

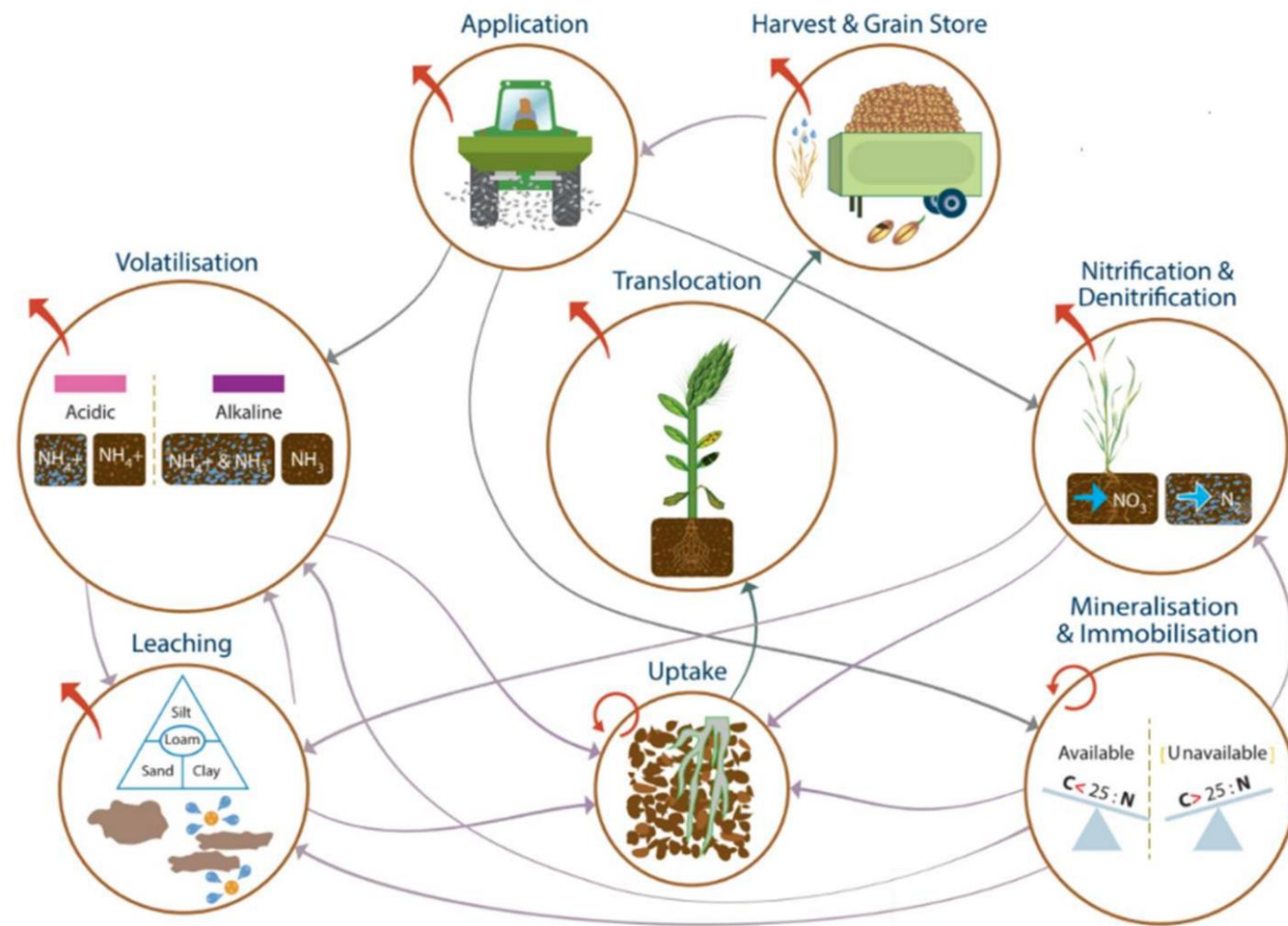
What you need to know about Nitrogen

Joel Williams

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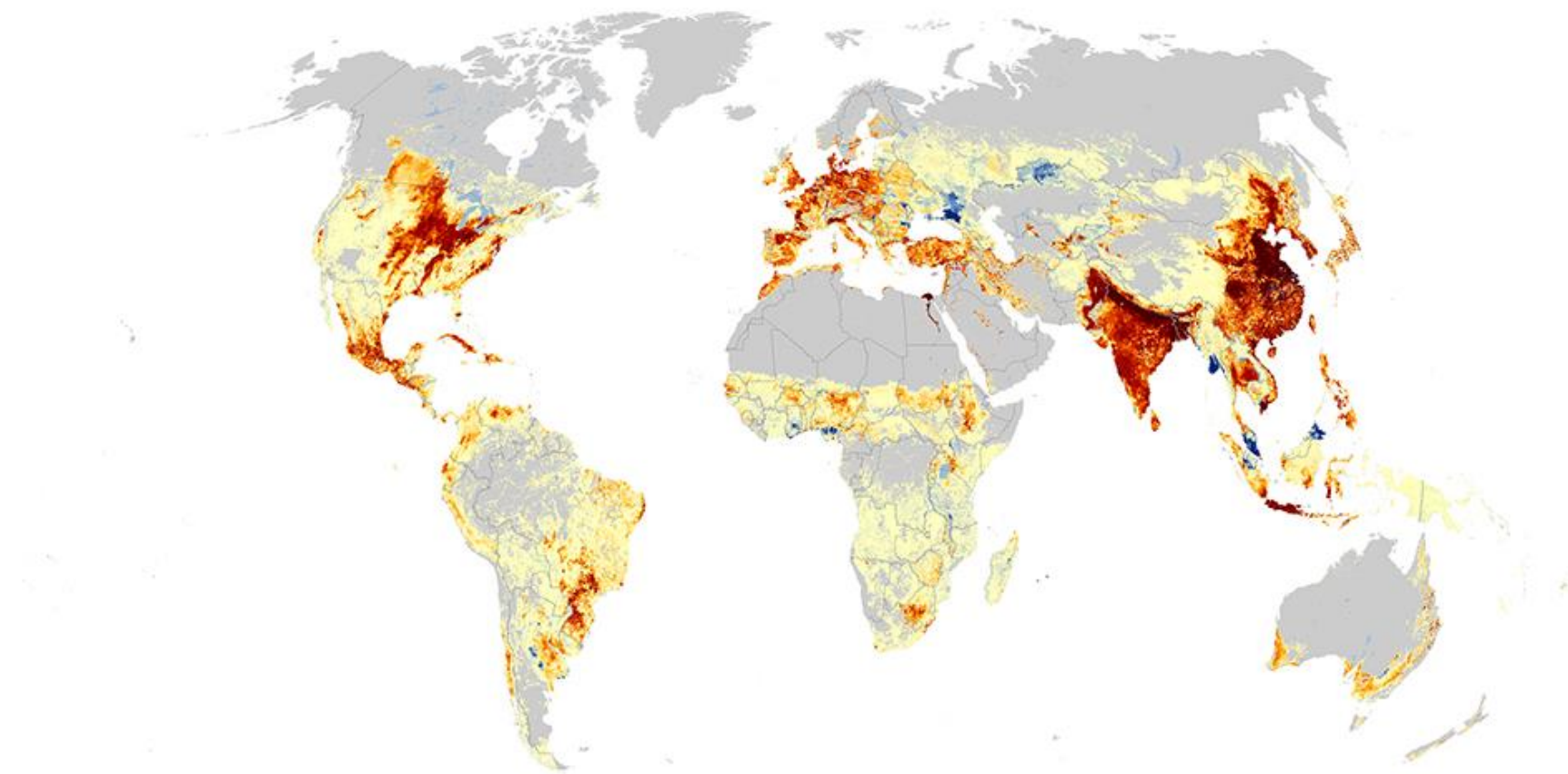




