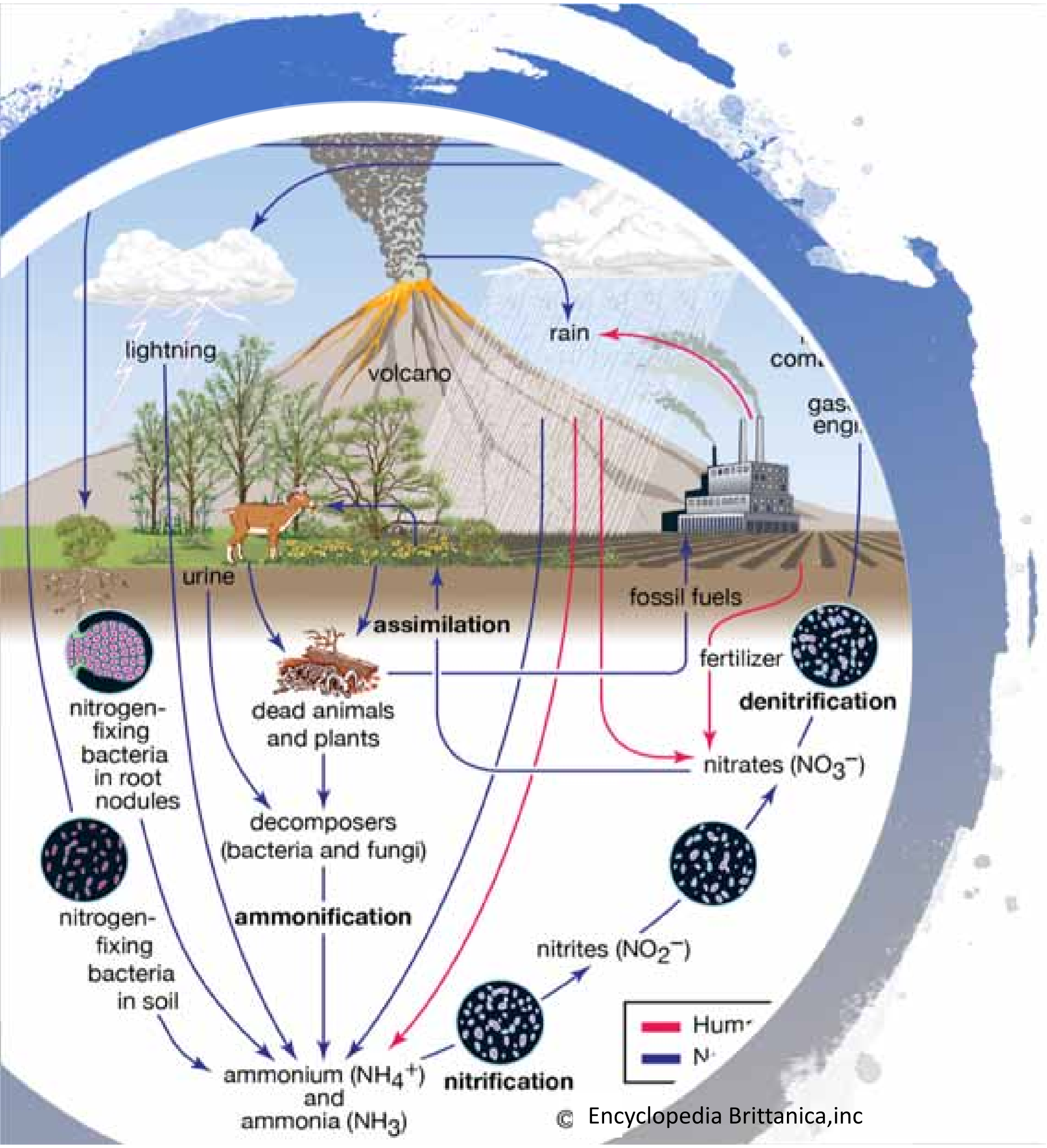

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Soil Nitrification and Inhibitors

Kody Oleson

March 25th 2020

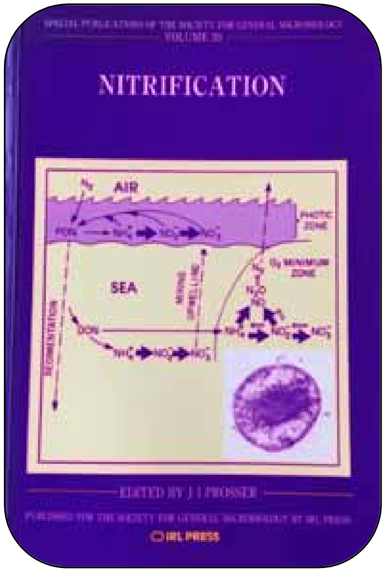
Soil Nitrification and Inhibitors

- **History and Biochemistry Nitrification**
- Nitrification in agricultural soils
- Nitrification Inhibitors
- Case studies

Nitrification

Historically…

- Gun Powder (KNO_3^-)
- Agriculture


NITRIFICATION

EDITED BY J I PROSSER

IRL PRESS

- Winogradsky proposed that nitrification happened in a single stage catalyzed by a genus he named *Nitrobacter* (Winogradsky. 1890; Prosser. 1986) *.*

- Miyake (1916) was the first to quantify soil nitrification, describing it as an autocatalytic reaction. (False)

- Buchanan (1917) classified ammonia and nitrite oxidizing bacteria as lithotrophic bacteria of the family Nitrobacteraceae. – Obligate aerobes

-Ammonia oxidizing bacteria into the beta sub-class of Proteobacteria.

-Nitrosolobus, Nitrosovibrio, Nitrosococcus are no longer distinct genera.

-Most soils dominated by Nitrosospira and archaea (Thaumarchaeota)

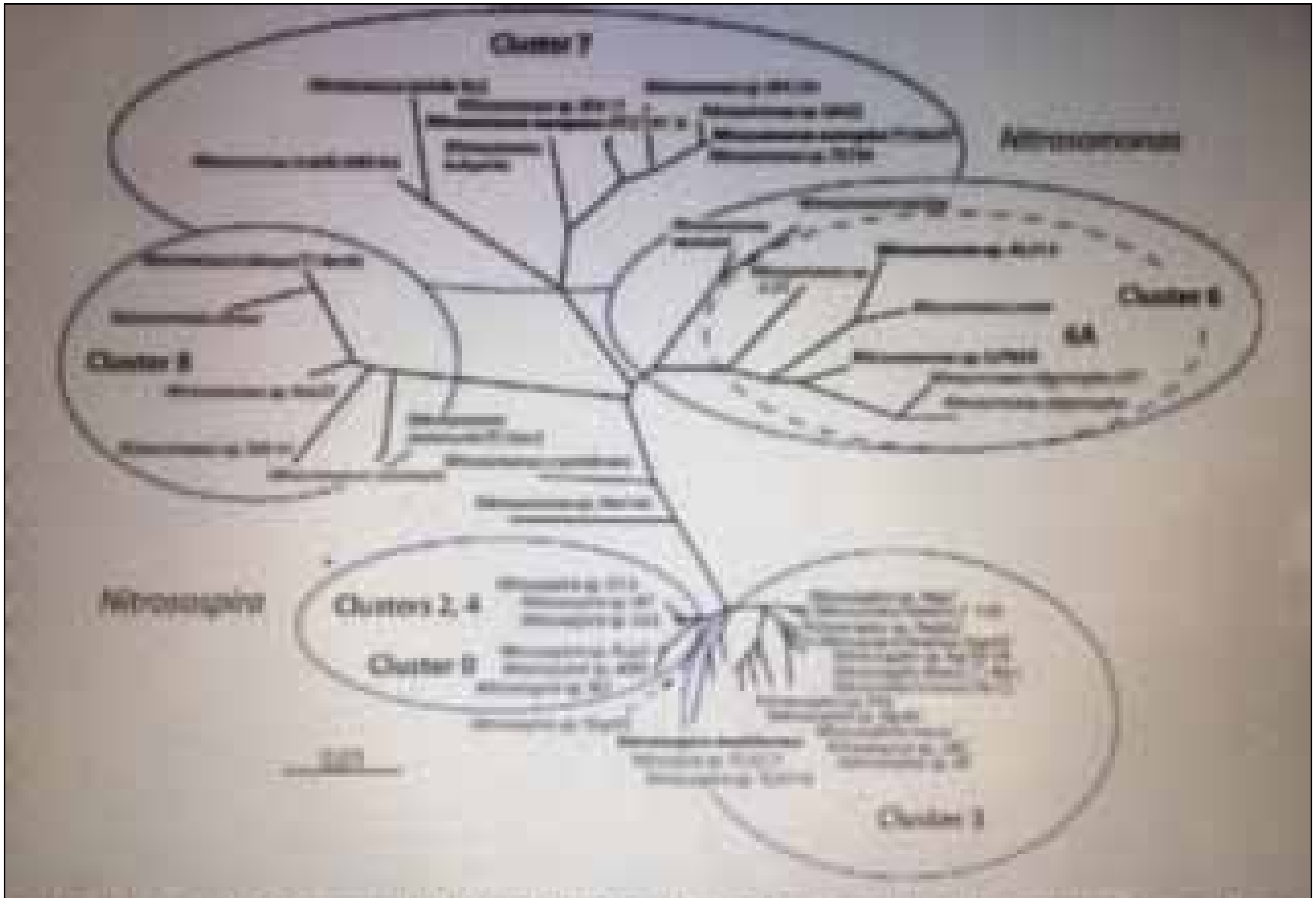


FIG. 14.4 A 16S ribosomal RNA guide tree for bacterial nitrifiers in the Betaproteobacteria based on isolates. The scale is substitutions per site. Redrawn from [illegible]

(Paul, 2014)

Autotrophic nitrification *… Chemolithoautotrophs*

Autotrophic nitrification is the microbial oxidation of NH_3^+ to NO_2^- and subsequently to NO_3^- , mediated by groups of bacteria and archaea known as ammonia and nitrite oxidizers.

