Agriculture and Food Development Authority

Assessing the impact of urease and nitrification inhibitor use on microbial community composition, diversity and function in grassland soil

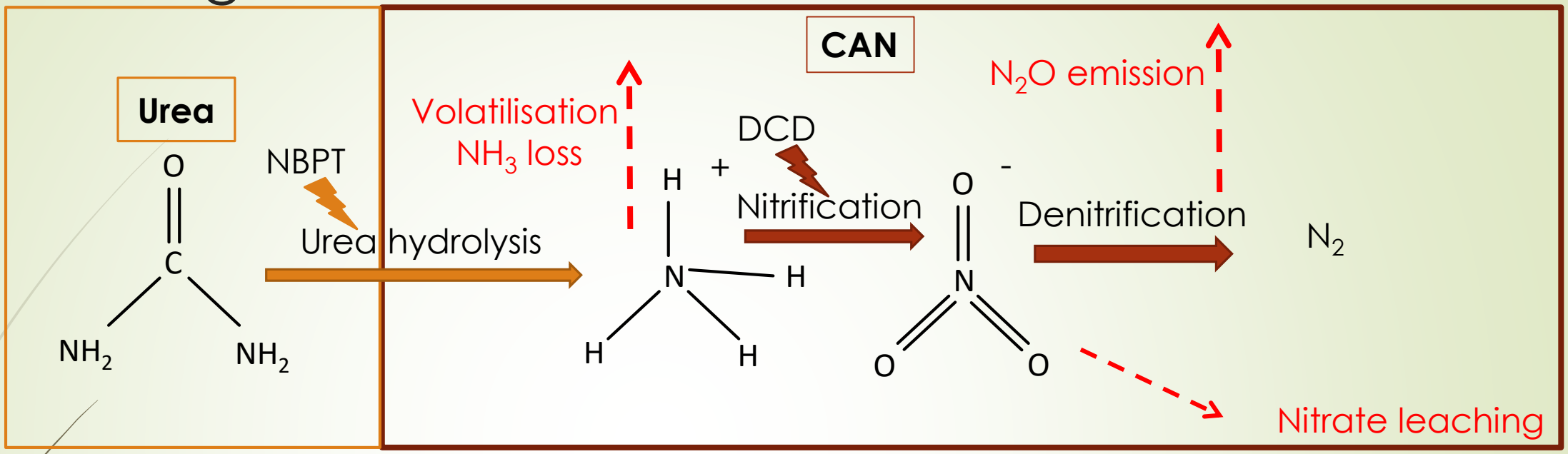
Aoife Duff; Patrick Forrestal; Israel Ikoyi and Fiona Brennan

EU sustainability targets

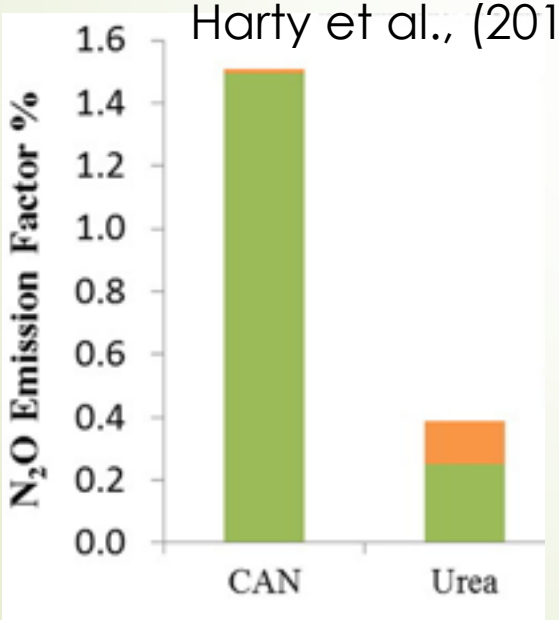
GHG emissions	↓	55% by 2030 compared to 1990 levels
Nutrient Loss	↓	50% by 2030 compared to 1990 levels
Fertiliser Use	↓	20% by 2030 compared to 1990 levels
Ammonia emissions	↓	exceeding the emission ceiling of 116 kilotonnes by 14.4%