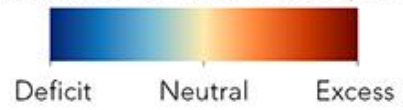


Nitrogen in the Soil

- Ammonia volatilisation
- Nitrous oxide denitrification
- Nitrate leaching



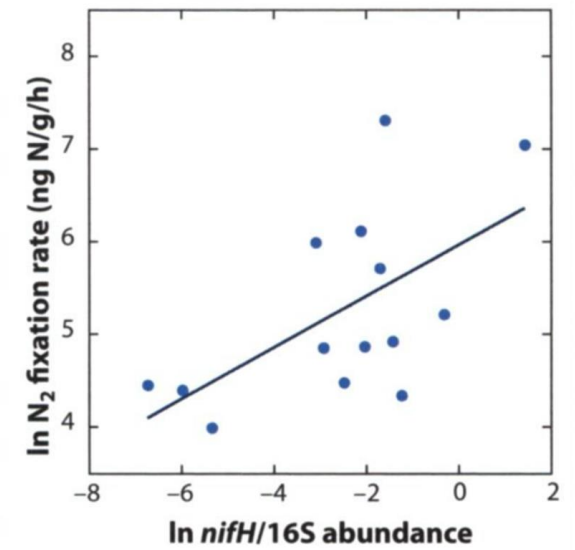
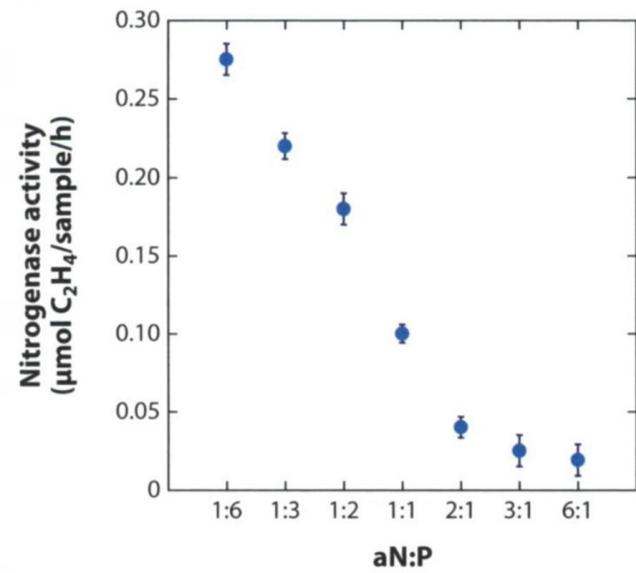
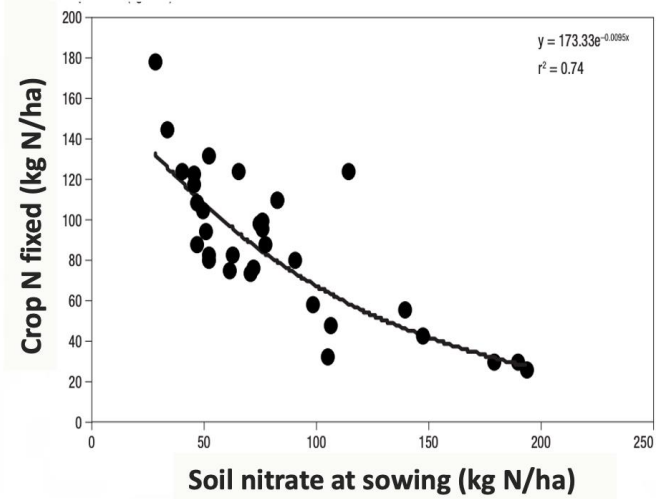
Nitrogen balance on the landscape: 140 crops





Excess N?

- Nutrient imbalances in plants
 - Decline in plant/community health (agricultural and ecological)
- Loss of biodiversity
- Soil carbon fluxes
- GHG
- Contamination of waterways
 - Low oxygen = algal blooms
 - Acidification of water bodies
- Human health (drinking water)



Suppressed N fixation and diazotrophs after four decades of fertilization

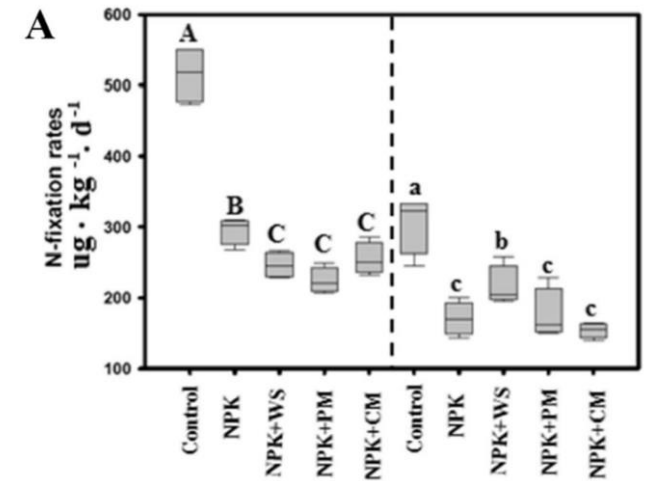
Abstract

Background: N fixation is one of the most important microbially driven ecosystem processes on Earth, allowing N to enter the soil from the atmosphere, and regulating plant productivity. A question that remains to be answered is whether such a fundamental process would still be that important in an over-fertilized world, as the long-term effects of fertilization on N fixation and associated diazotrophic communities remain to be tested. Here, we used a 35-year fertilization experiment, and investigated the changes in N fixation rates and the diazotrophic community in response to long-term inorganic and organic fertilization.

