
100
80
60
40
20
0
% NH4+ (Dissimilatory reduction)



http://www.advancedaquarist.com/2011/4/chemistry_album/Fig_4.jpg



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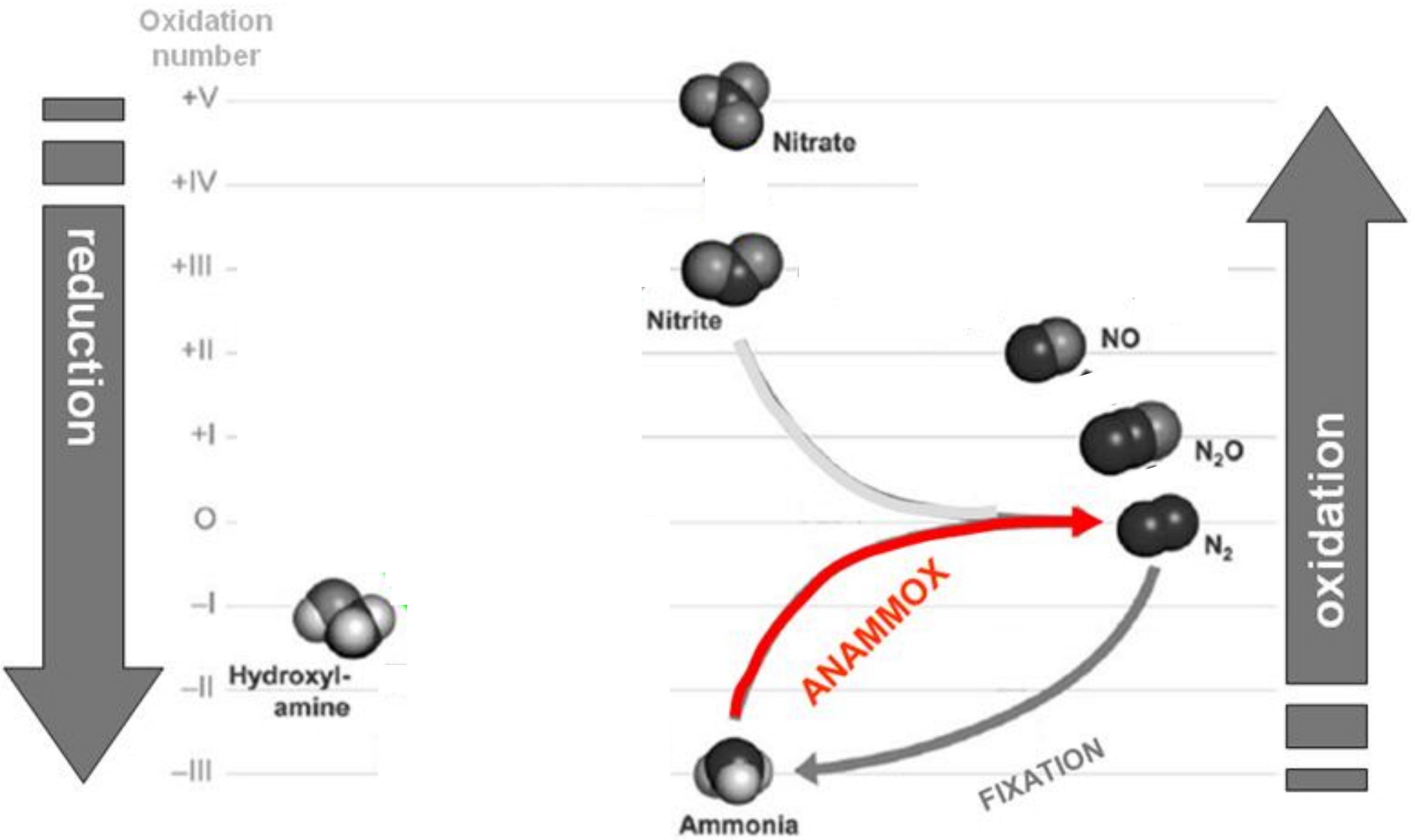
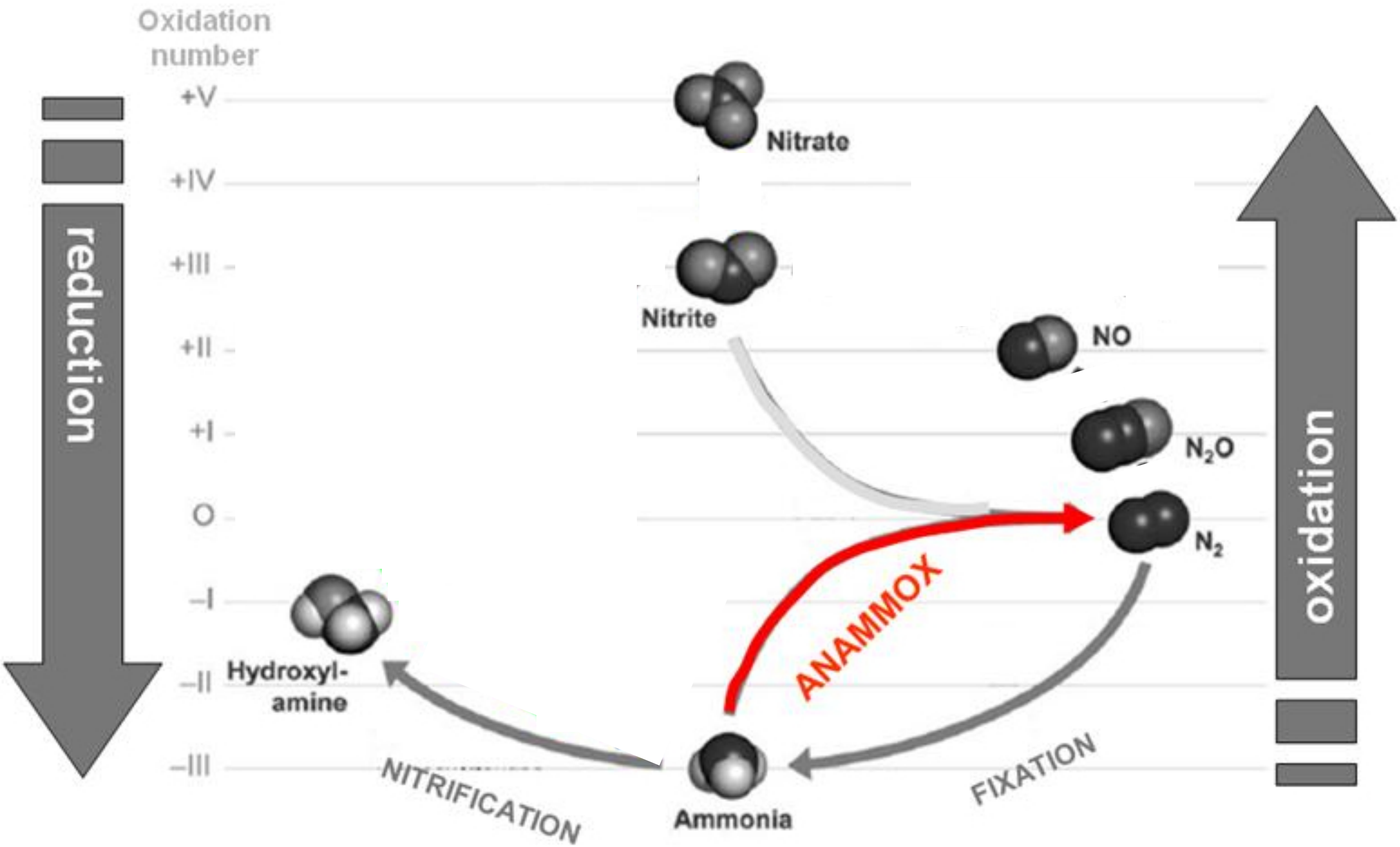
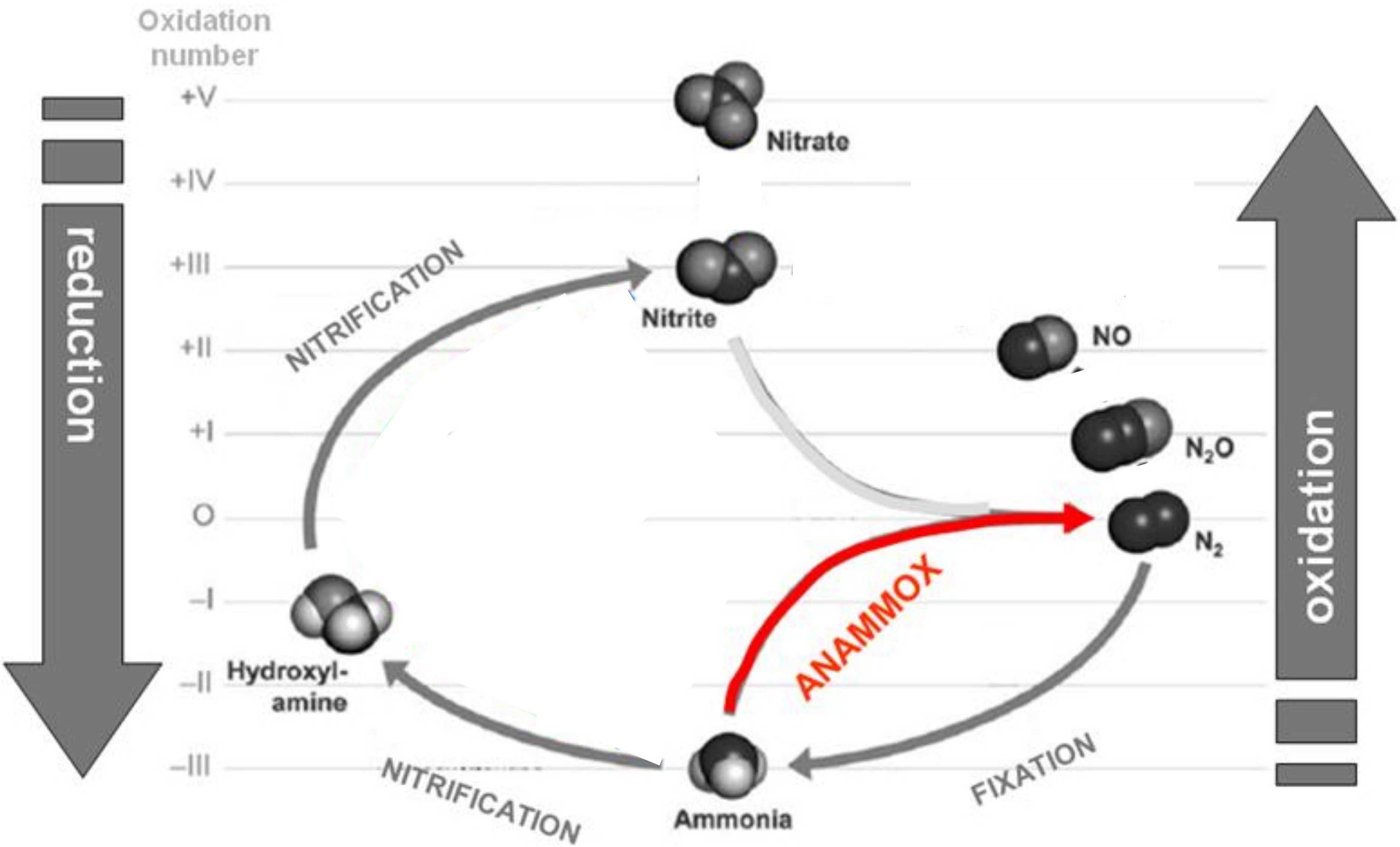