European Commission

Synthetic Nitrogen Fertilisers



Harty et al., (2016)



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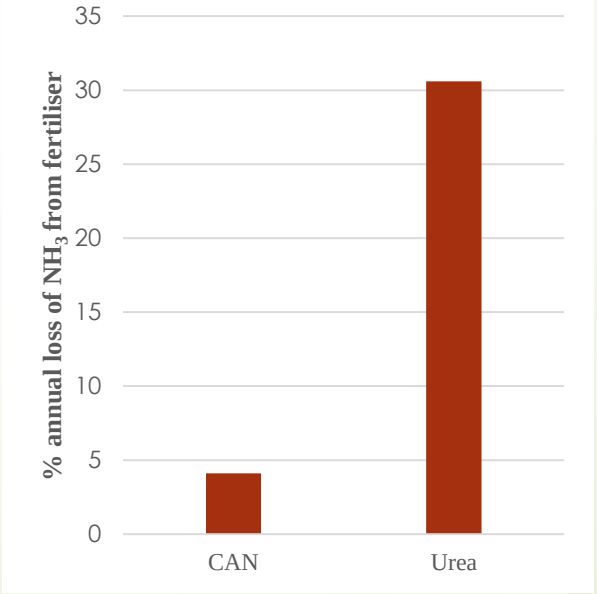
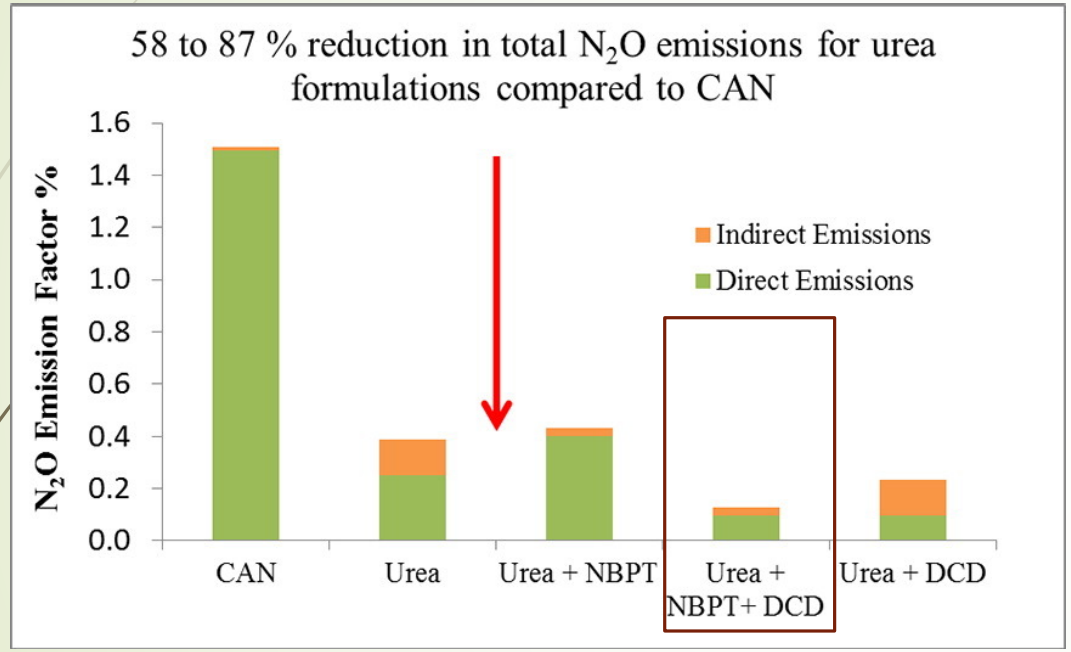