Results: It was found that N fixation was drastically reduced (dropped by 50%) after almost four decades of fertilization. Our results further indicated that functionality losses were associated with reductions in the relative abundance of keystone and phylogenetically clustered N fixers such as *Geobacter* spp.

Conclusions: Our work suggests that long-term fertilization might have selected against N fixation and specific groups of N fixers. Our study provides solid evidence that N fixation and certain groups of diazotrophic taxa will be largely suppressed in a more and more fertilized world, with implications for soil biodiversity and ecosystem functions.

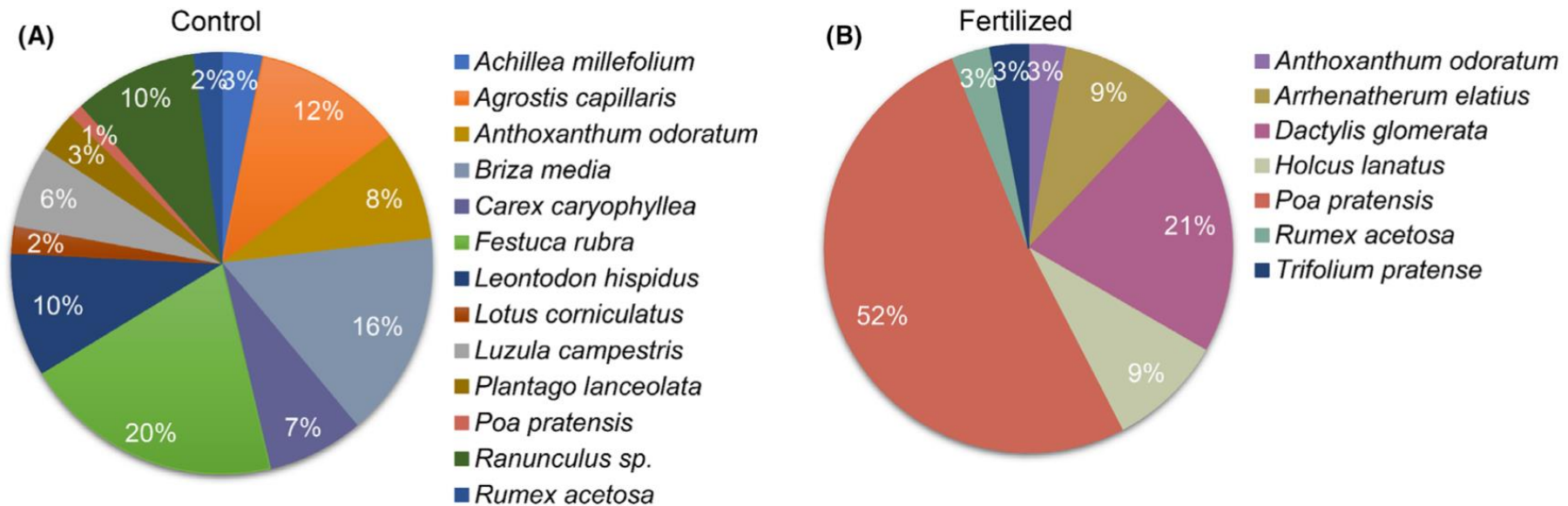
Keywords: Diazotrophs, Nitrogen fixation rates, Ecological clusters, Long-term fertilization



microbial biotechnology

Open Access

Plant–microbe networks in soil are weakened by century-long use of inorganic fertilizers



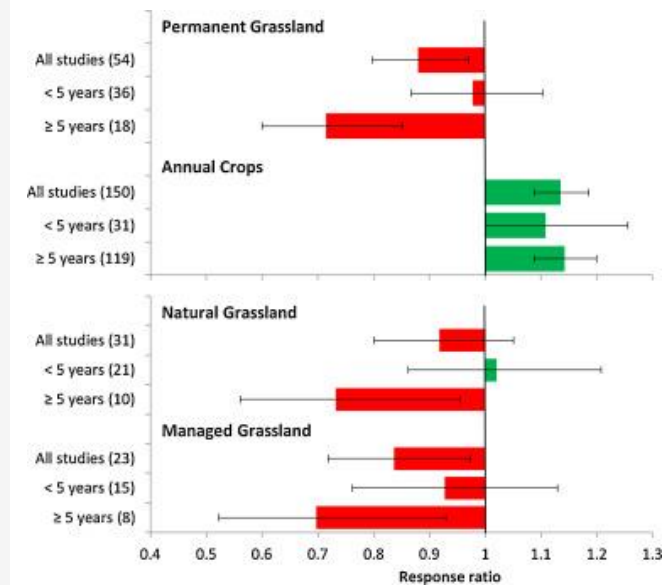
N inputs and SOM – Burn or Build?

- Both!
- Mixture of studies show N inputs can:
 - Increase SOM: greater residue input into the soil
 - Decrease SOM: increased mineralisation (C mining)
 - No effect
- The impact of N on SOM depends.

Mineral nitrogen input decreases microbial biomass in soils under grasslands but not annual crops

Highlights

- We studied the effect of N addition on soil microorganisms in a *meta-analysis*.
- Mineral N input decreased soil microbial biomass by 12% in grassland.
- The negative effect in grassland is likely due to reduced plant species richness.
- In annual cropping systems, mineral N input increased soil microbial biomass by 13.6%.
- Soil microbes benefit from higher residue inputs when annual crops are fertilized.