ANaerobic AMMonia OXidation

http://www.advancedaquarist.com/2011/4/chemistry_album/Fig_4.jpg



http://www.advancedaquarist.com/2011/4/chemistry_album/Fig_4.jpg



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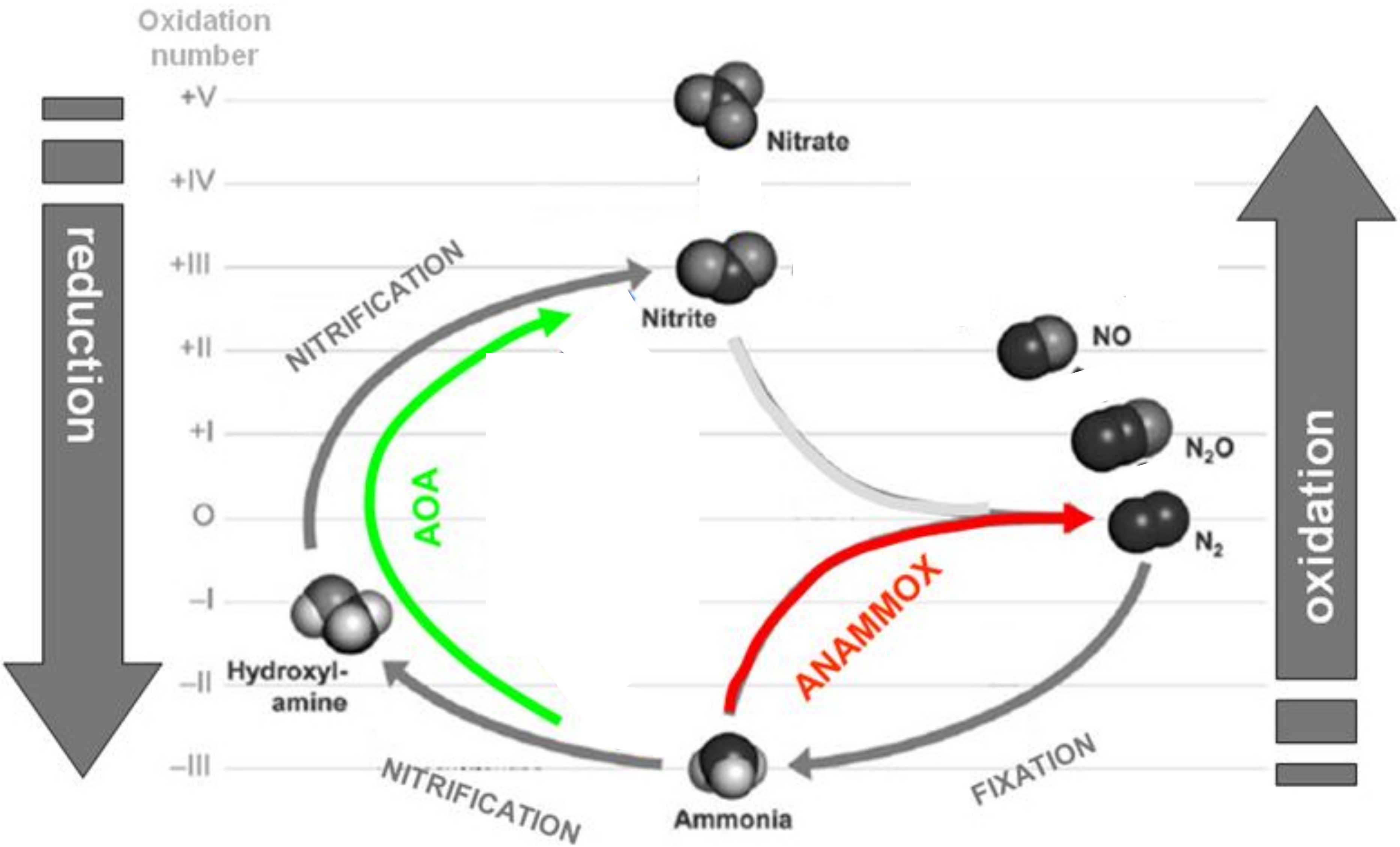