Step 1: Ammonia oxidation: $\mathbf{NH_3^+ + 1\frac{1}{2}\ O_2 \rightarrow NO^-_2 + H^+ + H_2O}$

$\mathbf{NH_3^+ + 2H^+ + O_2 + 2e^-}$ → ammonia mono-oxygenase → $\mathbf{NH_2OH + H_2O}$

$\mathbf{NH_2OH + H_2O}$ → hydroxylamine oxidoreductase → $\mathbf{NO^-_2 + 4e^- + 5H^+}$

$\mathbf{2H^+ + \frac{1}{2}\ O_2 + 2e^-}$ → Terminal oxidase → $\mathbf{H_2O}$ (Paul, 2014).

Ammonia oxidizing bacteria

With ammonia as an energy source, autotrophic growth with CO_2 or mixotrophically
Optimal growth at pH 7.5–8.0, 25–30 °C, ammonia conc. 2–10 mM. (Prosser, 1986)

Autotrophic nitrification

Step 2: Nitrite oxidation:

$$\mathbf{NO_2^- + H_2O} \rightarrow \text{Nitrite oxidoreductase} \rightarrow \mathbf{NO_3^- + 2\ H^+ + 2e^-}$$

This is a symbiotic relationship – substrate supply and detoxification (Daims et al, 2016)
Note: Nitrite oxidoreductase is a reversible enzyme, so dissimilatory nitrate reduction to nitrite can occur in anaerobic conditions (Paul, 2014)

Nitrite oxidizing bacteria
Nitrite as an energy source and CO_2 as a C source.
Optimal growth at pH 7.5—8.0, 25–30 ° C, nitrite conc. 2–30 mM.
Too high partial pressure of O2 can be inhibitory. (Prosser, 1986)
.

Heterotrophic nitrification

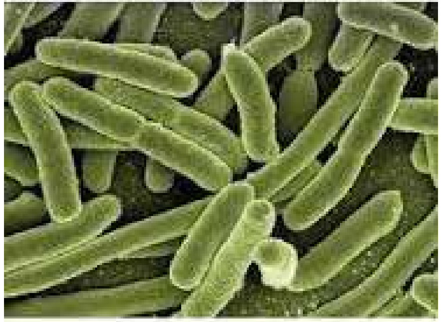
- Heterotrophic bacteria and fungi can undergo nitrification. However this is not linked to cell growth and produces no ATP or energy. This first pathway is like autotrophic nitrification, the second is organic and seems limited to fungi…

$$\mathbf{RNH_2 \rightarrow RNHOH \rightarrow RNO \rightarrow RNO_3 \rightarrow NO_3^-}$$

- Heterotrophic nitrification usually dominates in environments where autotrophic nitrification is inhibited. (Paul. 2014)

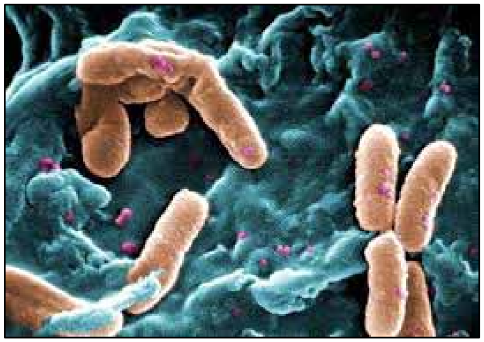
**Nitrobacter can use both autotrophic and heterotrophic pathways*

Nitrobacter sp.



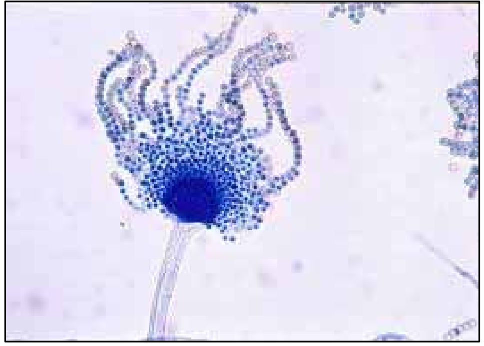
Indiamart.com

Pseudomonas sp.



Zmescience.com

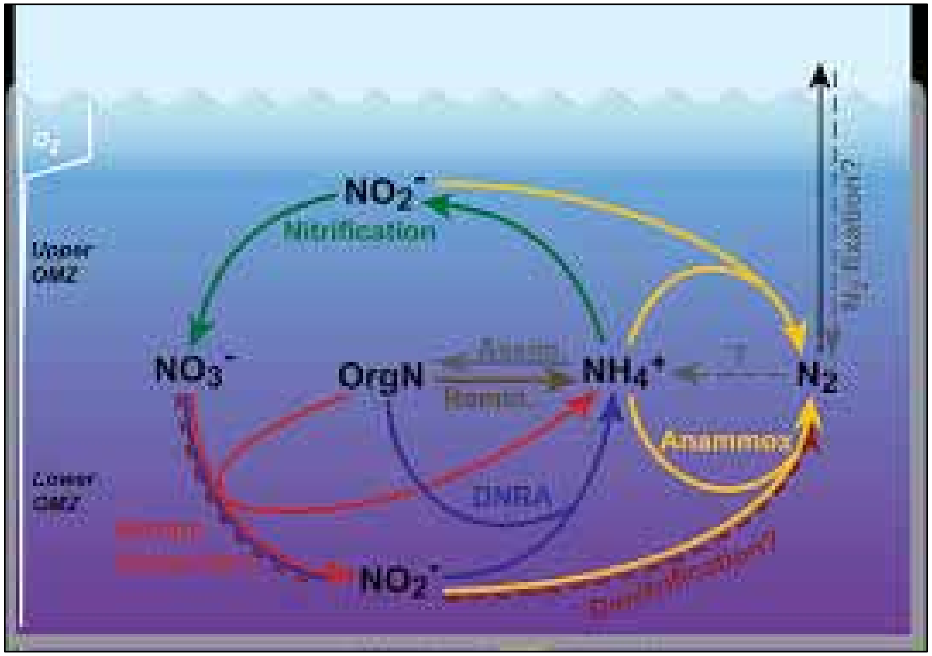
Aspergillus flavus



Aspergillus.org.uk

Anaerobic nitrification "Anammox"

- Anaerobic ammonia oxidizers are important for marine sediments, aquifers, wetlands, and saturated agricultural soils. (Bagnoud et al, 2020)