One way to try and meet the EU sustainability targets is to improve Nitrogen fertiliser efficiency

Harty et al., (2016)



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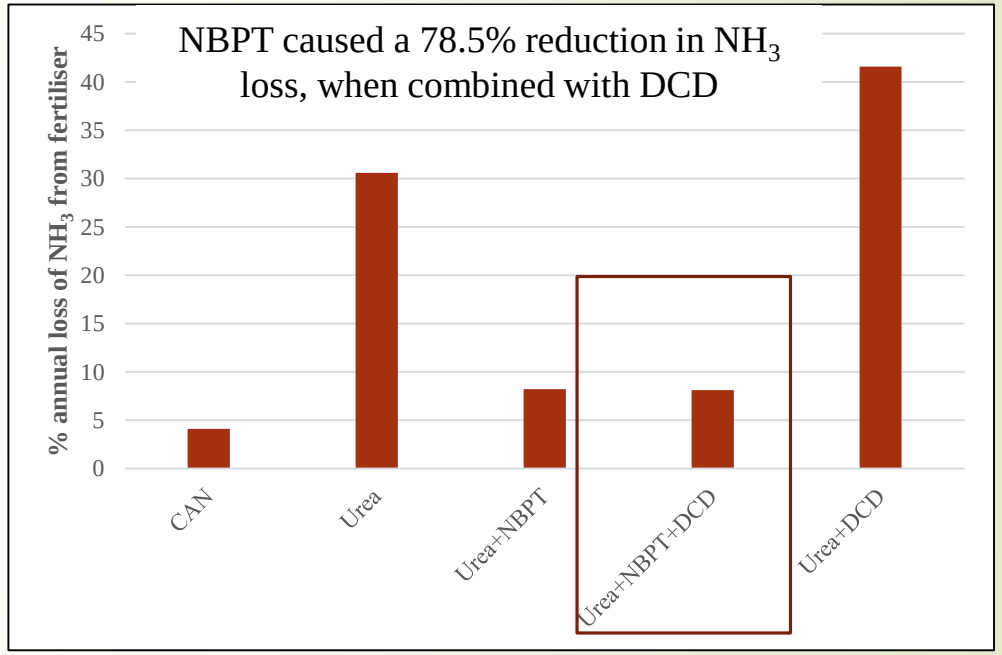


- **The use of inhibitors with Urea is mandated** in Ireland and other EU countries

Micro-organisms and Soil

CO_2

N_2O

Microbes in the soil deliver critical ecosystem functions:

- They mediate all steps of the N and C cycles
- They mediate GHG emissions
- C sequestration
- Plant soil microbial interactions
- Plant disease
- Plant health

Research questions

1. Is there an impact of **fertiliser** and or **inhibitor** use on overall microbial **abundance and function** in the **long-term**?

2. How is the nitrogen cycling **functional community** impacted by the use of N fertiliser and inhibitors?

3. What is the impact of N fertiliser formulation and inhibitor use on microbial **community composition and structure**?

Experimental design

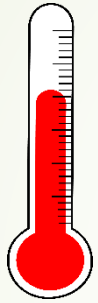
- **Experimental plot located in Johnstown castle research centre Wexford Ireland.**
- Randomised complete block with **five replicates**
- Fertiliser has been applied in **five equal split applications** during the growing season for **5 years** and continuing
- Total amount applied per year was 200 N kg/ha with 190 kg/ha in the year of sampling -year 5