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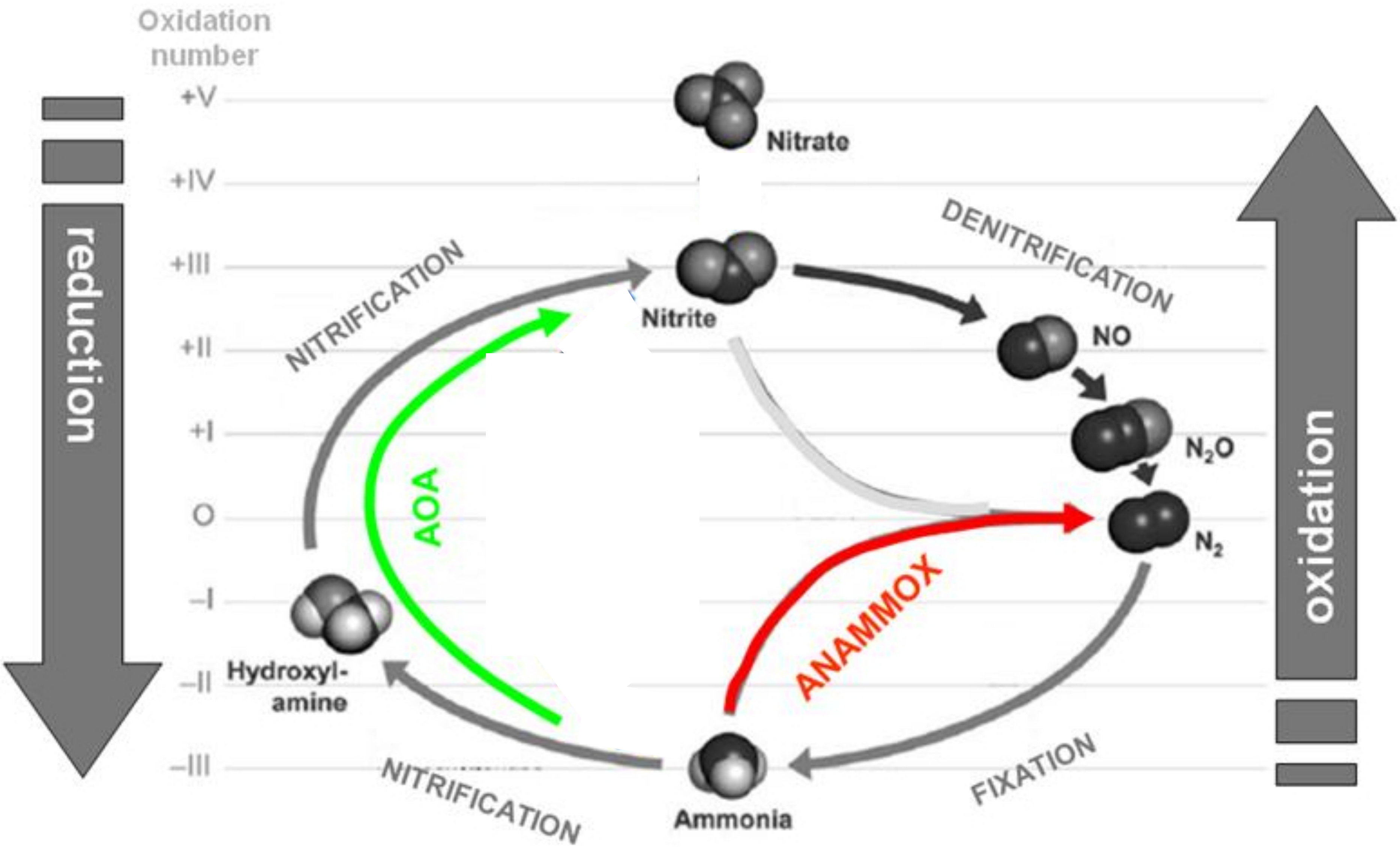