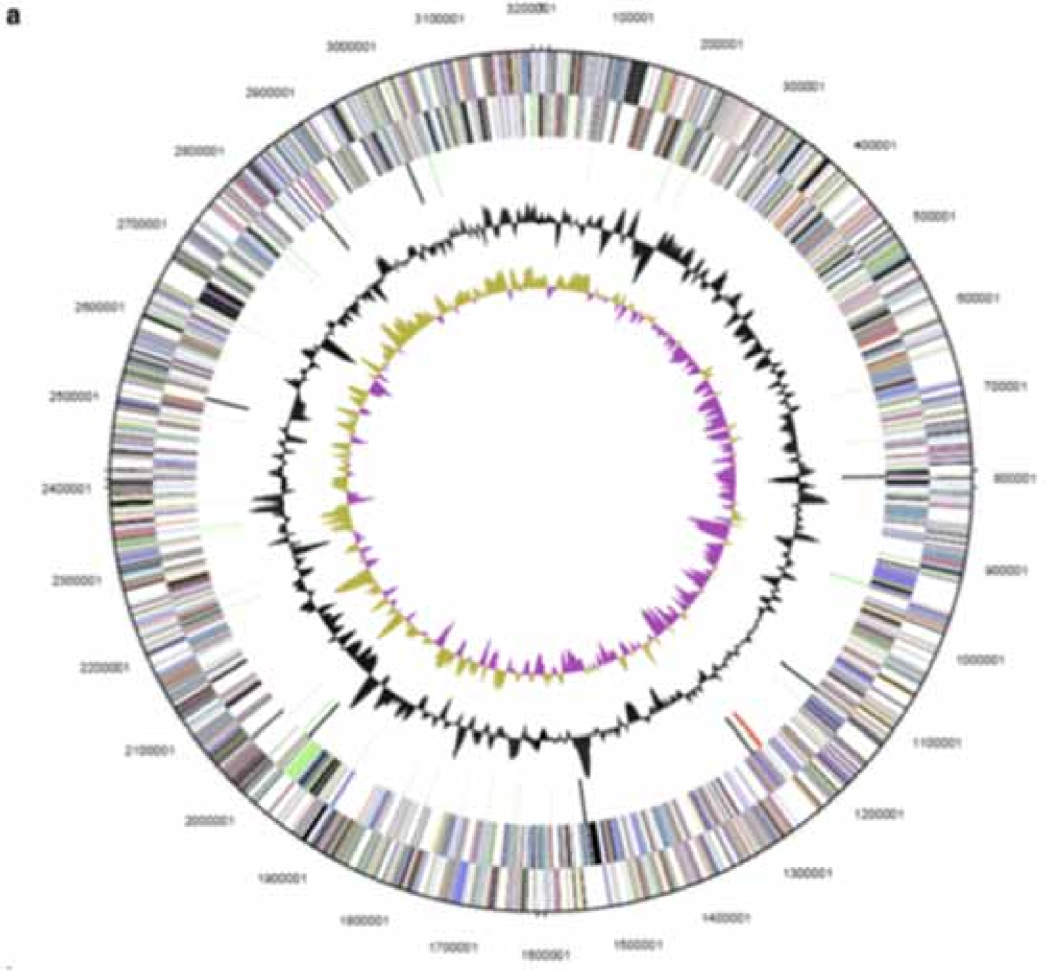
Mpi-Bremen.de

$$5NH_4^+ + 3NO_3^- = 4N_2 + 9H_2O + 2H^+$$

Or…

$$NH_4^+ + NO_2^- = N_2 + 2H_2O$$

Schepers & Raun (2008)



Graphical map of *Nitrosospira briensis C-128* genome
Rice et al (2016)

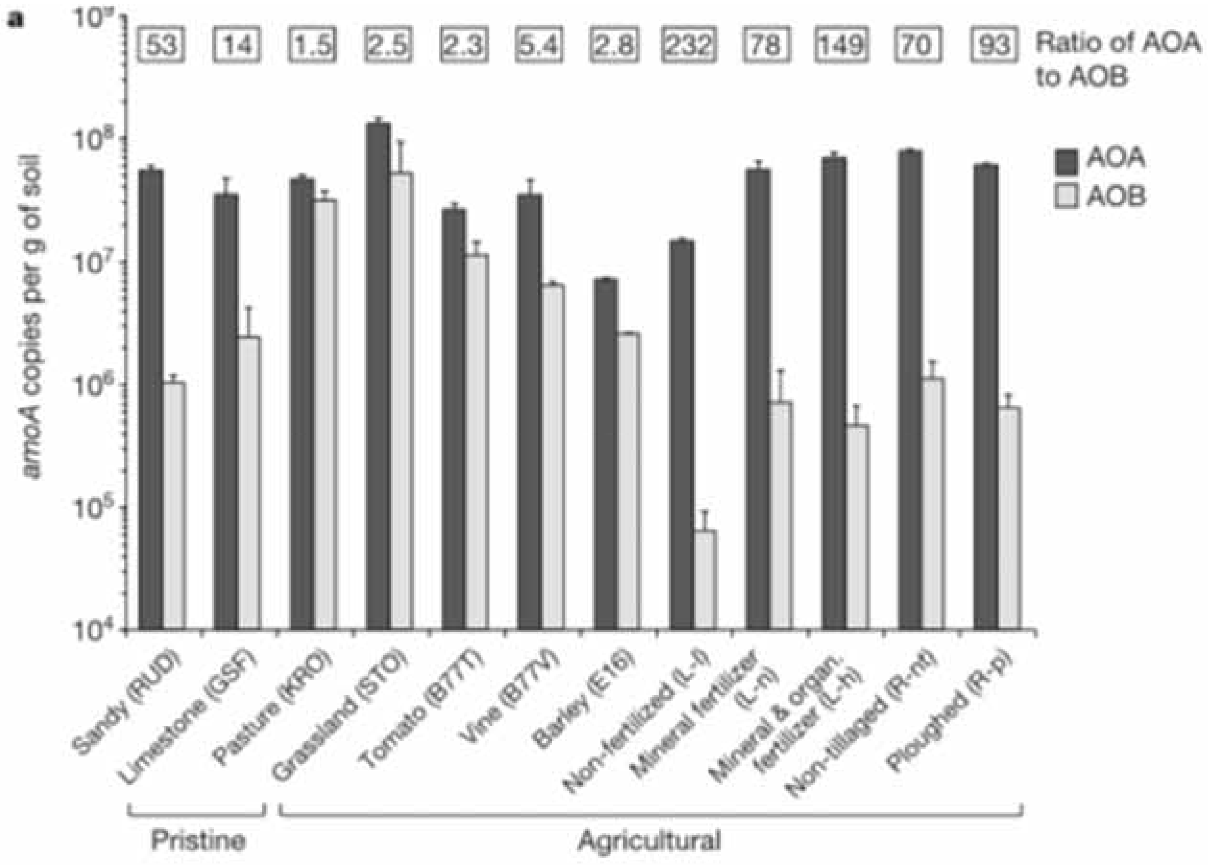
We have moved from morphological characterization toward DNA approaches that allow us to investigate certain gene clusters responsible for given functions (*amoA, hao, utp, nrxA,)*

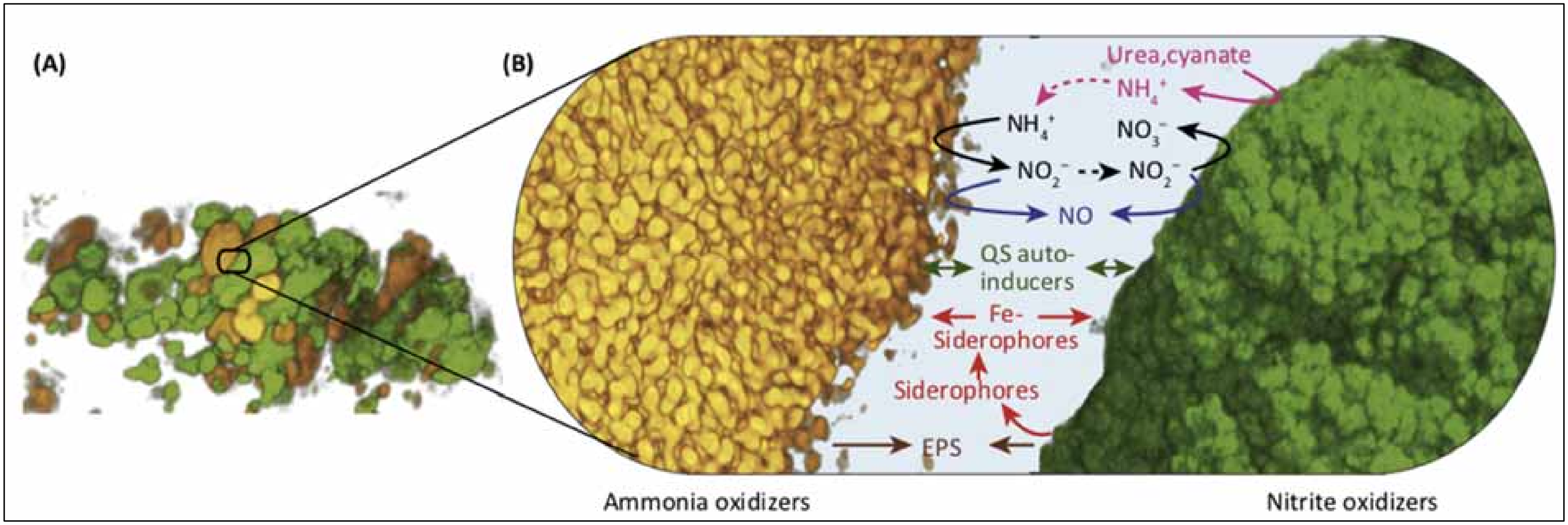
All genomes of AOB have genes encoding for four specialized proteins (Ward et al 2011)
-Ammonia monooxygenase (amo)
-hydroxylamine oxidoreductase (hao)
-cytochromes (cyt-c_{m554}) and (cyt-c_{m552})

Leininger et al (2006) “Archaea predominate among ammonia-oxidizing prokaryotes in soils”

Archaea (Crenarchaeota) are more abundant than AOB in many soils.