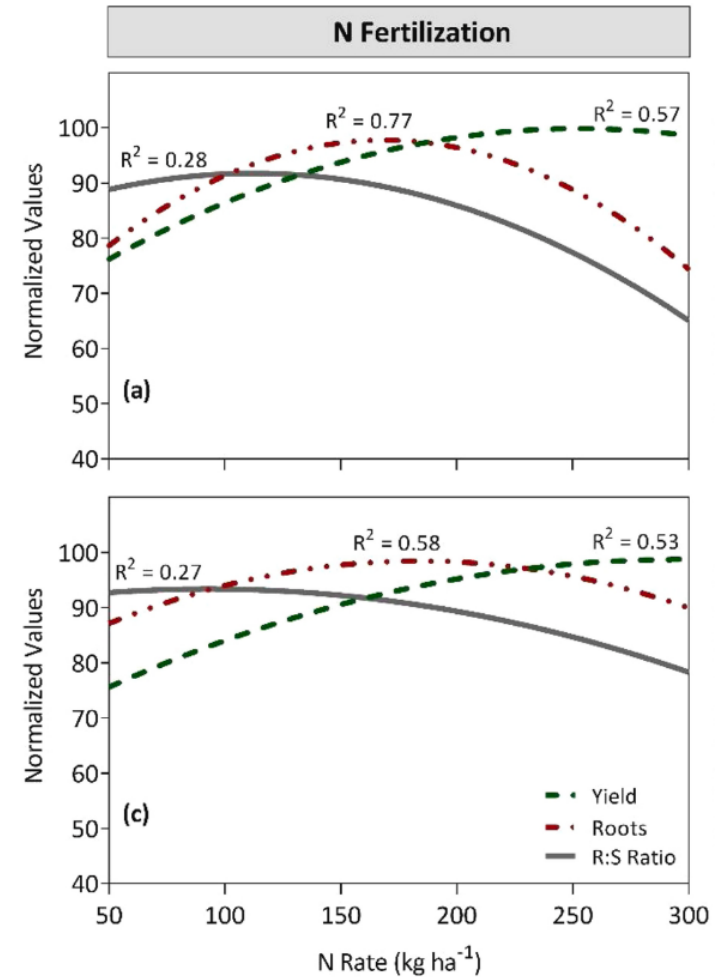


Insufficient and excessive N fertilizer input reduces maize root mass across soil types

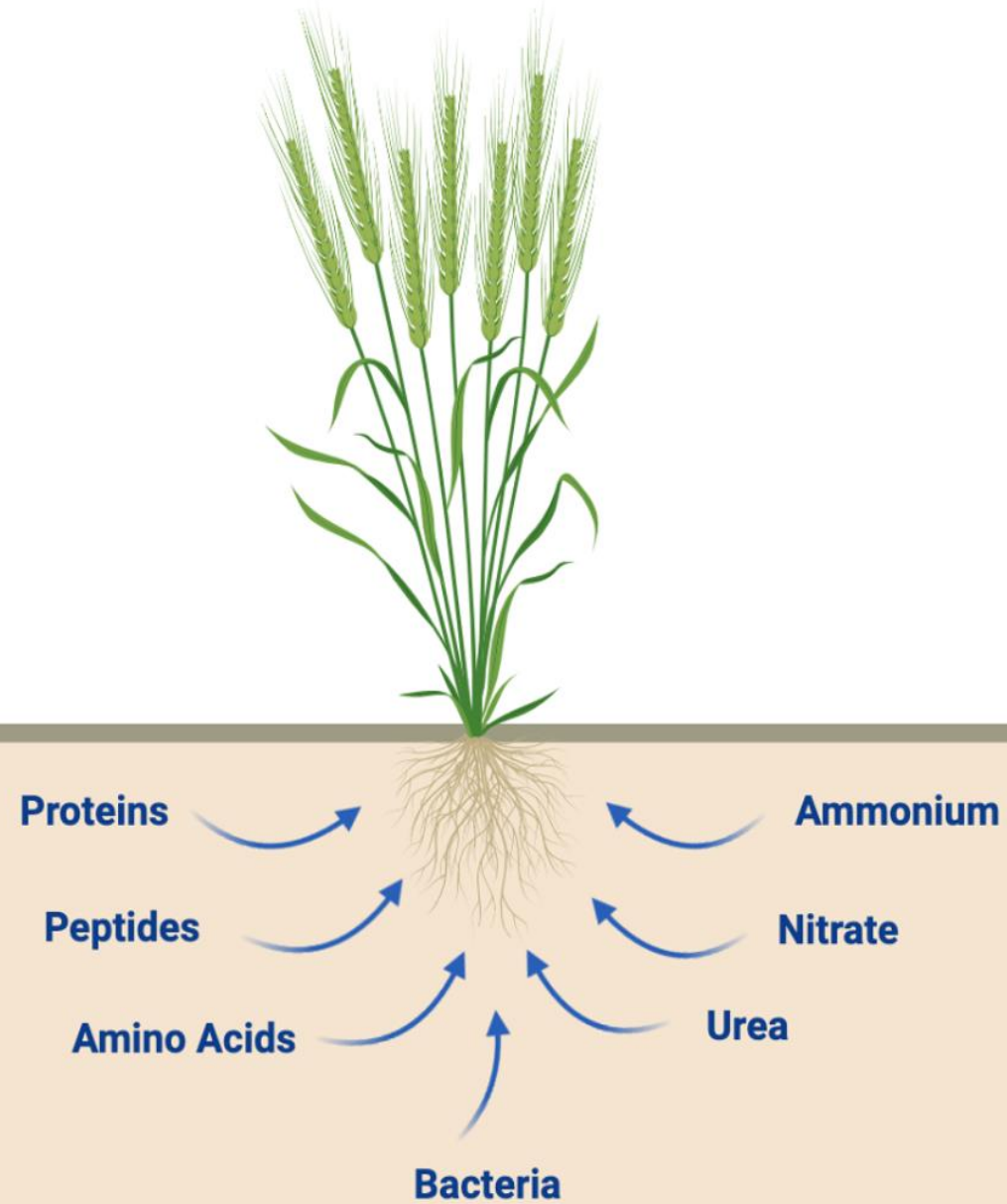
Highlights

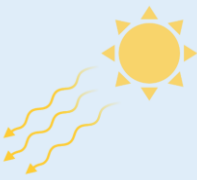
- Root mass was maximized near the agronomic optimum N rate.
- N fertilizer affected root traits in only the top 30 cm soil layer.
- Soil texture effects on root mass depend on soil moisture.
- The root to shoot ratio decreased with increasing yield levels.