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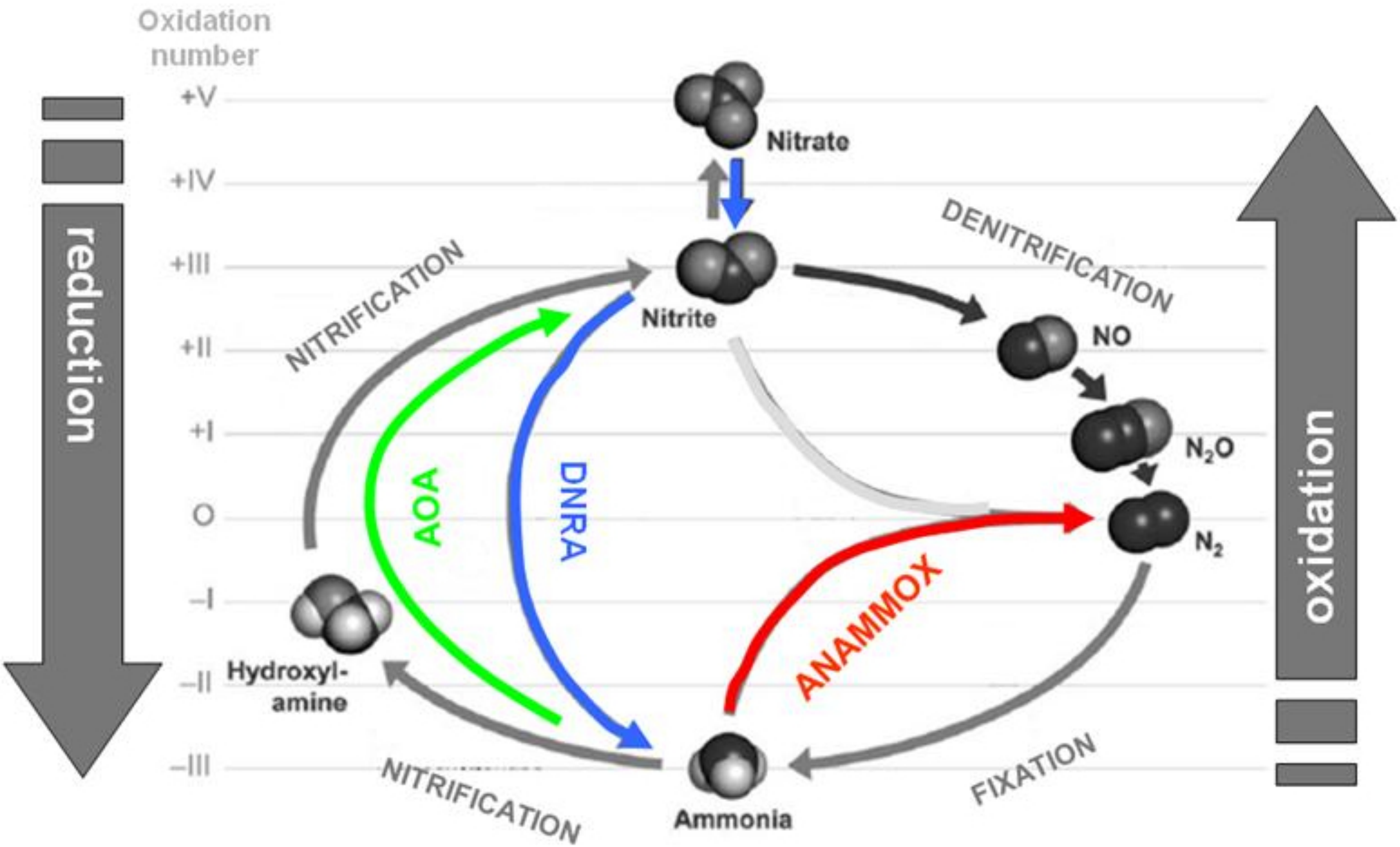
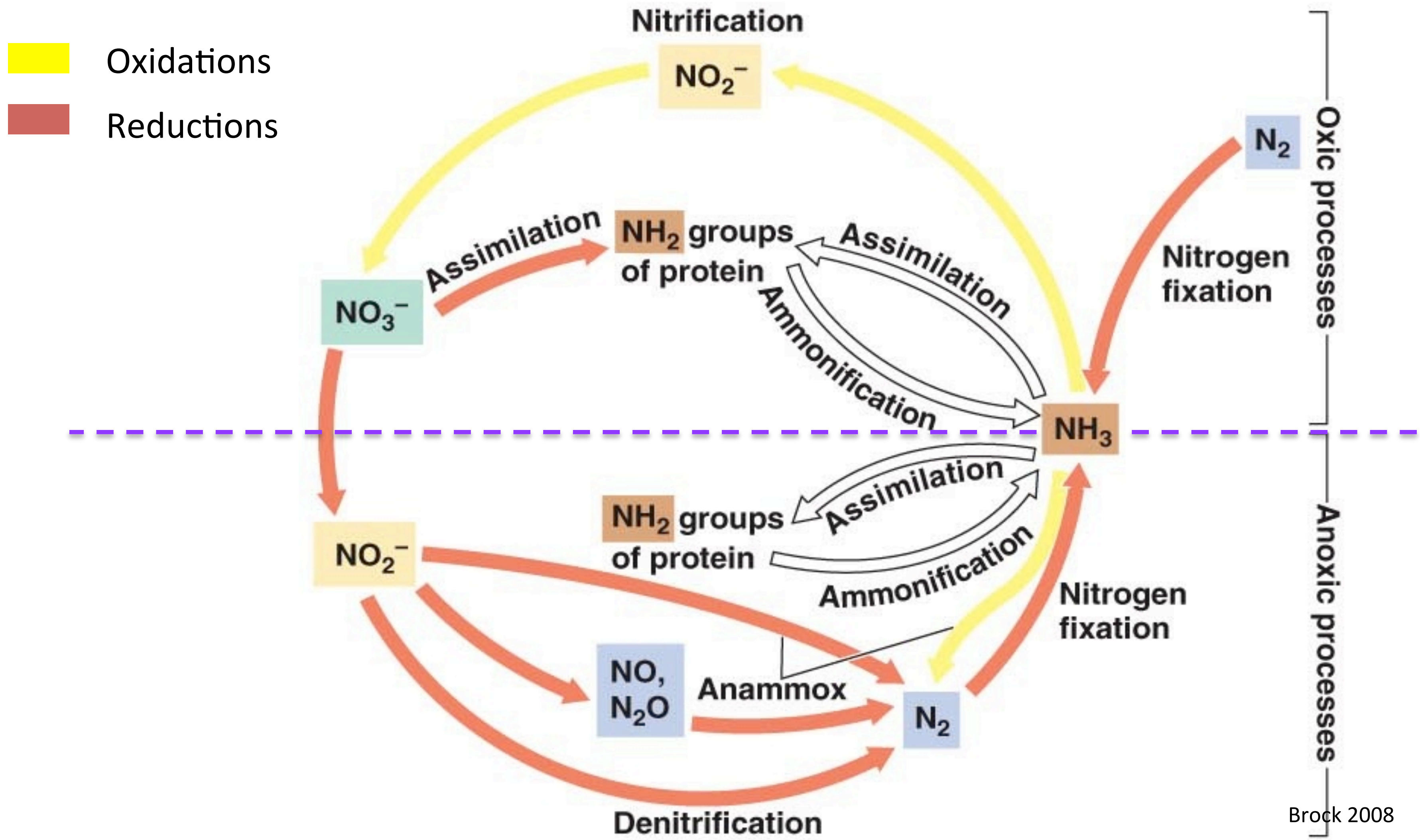