Some tolerate low O_2, Temp, extremes in pH, and have a broad range of substrate conc.



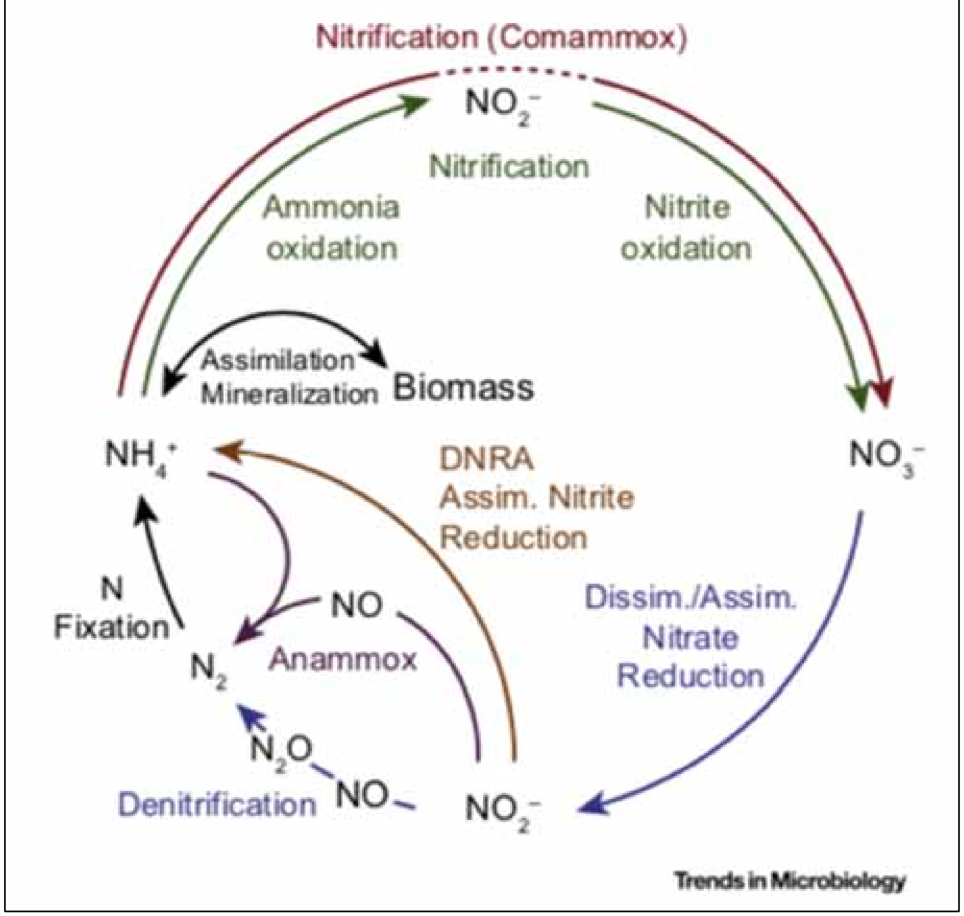


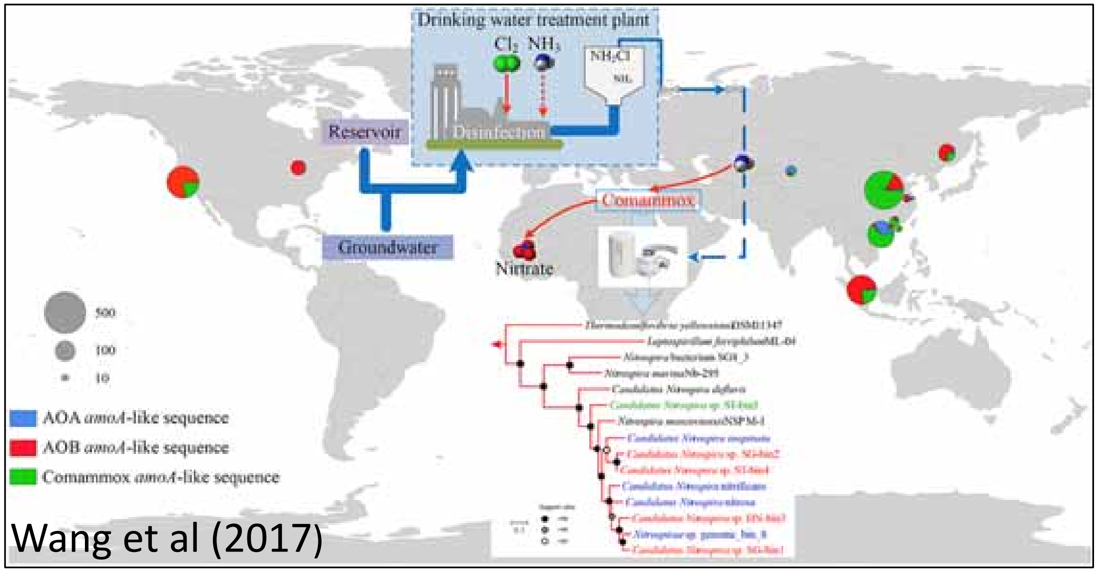
Visualization of a nitrification aggregate (Daims et al, 2016)

The *Nitrosomonas* genome does not have genes encoding for siderophores, however genes for siderophore receptors, while NOB such as *Nitrobacter* and *Nitrospira* can produce siderophores. (Fe)

New discoveries…

Daims et al, (2016)





Wang et al (2017)

- Comammox: Complete nitrifying bacteria of the genus *Nitrospira* could be a key player in the global N cycle, but there is not yet a way of quantifying contributions (Lan et al. 2020)

Soil Nitrification and Inhibitors

- History and Biochemistry Nitrification
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Influencing factors of soil nitrification

- Main factors influencing nitrification are nitrifier abundance, temperature, oxygen, moisture, pH, with substrate concentration and availability being of greatest importance. The majority of nitrification occurs in the upper soil horizons (O’Sullivan et al. 2013)

- Physical, and chemical soil properties influence microbiota at the ecosystem scale. eg: Macroaggregates, C:N ratio

- Nitrification is at maximum near field-capacity. And optimum temperatures vary between climates. Optimal pH ~8.5 (Sahrawat. 2008) .

Factors that influence nitrifier communities in soils are still being investigated…

O'Sullivan et al (2013) "*Factors affecting ammonia-oxidising microorganisms and potential nitrification rates in southern Australian agricultural soils*"

Analysis of 45 different sites in Australia concluded that

-AOA : AOB ratio depended on climate and sampling time

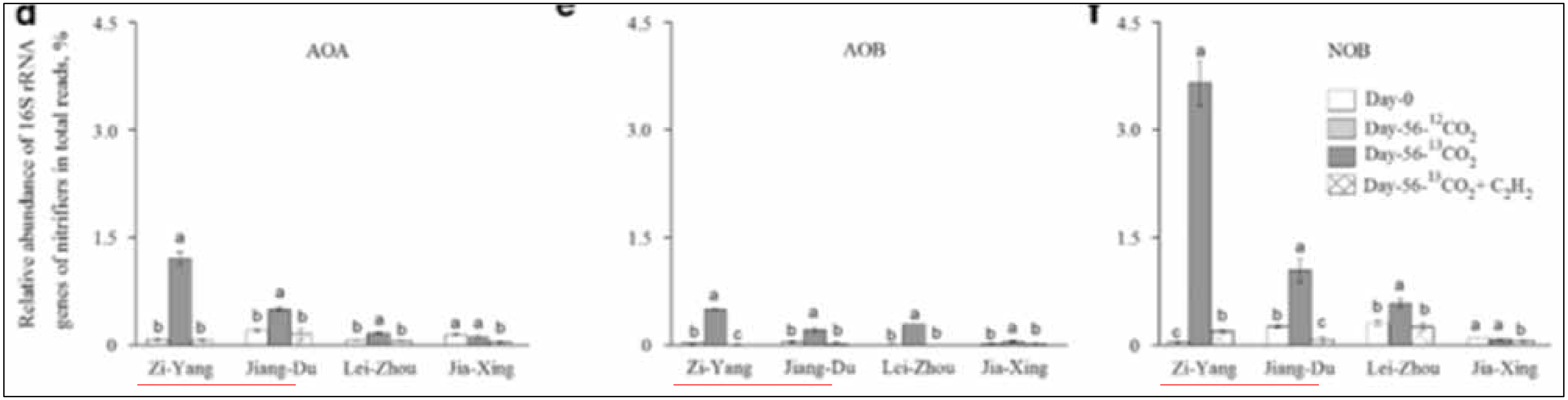
-AOB were correlated with coarse soil texture, AOA with fine texture

-No strong relationships with pH, even in acidic soils

Soil nitrification

- To investigate nitrification in soils, we can monitor changes in soil N pools and nitrifier abundance over time. Ex) Potential nitrification rates or N budget (Schepers & Raun. 2008)

- Real-time qPCR and specific *amoA* gene primers can be used to investigate which groups are most dominant in different ecosystems under certain conditions…

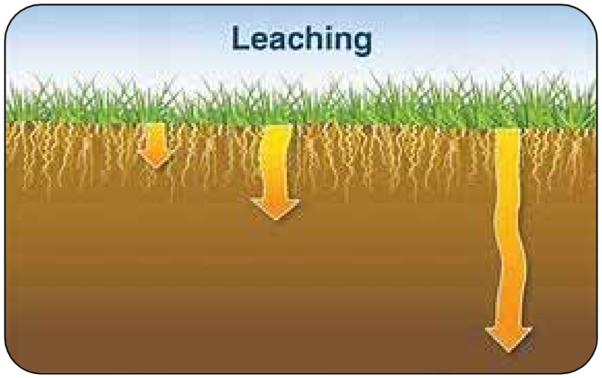


Greatest AOA labelling in high pH soil (8.23)

Wang et al, (2015)

Leaching and gaseous losses of N to the environment are highly dependent on microbial nitrification.