Methodology

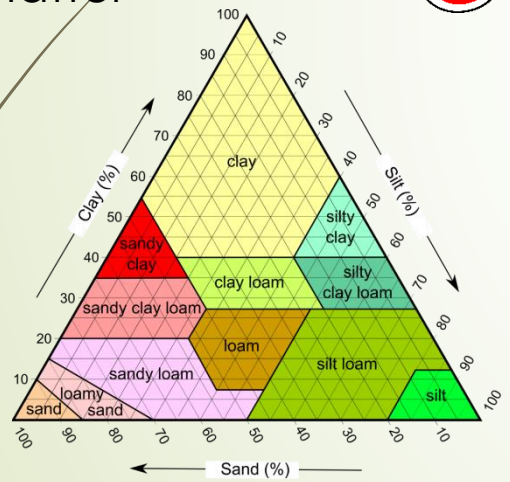
soil sample

Soil physicochemical parameters

N
P K



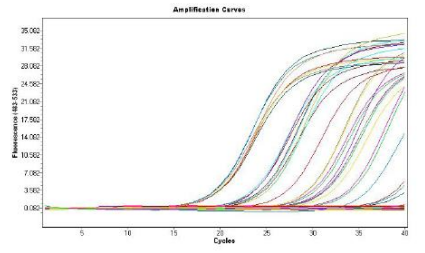
Organic matter



Soil classification

DNA extraction

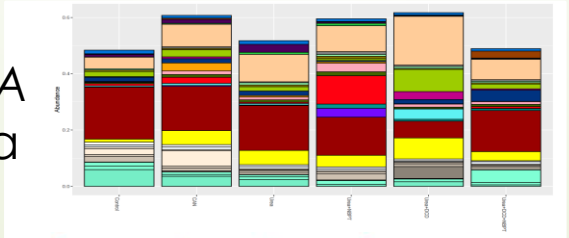
Gene abundances



Quantitative PCR

Amplicon sequencing

16S rRNA
Bacteria



ITS
Fungi

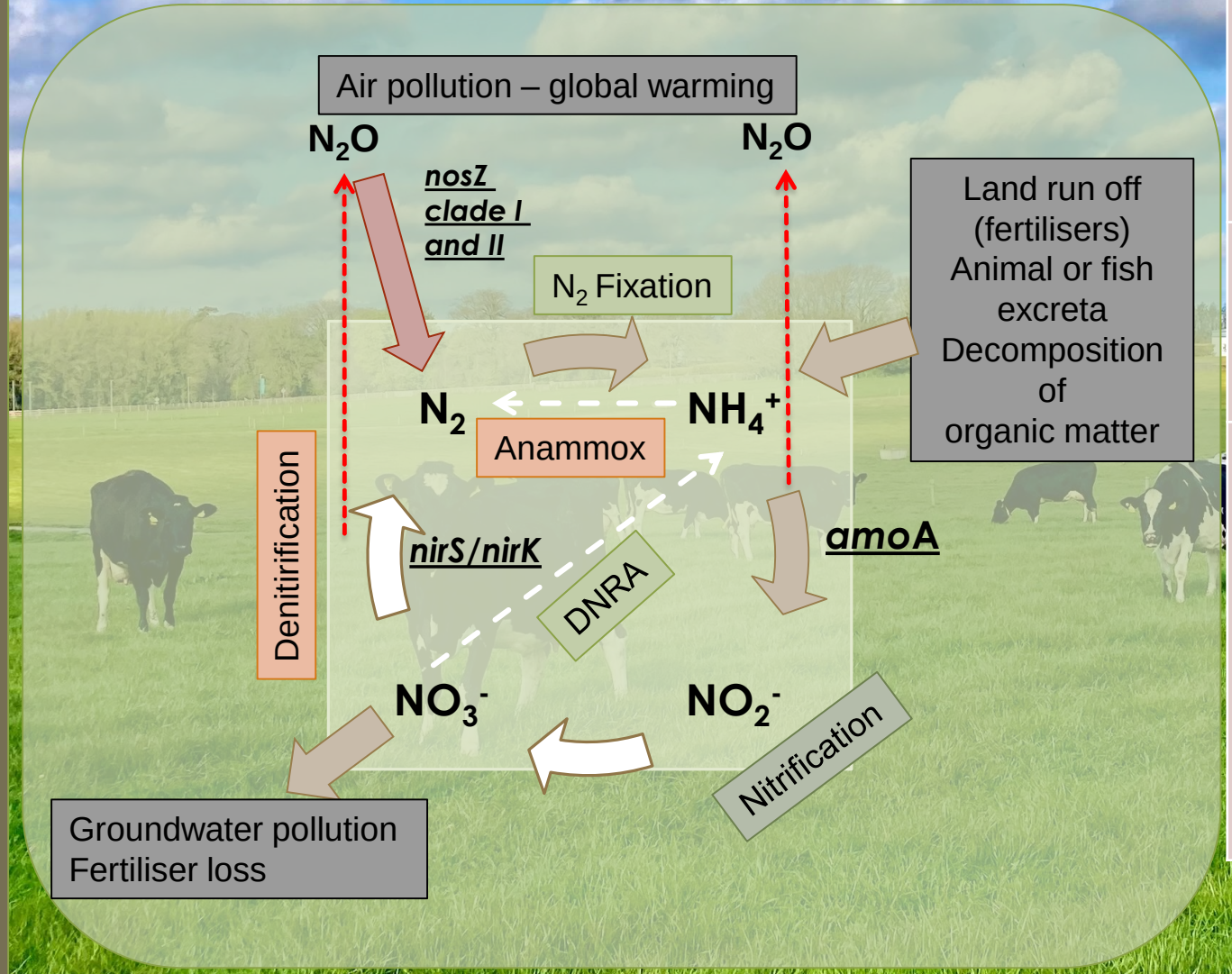
Functional assays

Potential Nitrification assay (PNA)

Potential Denitrificiation assay (PDA)

1. Is there an impact of fertiliser and or inhibitor use on overall microbial abundance and function in the long-term?

Nitrogen cycle & genes



	Gene	Function	Organism
Total Community	16S *rRNA*	ribosomal RNA	Bacteria & Thaumarchaea
	ITS	Internal transcribed spacer	Fungi
Nitrifier Community	*amoA*	RLS of 2- step nitrification	Archaea and Bacteria
	amoA	COMMAMOX 1-step nitrification	Bacteria
Denitrifier Community	*nirS*	Nitrite reducer	Bacteria
	nirK	Nitrite reducer	Bacteria
	nosZ clade I	Nitrous oxide reducer	Bacteria
	nosZ clade II	Nitrous oxide reducer	Bacteria