“Root mass was maximized at 168 kg/ha N; zero and excessive N fertilization decreased root mass by 33 and 17 %, respectively”



Nitrogen Uptake



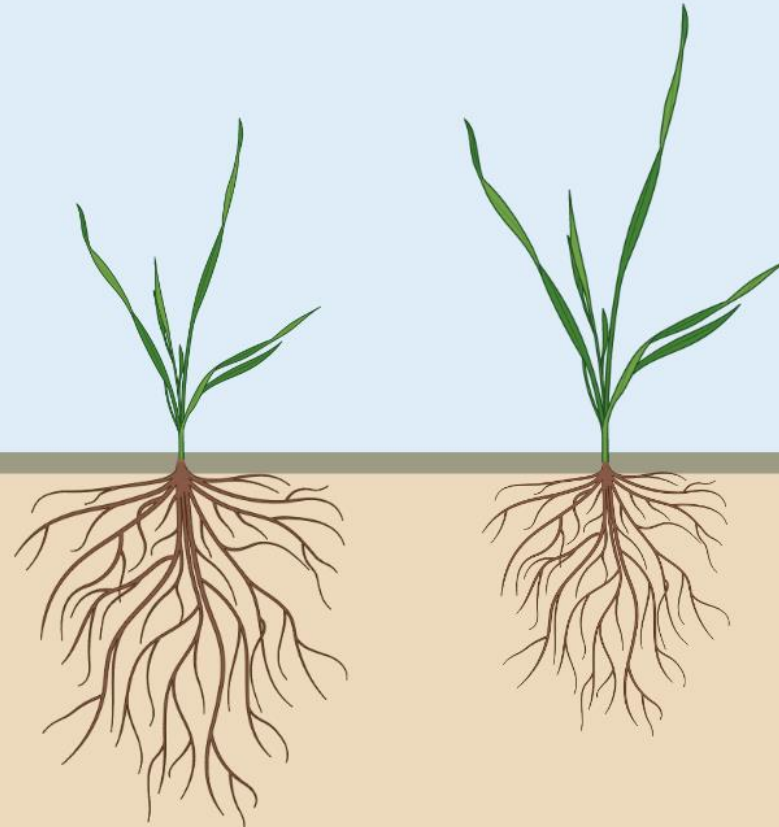


Nitrate

Metabolised in leaf

Encourages shoot biomass

Sunlight dependent
(nitrate reductase)