(Qiao et al. 2015)



$NH_4^+ \rightarrow NO_3^-$

Immobile → Mobile

Photos from :Plantcaretoday.ca , dreamstime.com, & nysgolfbmp.cals.cornell.edu/leaching

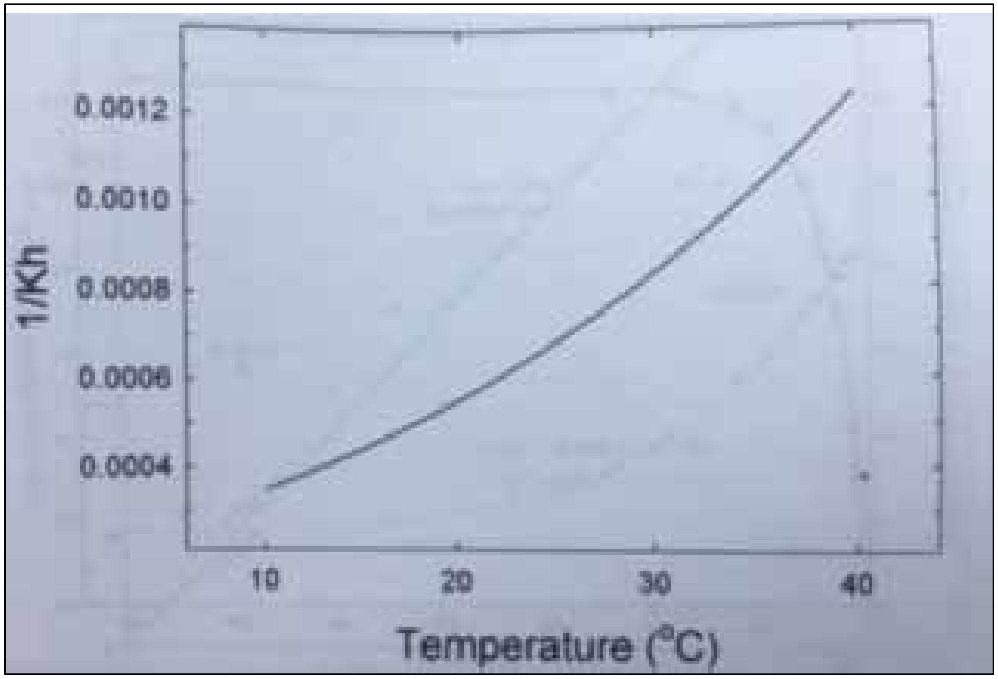
Ammonia volatilization

All N fertilizers have potential for volatilization, however urea-based fertilizers increase pH, ammonia concentration and thus volatilization (Jones et al. 2007

Moisture, Temperature, partial pressure, concentration, depth, soil organic matter, and CEC are influential factors (Jones et al. 2007)

$CO(NH_2)_2 + H^+ + 2H_2O \rightarrow$ urease $\rightarrow 2NH_4^+ + HCO_3^-$

$NH4^+ = NH_3 + H^+$



Effect of Temperature on $1/k_h$, where k_h is Henry's constant for partitioning NH_3 between solution and gas phases. (Schepers & Raun. 2008)

P/K_h = Concentration

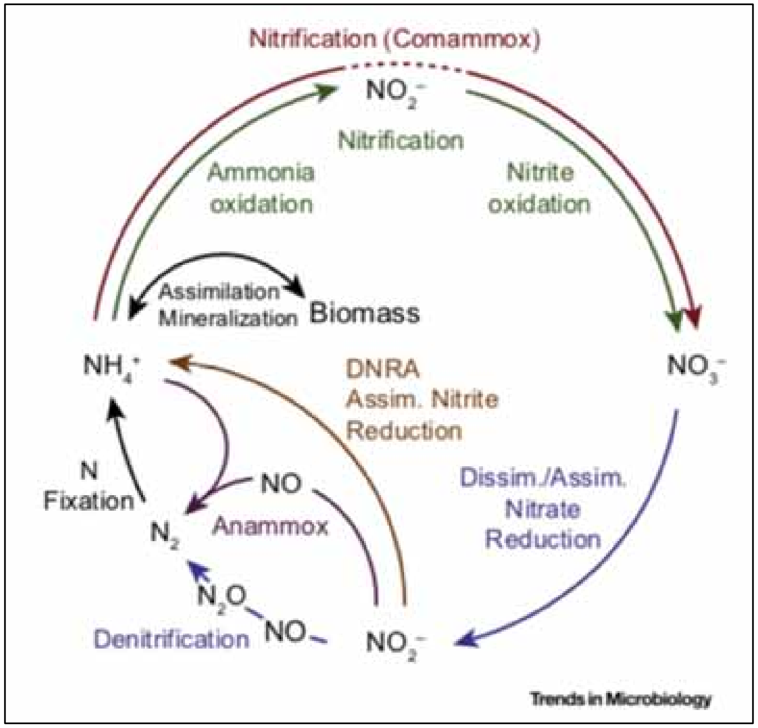
GHG emissions

N_2O is produced in small quantities via nitrite reduction. Nitrous oxide is a powerful greenhouse gas.

N_2O production tends to increase as O_2 availability decreases (Prosser, 1986)

N_2O accounts for ~3% of Canada's GHG emissions and 1-4% of applied N either directly or indirectly. (Amiro, B., Tenuta, M., Hanis-Gervais, K., Gao, X., Flaten, D., & Rawluk, C. (2017)

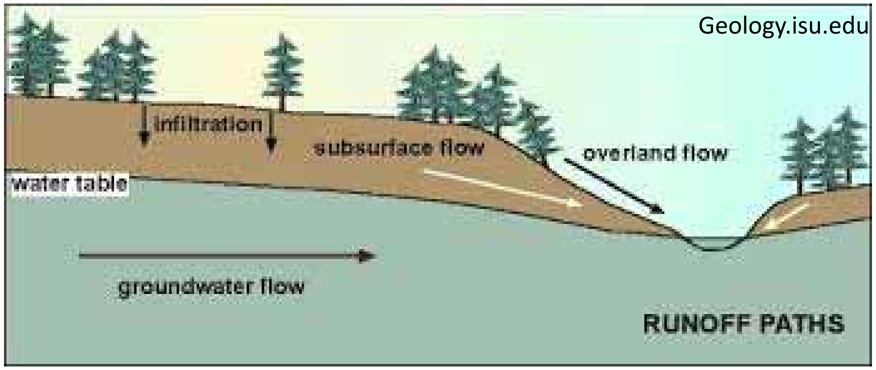
Daims et al, (2016)



Leaching and surface flow

Nitrification transforms held NH_4^+ to more mobile NO_3^-, posing risks to ground and surface water resources.

Ex) N from manure and fertilizer applications are significant nutrient inputs to Lake Winnipeg (Board. 2006).

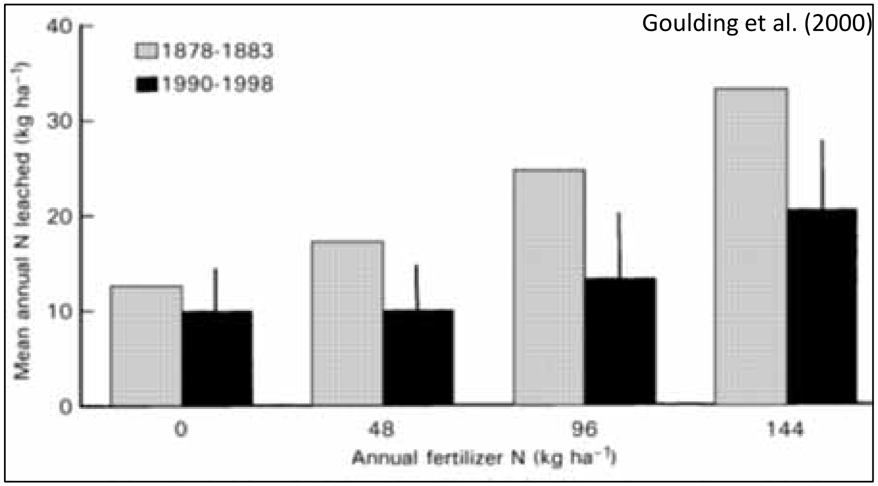


Geology.isu.edu

Goulding et al. (2000) observed higher NO_3^- concentration in spring drainage.