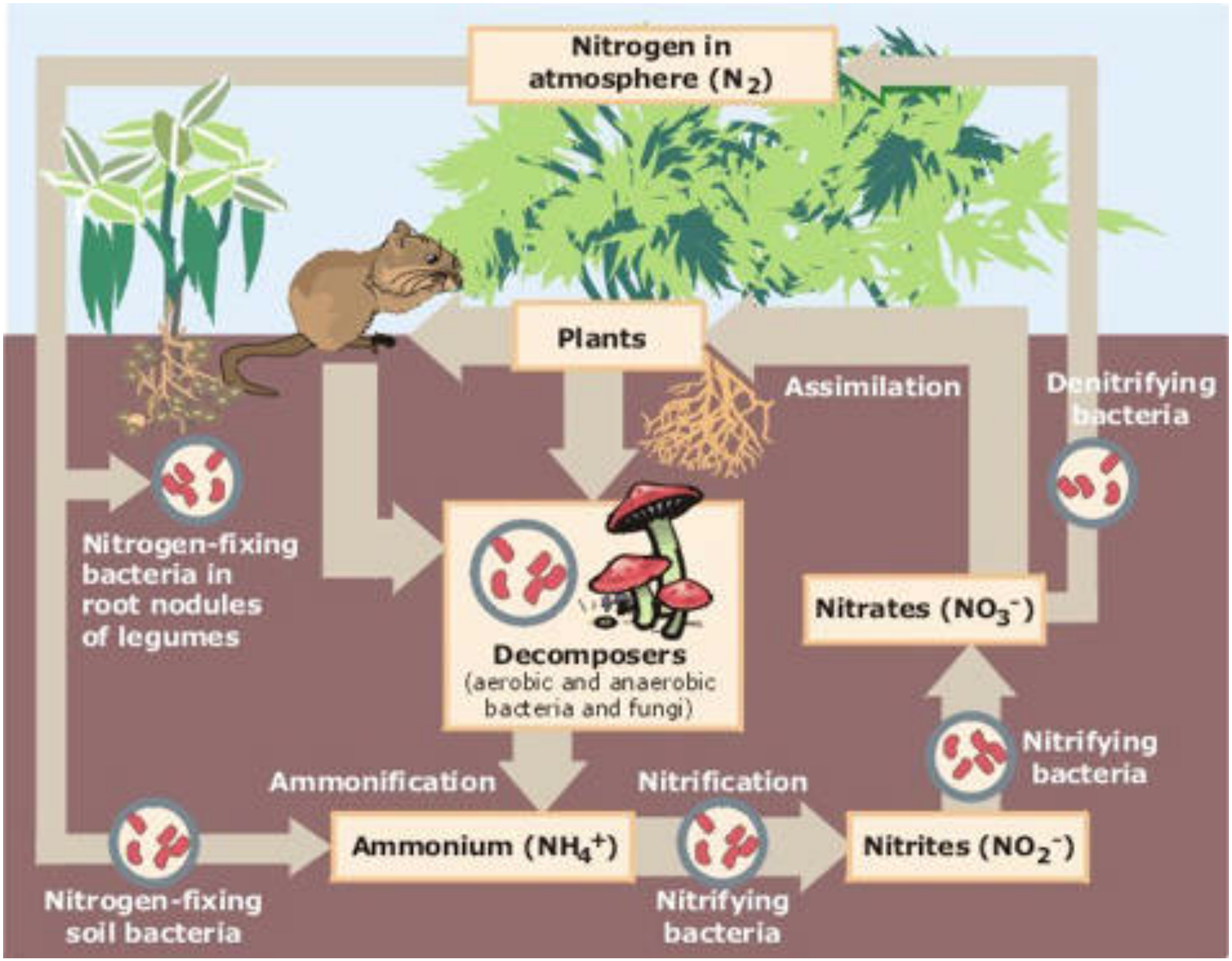
http://www.advancedaquarist.com/2011/4/chemistry_album/Fig_4.jpg