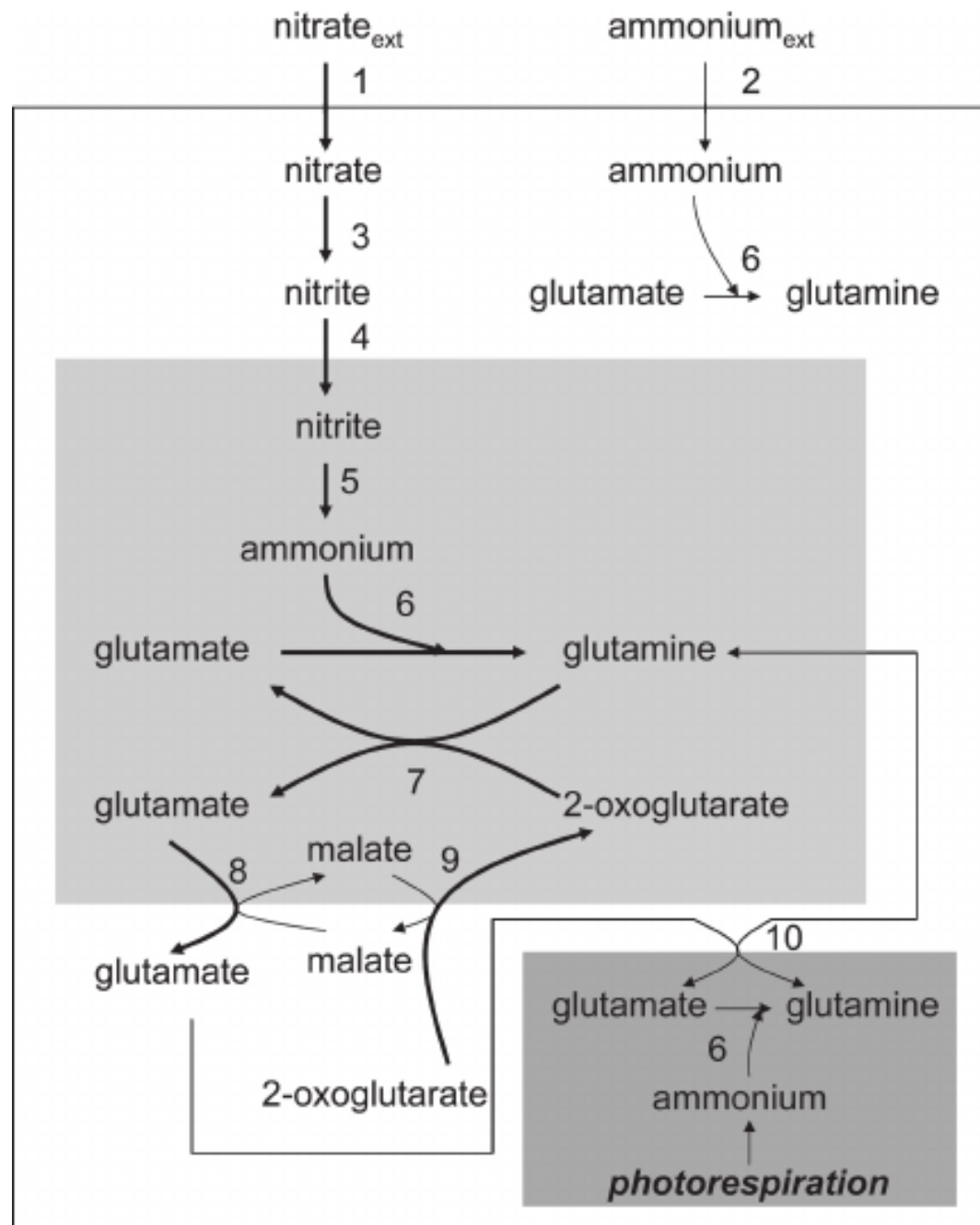
Ammonium

Metabolised in roots

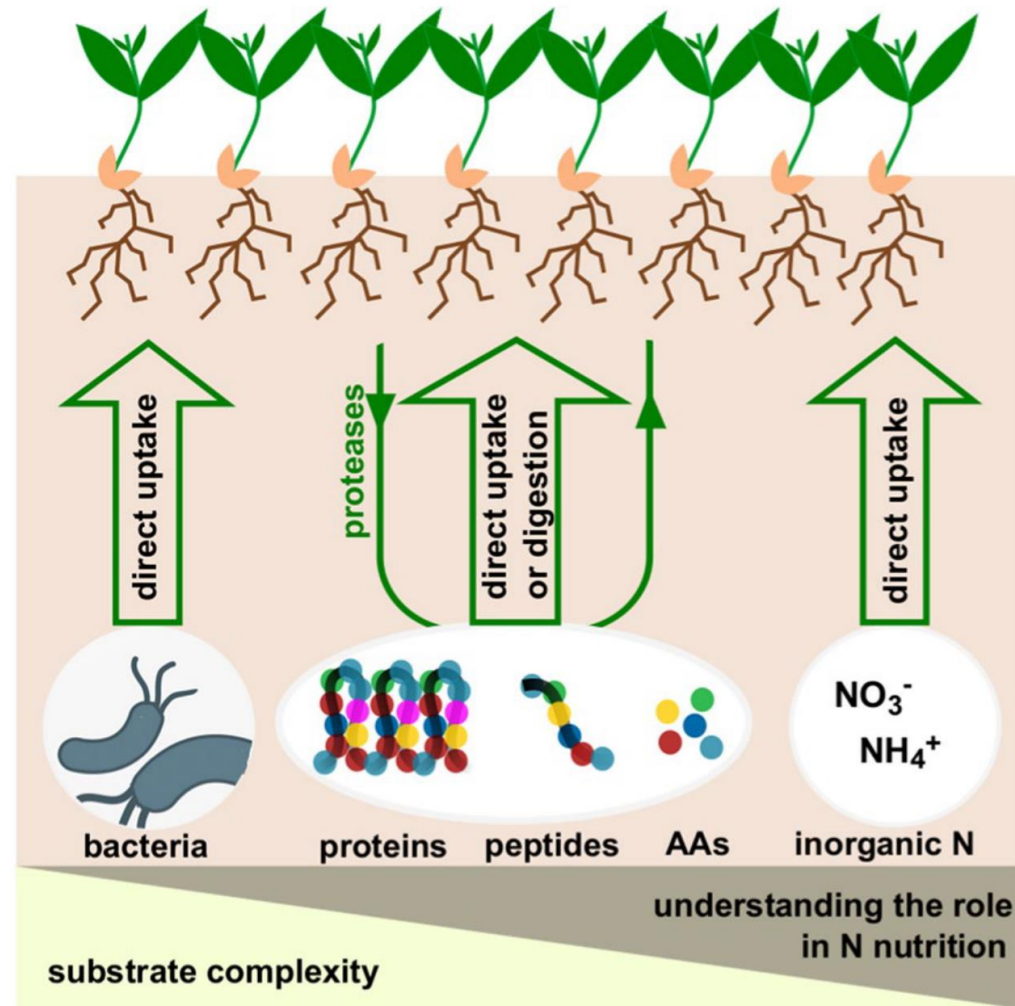
Encourages root biomass

More roots enhance above
ground biomass later in season

Ammonium VS Nitrate



How do terrestrial plants access high molecular mass organic nitrogen, and why does it matter for soil organic matter stabilization?



Soil organic nitrogen: an overlooked but potentially significant contribution to crop nutrition

Background

For more than a century, crop N nutrition research has primarily focused on inorganic N (IN) dynamics, building the traditional model that agricultural plants predominantly take up N in the form of NO_3^- and NH_4^+ . However, results reported in the ecological and agricultural literature suggest that the traditional model of plant N nutrition is oversimplified.

Scope

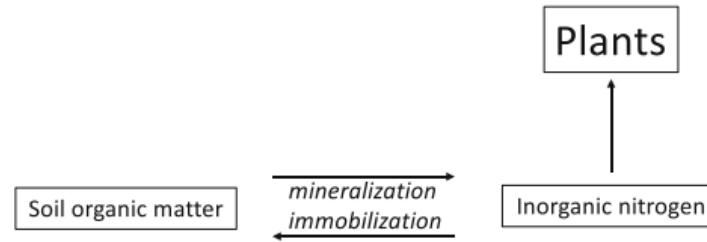
We examine the role of organic N (ON) in plant N nutrition, first by reviewing the historical discoveries by ecologists of plant ON uptake, then by discussing the advancements of key analytical techniques that have furthered the cause (stable isotope and microdialysis techniques). The current state of knowledge on soil ON dynamics is analyzed concurrently with recent developments that show ON uptake and assimilation by agricultural plant species. Lastly, we consider the relationship between ON uptake and nitrogen use efficiency (NUE) in an agricultural context.

Conclusions

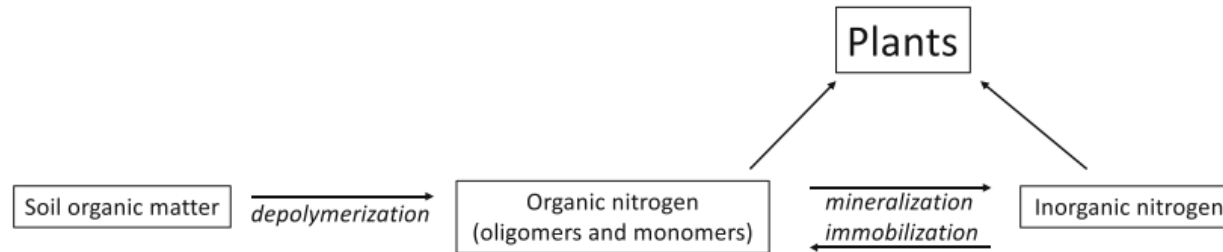
We propose several mechanisms by which ON uptake and assimilation may increase crop NUE, such as by reducing N assimilation costs, promoting root biomass growth, shaping N cycling microbial communities, recapturing exuded N compounds, and aligning the root uptake capacity to the soil N supply in highly fertilized systems. These hypothetical mechanisms should direct future research on the topic. Although the quantitative role remains unknown, ON compounds should be considered as significant contributors to plant N nutrition.