-Higher losses after water stressed years.

-Leaching varied greatly between years, influenced by high amounts of continuous, intense precipitation.



Goulding et al. (2000)

Soil Nitrification and Inhibitors

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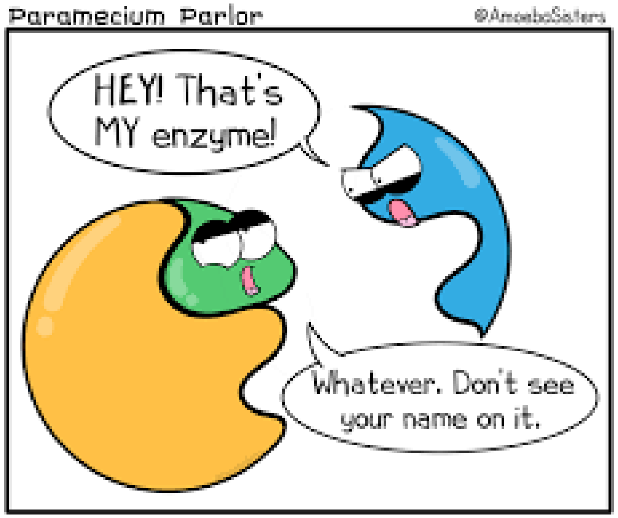
Nitrification inhibitors

- Modes of action

1) binding directly to AMO, on the active site if acting competitively
2) interference with reductant supply for oxidation (non-specific)
3) inactivation of AMO by other reactive products (McCarty, 1999)

Ex) Nitrapyrin “N-Serve” inhibits nitrification by AMO, binding to the Cu component of cytochrome oxidase.

Also inhibits methanogenesis



Competitive Inhibitors: If it fits, it sits.

amoebasisters.com

Nitrification inhibitors

- Other inhibitors…   Bactericidal vs. Bacteriostatic

Acetylene (C_2H_2) - Bacteriostatic

- Acts as a non - competitive inhibitor for autotrophic bacteria (Hynes & Knowles. 1981)
- Acetylene is an example for the third mode of action, which can be oxidized to reactive products that form covalent bonds with AMO, causing an irreversible inhibition (McCarty, 1999).

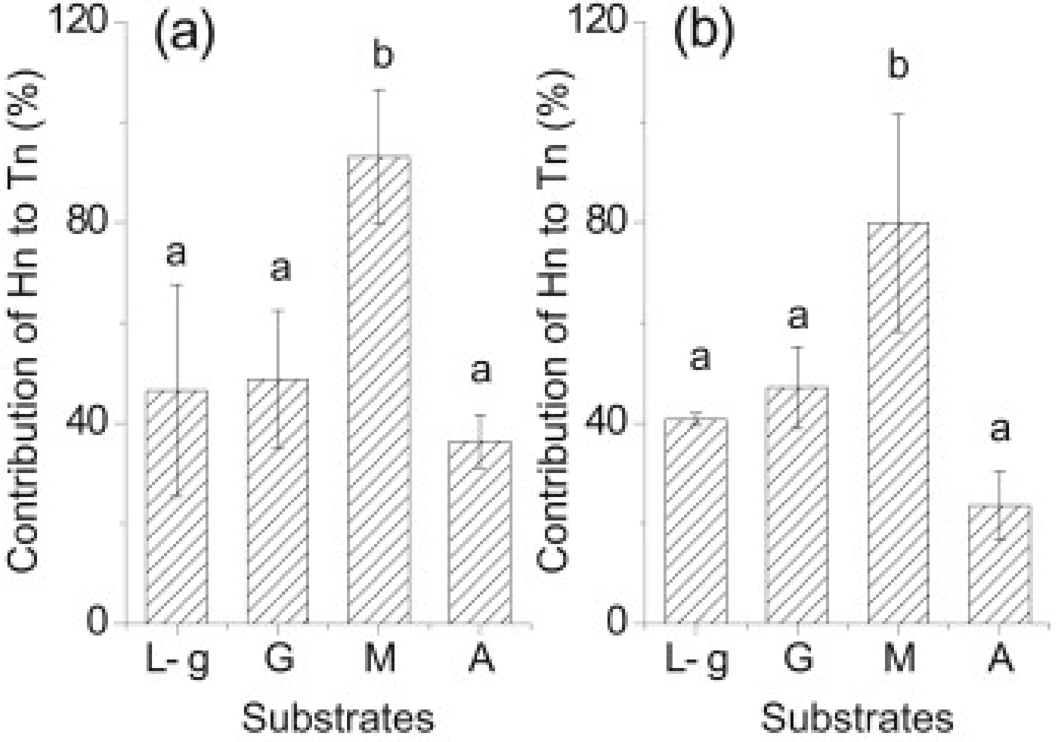
Dicyandiamide (DCD) : Bacteriostatic

- Poorly characterized MOA - suggested to prevent ammonia uptake or Cu chelation (Lehtovirta-Morley et al. 2013)

2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) : Bacteriostatic

- Reduces ecotoxicological burden with ~1/10 lower concentration compared to DCD, mode of action still under review, however function is related to AMO inhibition (Torralbo et al. 2017)

Zhang et al 2014 ” The substrate is an important factor in controlling the significance of heterotrophic nitrification in acidic forest soils”



Treatments for 2 acidic soils in China incubated with 1 Kpa acetylene for one day. (Glutamic acid, glycine, maize straw, ammonium sulphate

Acetylene has been proven to inhibit autotrophic but not heterotrophic nitrification.

High C/N ratio may enable fungal heterotrophs to outcompete autotrophic population.

Efficacy of nitrification Inhibitors

The efficacy of various inhibitors can be evaluated through persistence and bioactivity (Prosser. 1986).

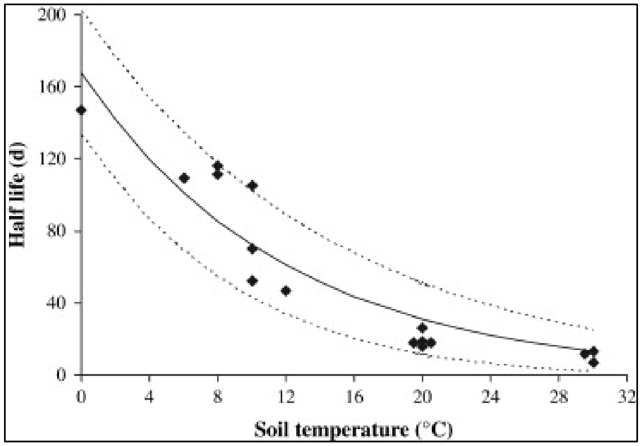
Inhibitor properties – Water solubility, volatility, sorption, stability

Soil chemical and physical properties – pH, texture, available N

Soil biological properties – Abundance, energy

Abiotic factors – temperature, moisture

Management – form of fertilizer, mode of application



Half life of DCD with temperature for incubated soils. Kelliher et al. (2008)