Redox Cycle for Nitrogen



Anammox = **an**aerobic **amm**onia **ox**idation

Assimilation vs dissimilation



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transformation	pathway	environmental effect
N_2 fixation: Haber–Bosch process $N_2 \longrightarrow NH_3$	industry	energy intensive process, production of CO_2 plus all the consequences of the N_r as it cascades through soils, the atmosphere and aqueous phases
N fertilizer on crops	agricultural lands	provision of food for human consumption
NH_4 nitrified to $\longrightarrow NO_3$ NO in soil $\longrightarrow$ atmosphere oxidation of NO $\longrightarrow NO_2 \longrightarrow HNO_3$	NO emission from soil to atmosphere and ozone production during volatile organic compound degradation	ozone effects on vegetation or human health [48,49]
aerosol formation, $HNO_3 \longrightarrow NO_3$	in atmosphere	planetary albedo, human health [50]
wet + dry deposition NO_3 to soil $\longrightarrow$ vegetation $NO_3 \longrightarrow R\text{-}NH_2$	removal from atmosphere and transfer to plant biomass	eutrophication, acidification [51]
consumption by herbivores: excreted as urea $R\text{-}NH_2 \longrightarrow CO(NH_2)_2$	plant biomass $\longrightarrow$ animal protein $\longrightarrow$ excreted and returned to soil	eutrophication [51]
urea converted to NH_3 in soil and released to atmosphere	soil to atmosphere flux of NH_3	eutrophication
NH_3/NH_4 uptake by vegetation	removal from atmosphere by dry deposition to vegetation	eutrophication
decomposition $R\text{-}NH_3 \longrightarrow NH_4$	vegetation to soil	eutrophication
NH_4 nitrified to NO_3 transferred to river/estuary/open ocean	soil to ground water $\longrightarrow$ river $\longrightarrow$ ocean	eutrophication
ocean uptake in phyto/zooplankton	shelf seas to open ocean	eutrophication
denitrification in ocean sediments $NO_3 \longrightarrow N_2$	returns to atmosphere as N_2 and N_2O	climate change