Soil organic nitrogen: an overlooked but potentially significant contribution to crop nutrition

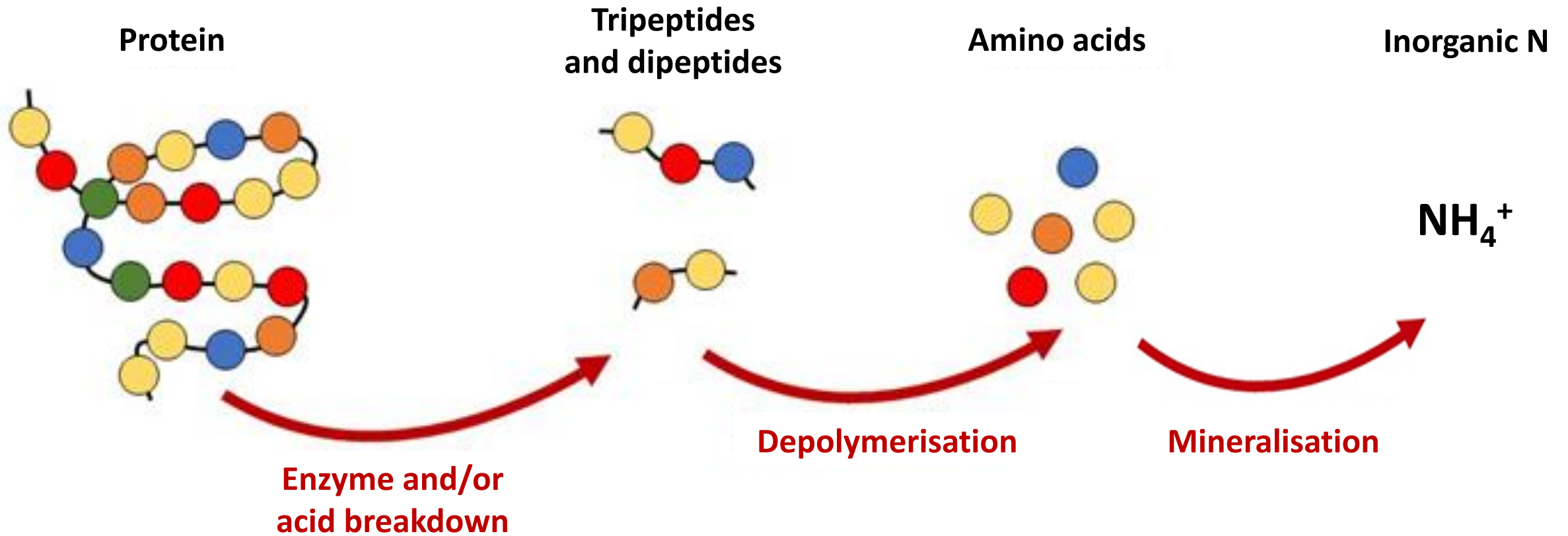
a Traditional model of agricultural plant nitrogen nutrition



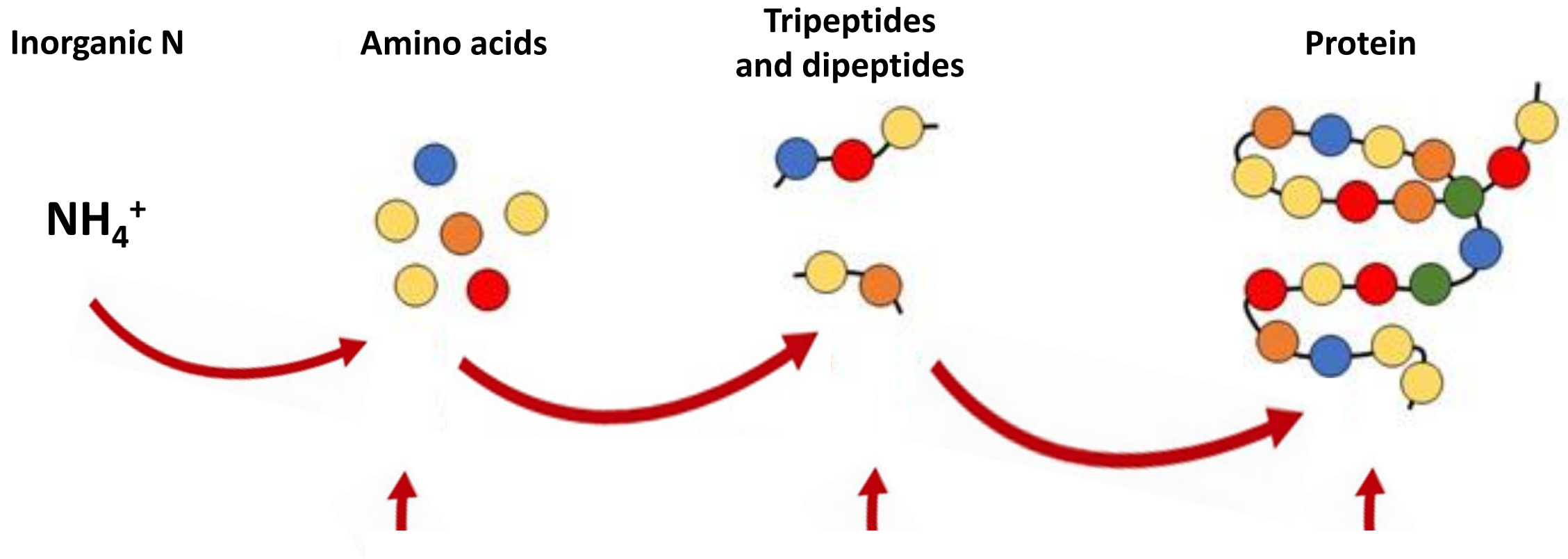
b Current model of agricultural plant nitrogen nutrition



Decomposition / Mineralisation



Metabolic Shortcutting



The carbon bonus of organic nitrogen enhances nitrogen use efficiency of plants

The importance of organic nitrogen (N) for plant nutrition and productivity is increasingly being recognized. Here we show that it is not only the availability in the soil that matters, but also the effects on plant growth. The chemical form of N taken up, whether inorganic (such as nitrate) or organic (such as amino acids), may significantly influence plant shoot and root growth, and nitrogen use efficiency (NUE). We analysed these effects by synthesizing results from multiple laboratory experiments on small seedlings (Arabidopsis, poplar, pine and spruce) based on a tractable plant growth model. A key point is that the carbon cost of assimilating organic N into proteins is lower than that of inorganic N, mainly because of its carbon content. This carbon bonus makes it more beneficial for plants to take up organic than inorganic N, even when its availability to the roots is much lower – up to 70% lower for Arabidopsis seedlings. At equal growth rate, root:shoot ratio was up to three times higher and nitrogen productivity up to 20% higher for organic than inorganic N, which both are factors that may contribute to higher NUE in crop production.