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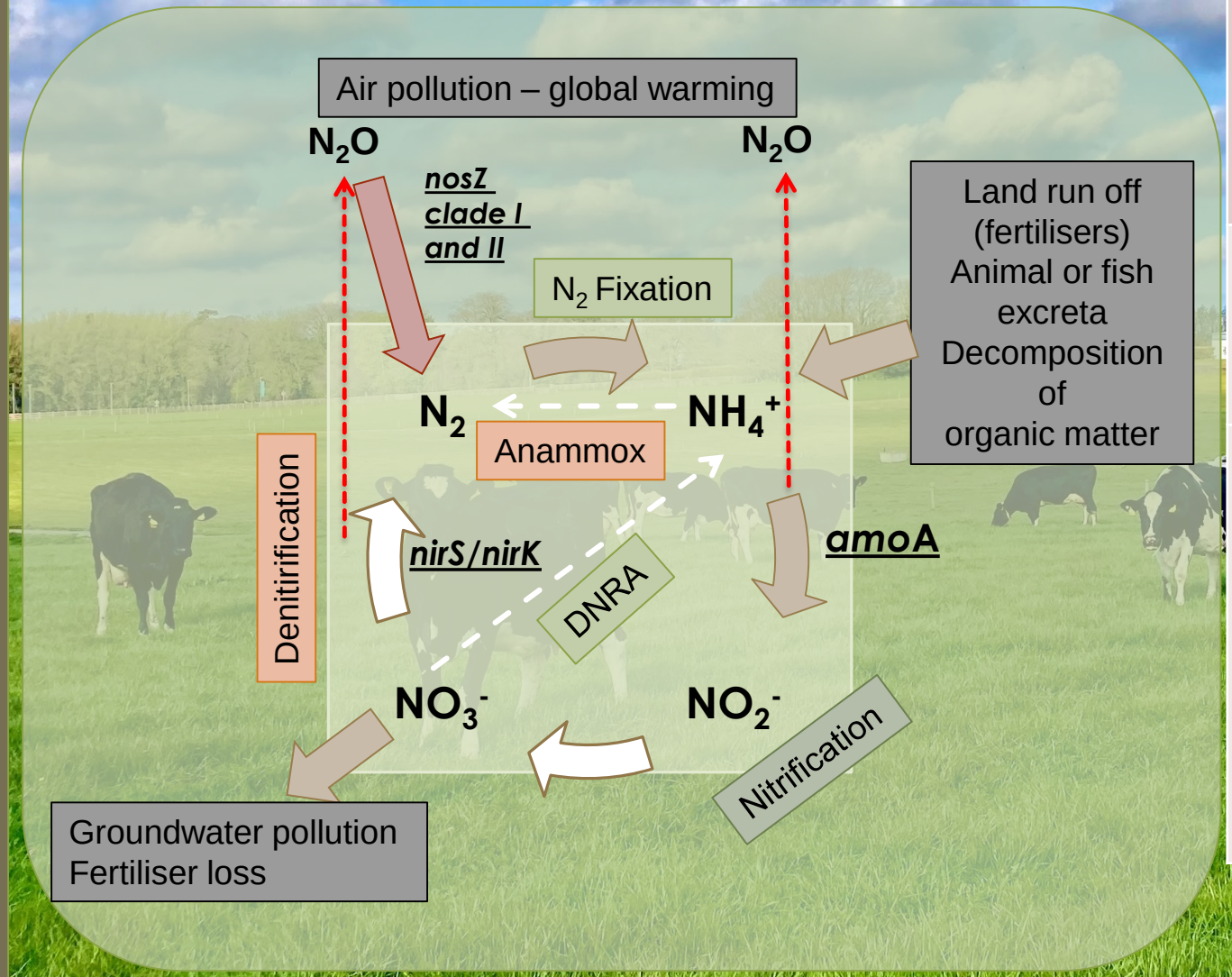