Table 3. Fowler et al 2013 PhilTransB

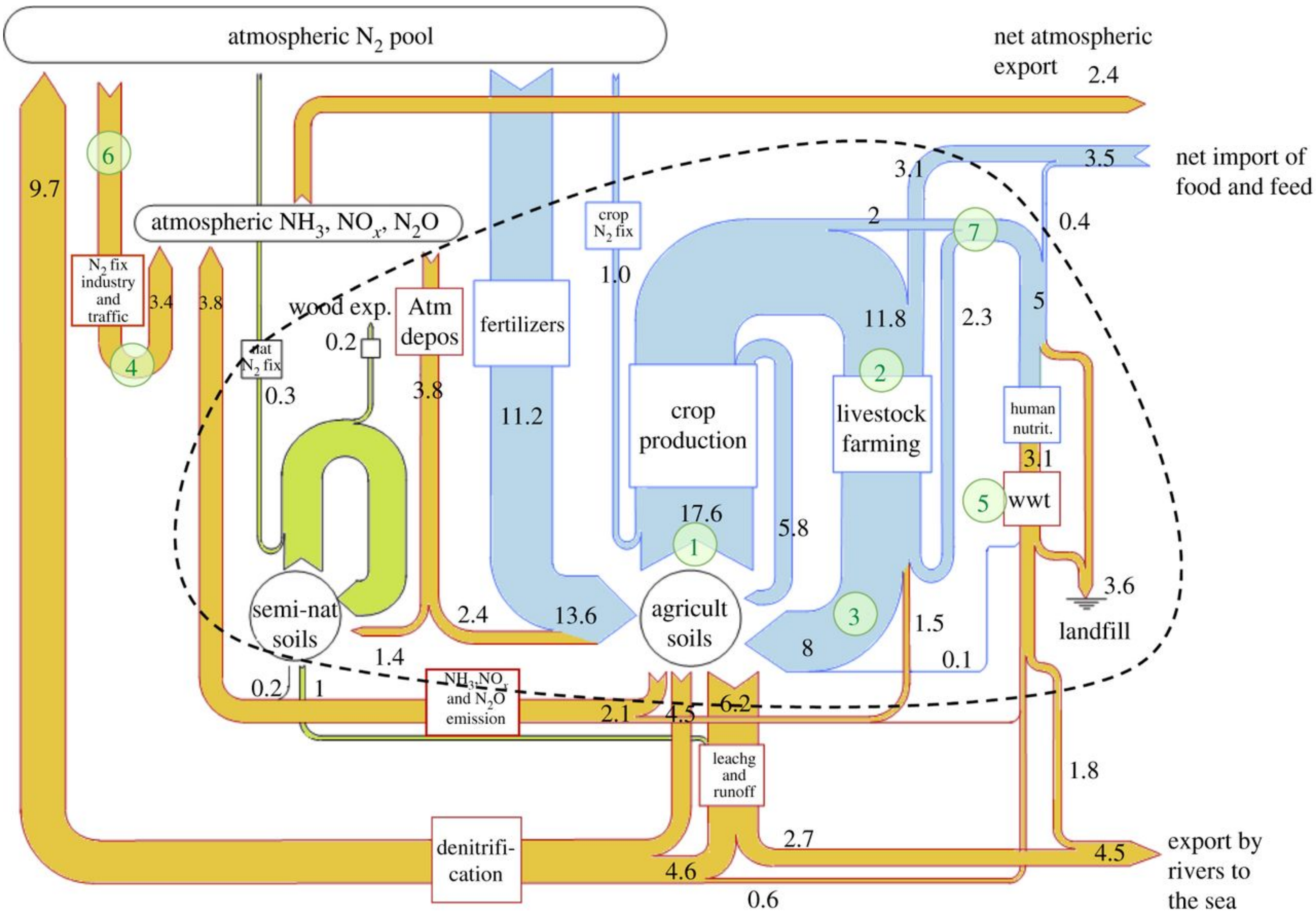

Figure 4. Fowler et al 2013 PhilTransB The nitrogen cycle within the EU-27 showing natural fluxes (Tg N) in green, (intentional) anthropogenic fluxes as blue and (unintentional) as orange adapted from the ENA [69]. The terrestrial component of the cycle is delineated by the dotted ellipse.

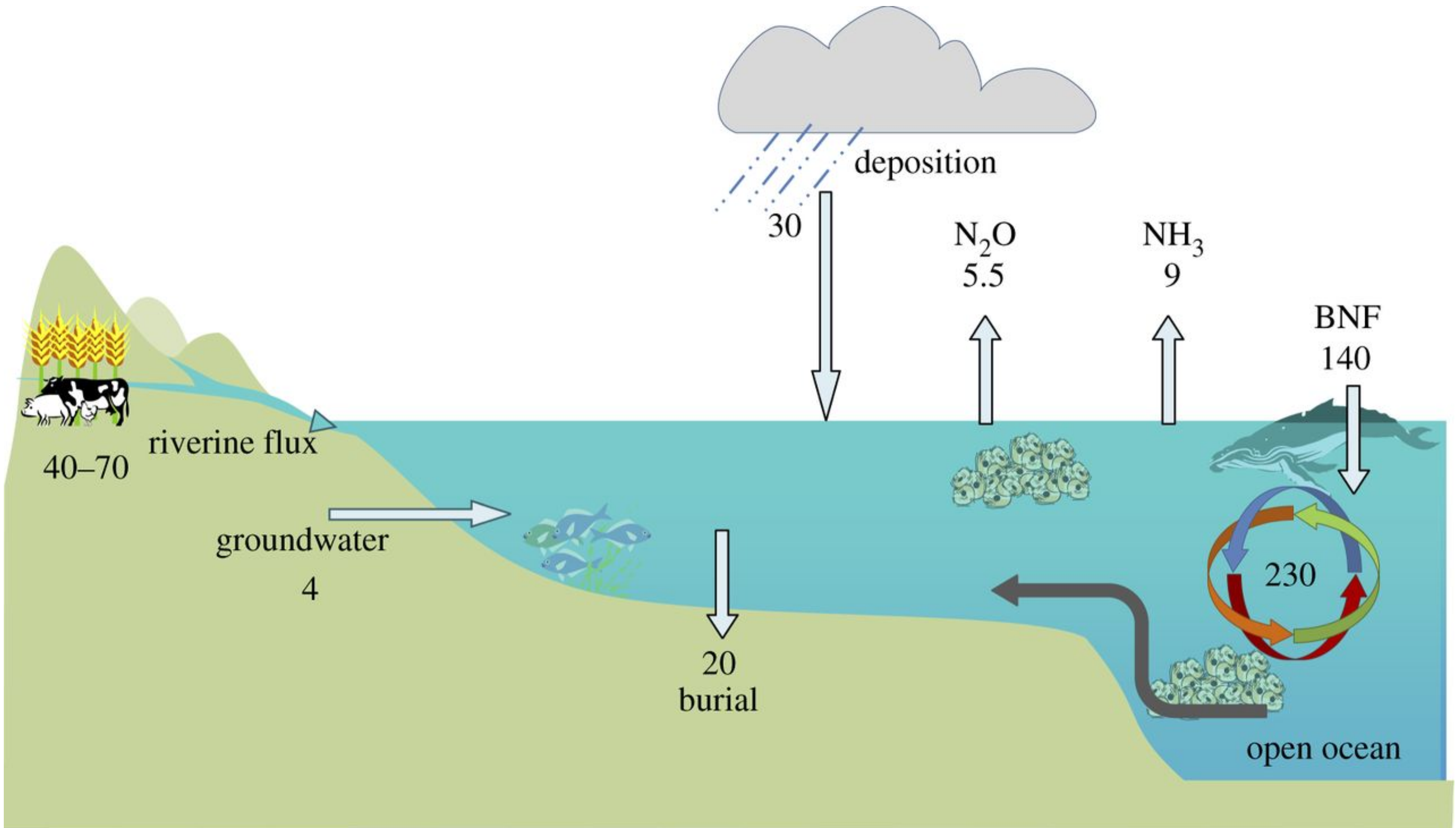

Figure 5. Fowler et al 2013 PhilTransB A simplified schematic of nitrogen cycling in the global oceans (adapted and simplified from [17]). The fluxes are as detailed in the text to be consistent with figure 2.