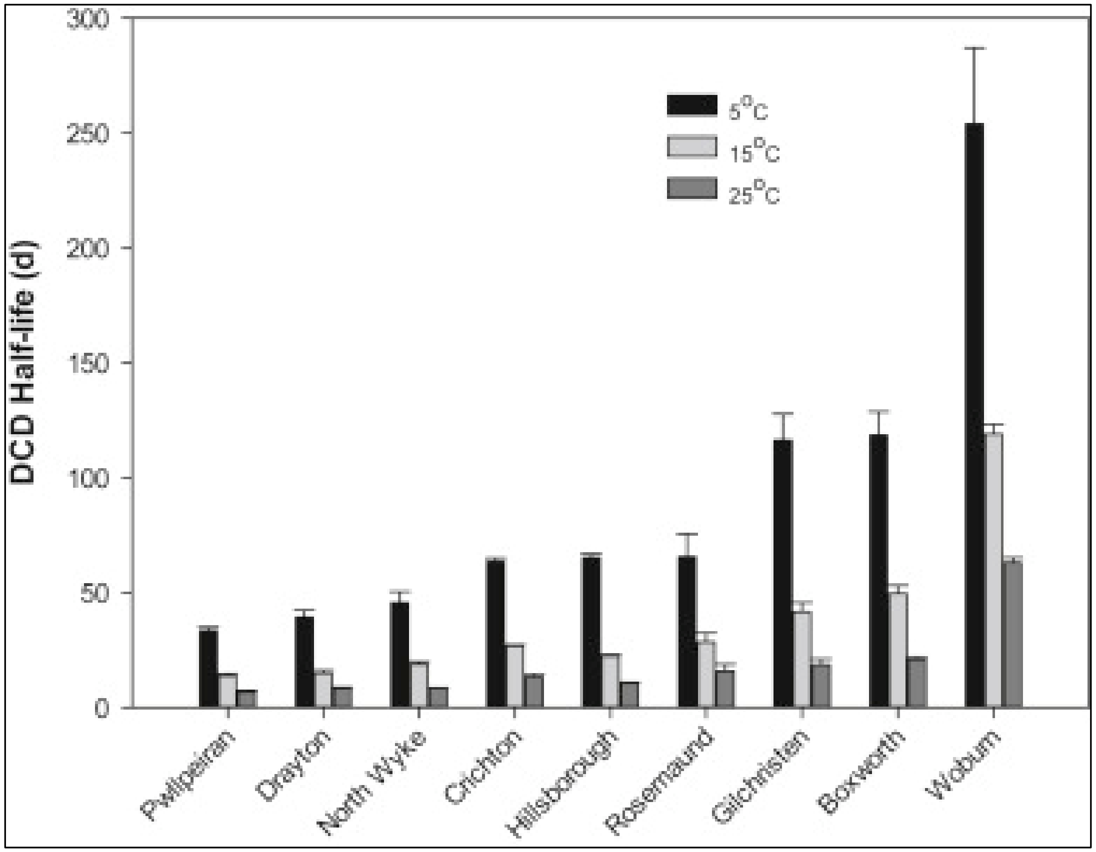
Efficacy of nitrification Inhibitors

DCD efficacy best correlated with soil Cu (-0.82) while Cu, oxalate extractable Fe and Al explained 85% of variation in efficacy.

DCD decomposition highly correlated with oxalate extractable Fe (0.82).

ex) *Nitrosomonas europaea* have a high requirement for Fe, with 90 different genes for acquisition. (Wei et al. 2006)

DCD half life for UK soils at different temperatures.



(McGeouhg et al. 2016)

Nitrification inhibitors

- Inhibitors may be useful to mitigate environmental losses if we can match the inhibitor with N applications. Can they maintain yield and give economic incentive for growers to use them?

- A small survey showed a small percentage of agronomist in Manitoba (13%) would use and/or recommend slow release, urease, or nitrification inhibitor N sources. (Amiro et al. 2017)

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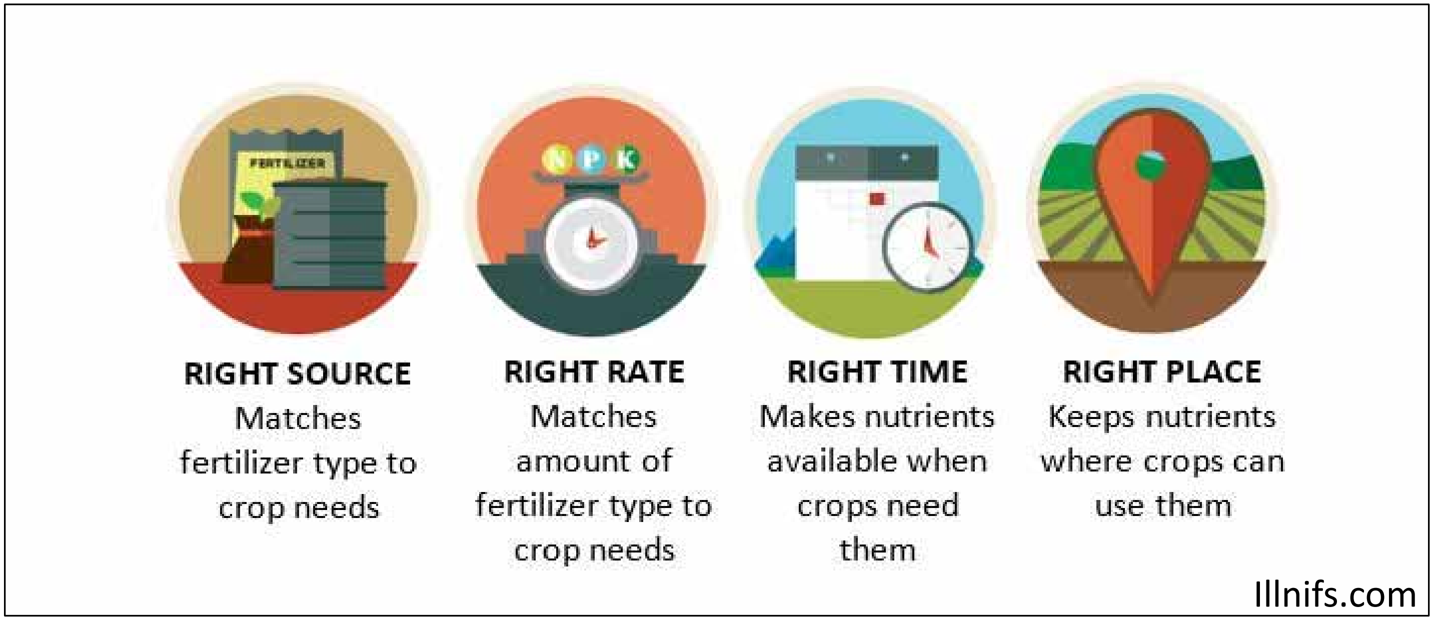
4R N management

- Guides Best Management Practices in different regions to boost nutrient use efficiency, sustainability, and profitability in ag productions (Johnston & Bruulsema. 2014)

- Due to low cost and availability, urea is the most widely used fertilizer applied to Corn, globally . (Pawlick et al. 2019)



RIGHT SOURCE
Matches fertilizer type to crop needs

RIGHT RATE
Matches amount of fertilizer type to crop needs

RIGHT TIME
Makes nutrients available when crops need them

RIGHT PLACE
Keeps nutrients where crops can use them

Illnifs.com

Inhibitor case studies (GHG)

- Polyolefin-coated Urea at 280 kg N ha-1 reduced NO3- leaching by 34–49% and in wet years increased tuber yield by 12–19% in comparison to three applications of urea. (Zvomuya et al. 2003)

- In a grazed diary pasture, DCD reduced NO_3^- leaching by 76% and N2O emissions by 82%, also increasing forage yield by more that 30%. (Di & Cameron. 2005)

- Reduction of NH3 volatilization significantly greater in fall (65%) than in spring (40%) and was more effective on urea (61%) compared to UAN (43%). (Lasisi et al. 2020)

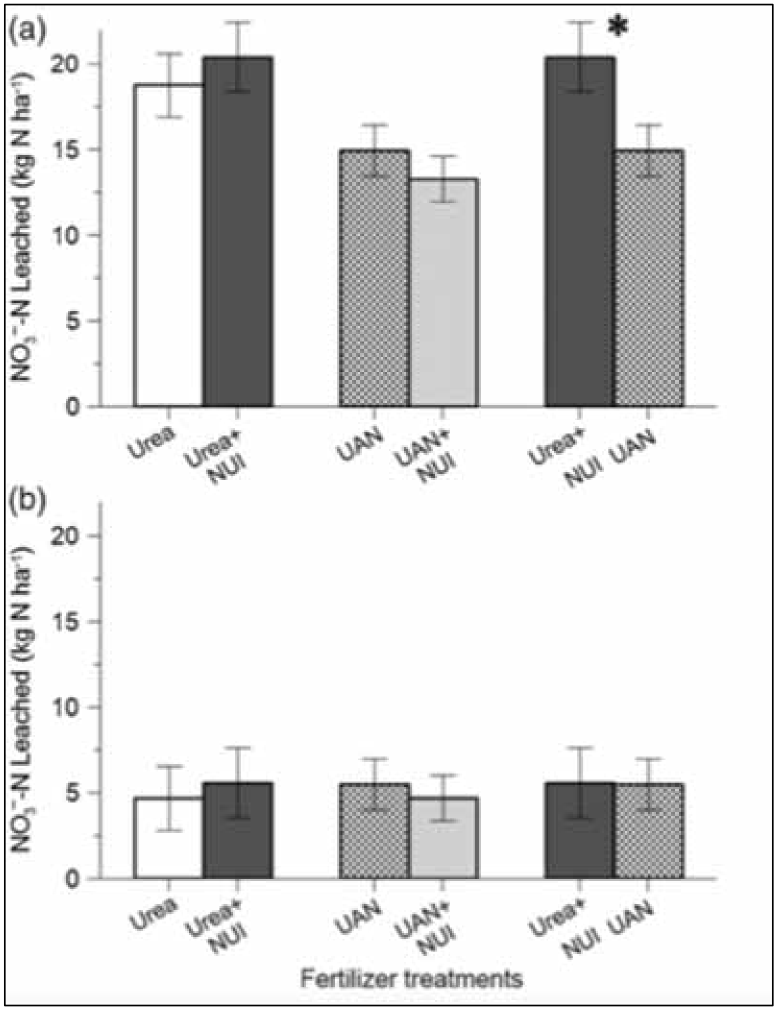
Pawlick et al (2019)