	Gene	Function	Organism	Impact	
Total Community	16S *rRNA*	ribosomal RNA	Bacteria & Thaumarchaea	**Fert**	**Inhi**
				No	No
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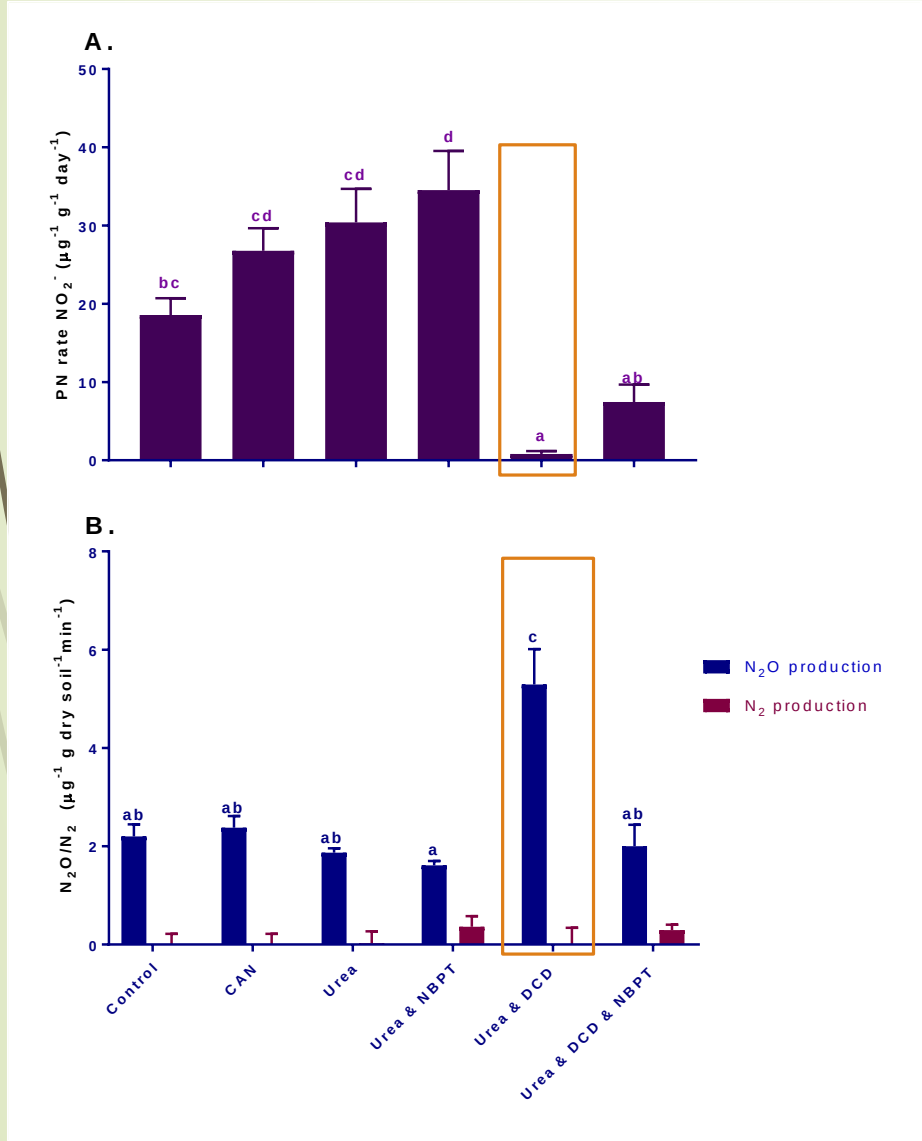
	nirK	Nitrite reducer	Bacteria	No	No
	nosZ clade I	Nitrous oxide reducer	Bacteria	No	No
	nosZ clade II	Nitrous oxide reducer	Bacteria	No	No