Water balance method : D = P − ET − ΔS

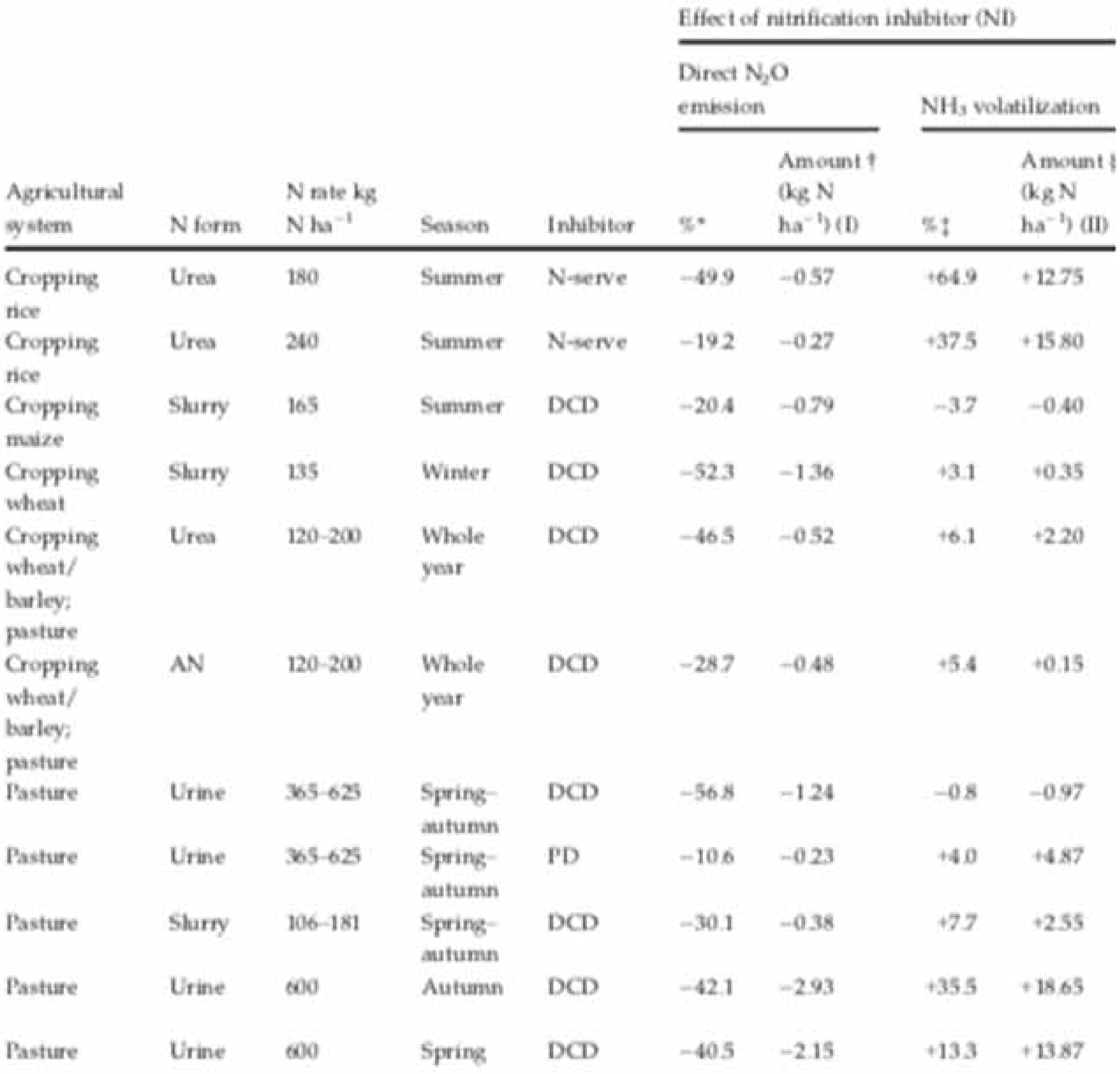
-Pore-water samplers, water capacitance probes and Eddy-covariance were used to estimate drainage and nitrate leaching from corn with applied Urea, UAN, Urea+ NUI, UAN +NUI.

-Significant differences in crop yield resulted in greater NO_3^- concentration in soil water for 2015 compared to 2016.

-Delayed side-dress UAN applications significantly decreased leaching compared to broadcast incorporated Urea + NUI.



Lam et al. (2017)



					Effect of nitrification inhibitor (NI)			
					Direct N_2O emission		NH_3 volatilization	
Agricultural system	N form	N rate kg N ha^{-1}	Season	Inhibitor	%*	Amount † (kg N ha^{-1}) (I)	%‡	Amount (kg N ha^{-1}) (II)
Cropping rice	Urea	180	Summer	N-serve	−49.9	−0.57	+64.9	+12.75
Cropping rice	Urea	240	Summer	N-serve	−19.2	−0.27	+37.5	+15.80
Cropping maize	Slurry	165	Summer	DCD	−20.4	−0.79	−3.7	−0.40
Cropping wheat	Slurry	135	Winter	DCD	−52.3	−1.36	+3.1	+0.35
Cropping wheat/barley; pasture	Urea	120-200	Whole year	DCD	−46.5	−0.52	+6.1	+2.20
Cropping wheat/barley; pasture	AN	120-200	Whole year	DCD	−28.7	−0.48	+5.4	+0.15
Pasture	Urine	365-625	Spring-autumn	DCD	−56.8	−1.24	−0.8	−0.97
Pasture	Urine	365-625	Spring-autumn	PD	−10.6	−0.23	+4.0	+4.87
Pasture	Slurry	106–181	Spring-autumn	DCD	−30.1	−0.38	+7.7	+2.55
Pasture	Urine	600	Autumn	DCD	−42.1	−2.93	+35.5	+18.65
Pasture	Urine	600	Spring	DCD	−40.5	−2.15	+13.3	+13.87

Nitrification inhibitors reduced N2O emissions in many cases, however NH_3 volatilization increased in-turn.

This has implications for indirect GHG emissions with NH_3 deposition and denitrification of leached nitrate.

Qiao et al. 2015

Examined results from 62 nitrification inhibitor studies

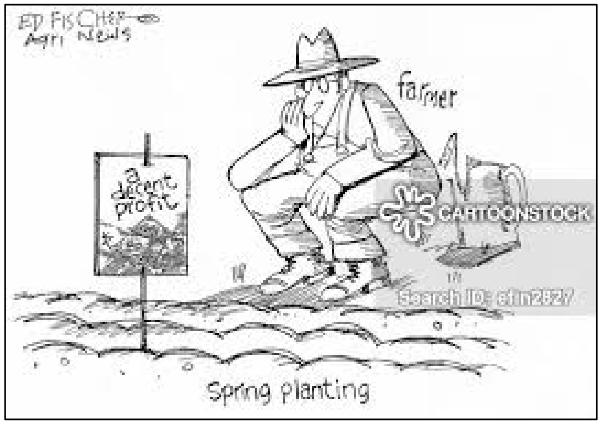
Overall increased NH_3 volatilization (20% mean)

Decreased N leaching (48%)

Decreased N_2O emissions (44%) and NO emissions (24%)

Total 16.5% decrease of N to the environment

Estimates that applying nitrification inhibitors to a typical Corn field could increase revenues ~9%.



In Conclusion

- Its complicated…

- With more advanced technologies we know more about nitrifier communities and inhibitors than in the past, however it seems there is still much to learn.

- Inhibitors can reduce losses to the environment and boost yields in many circumstances.

- Further research on microbial communities and interaction with various inhibitors, alongside research that combines this information with management practices in the field will move us further toward our goals.

We need a lot in life, but Nitrogen is vital
If we don’t get it right, we all might go in cycles
I need some fresh air, not the type to go in rifles
It’s easy to lose it we have no right to be entitled

It’s in this very place, in the ceiling, in my head
In many ways we take it from beginning to the end
From the mind, body, soul, from the soil into the stem
Most of them get older and don’t focus on their friends

But maybe we can grow, we can get the world to size up
Get some seed sowing, need a bit of fertilizer
Maybe it’s the season we can show them the horizons
Making these synthetic relationships mycorrhizal

I don’t know my fate but I’ll make it with the right goals
Sources say this place doesn’t wait at the rate that time goes
By and though I’m needed I keep leaving on a high note
Seeking innovation and that’s really all that I wrote

Kody O

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