2. How is the nitrogen cycling **functional community** impacted by the use of N fertiliser and inhibitors?

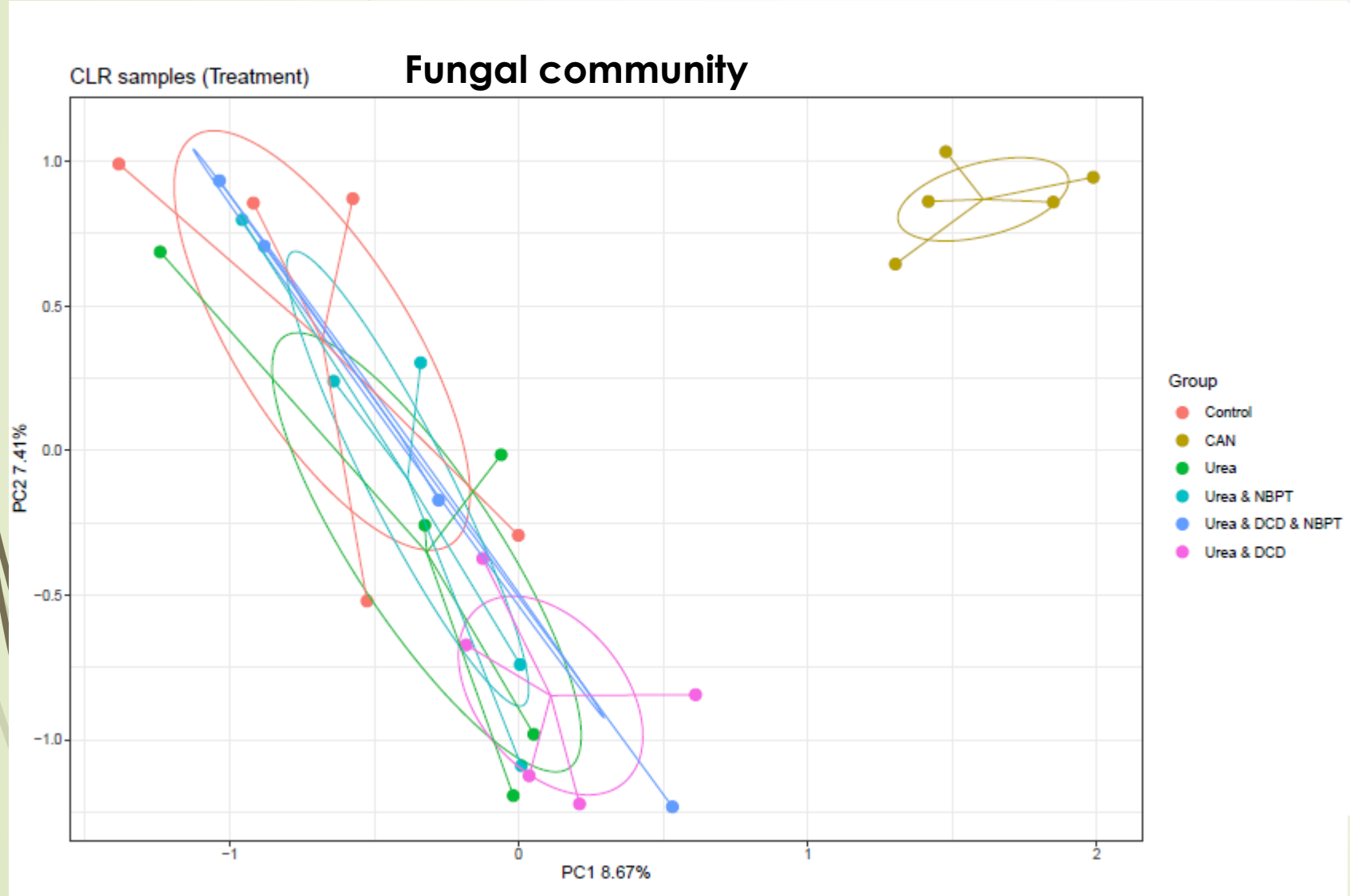


- No fertiliser effect
- Significant effect of DCD inhibitors on the potential to nitrify and denitrify
- No effect of NBPT on potential nitrify and denitrify
- N_2 production overall is quite poor

3. What is the impact of N fertiliser formulation and inhibitor use on microbial **community composition and structure**?

PCA

- There was no significant differences between treatments for 16S rRNA
- For ITS we see a significant difference between the control, CAN and each treatment - pairwise Adonis $P < 0.05$



Some examples of significantly different genus between treatments are:

- ***Clonostachys*** - has biological control ability against numerous fungal plant pathogens
- ***Glomus*** - form arbuscular mycorrhizae
- **Tylospora –** ectomycorrhizal fungi that includes species that can grow on nitrogen sources and produce N_2O and CO_2

Conclusion

- Bacterial and Fungal communities **were not** significantly affected by inhibitor treatments with long-term usage
- Microbial function and the abundance of nitrogen cycling communities were **mainly unaffected** by fertiliser or inhibitors
- The application of Urea or CAN **did not** change bacterial composition or diversity, but **did** alter fungal community structure
- Overall, we find that the effect of fertilisation on the microbial community is greater than the impact of inhibitor use.

Duff et al., (2022) - *Soil Biology and Biochemistry*

Thank you for listening