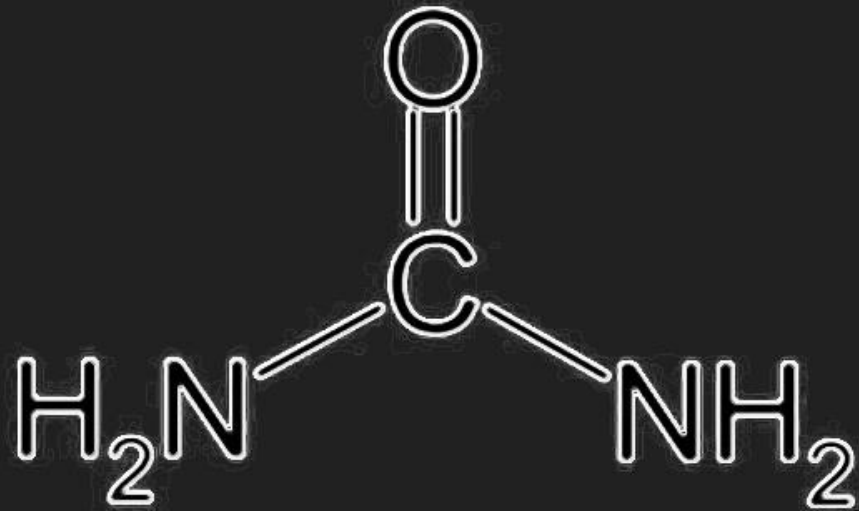
Table 2. Biochemically calculated assimilation costs for different N sources in gC gN⁻¹ according to Zerihun *et al.* (1998)

N source	Gross C costs	C bonus ^a	Net N assimilation C cost
NO ₃	5.81	0	5.81
NH ₄	4.32	0	4.32
Gln	4.30	2.14	2.16
Arg	4.30 ^b	1.29	3.02 ^b

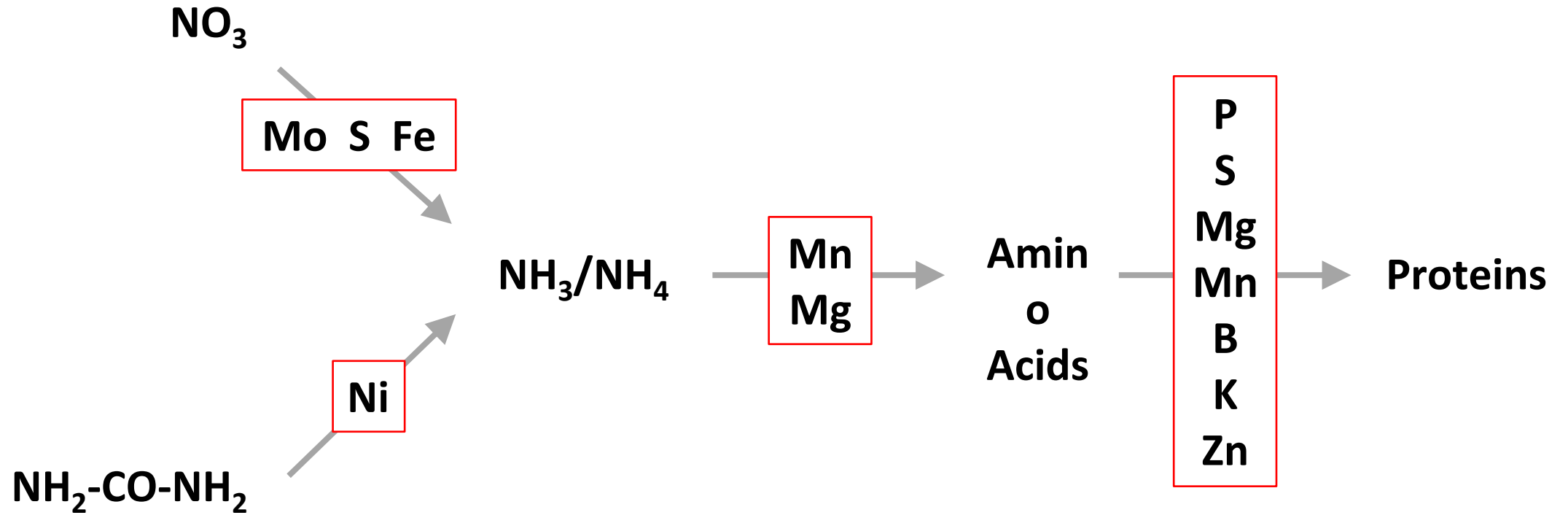
^a C bonus is equal to the molecular gC per gN.

^b Calculated assuming gross C costs (without C bonus) for N assimilation are equal to Gln.

Urea: C-based N



Converting N



Mineral		Current level			
Total Sugars	%	0,8	¹		
	%	0,2	²		
pH		5,8	¹		
		5,5	²		
EC	mS/cm	12,2	¹		
	mS/cm	17,7	²		
K - Potassium	ppm	3997	¹		
	ppm	4461	²		
Ca - Calcium	ppm	551	¹		
	ppm	1773	²		
K / Ca		7,25	¹		
		2,52	²		
Mg - Magnesium	ppm	380	¹		
	ppm	553	²		
Na - Sodium	ppm	22	¹		
	ppm	35	²		
NH4 - Ammonium	ppm	54	¹		
	ppm	68	²		
NO3 - Nitrate	ppm	3138	¹		
	ppm	8032	²		
N in Nitrate	ppm	708	¹		
	ppm	1813	²		
N - Total Nitrogen	ppm	1458	¹		
	ppm	2323	²		
Cl - Chloride	ppm	417	¹		
	ppm	178	²		
S - Sulfur	ppm	628	¹		
	ppm	1187	²		
P - Phosphorus	ppm	591	¹		
	ppm	626	²		
Si - Silica	ppm	7,2	¹		
	ppm	13,6	²		
Fe - Iron	ppm	0,80	¹		
	ppm	0,45	²		
Mn - Manganese	ppm	4,03	¹		
	ppm	7,86	²		
Zn - Zinc	ppm	1,79	¹		
	ppm	0,88	²		
B - Boron	ppm	0,72	¹		
	ppm	1,85	²		
Cu - Copper	ppm	1,84	¹		
	ppm	3,20	²		
Mo - Molybdenum	ppm	0,06	¹		
	ppm	0,05	²		
Al - Aluminium	ppm	<0,50	¹		
	ppm	<0,50	²		

Integrated N Management

INM - Designing integrated strategies to manage N

Foliar Nitrogen

Carbon stabilisers

Organic amendments

Biofertilisers – N fixers, endophytes etc

Plant breeding for NUE

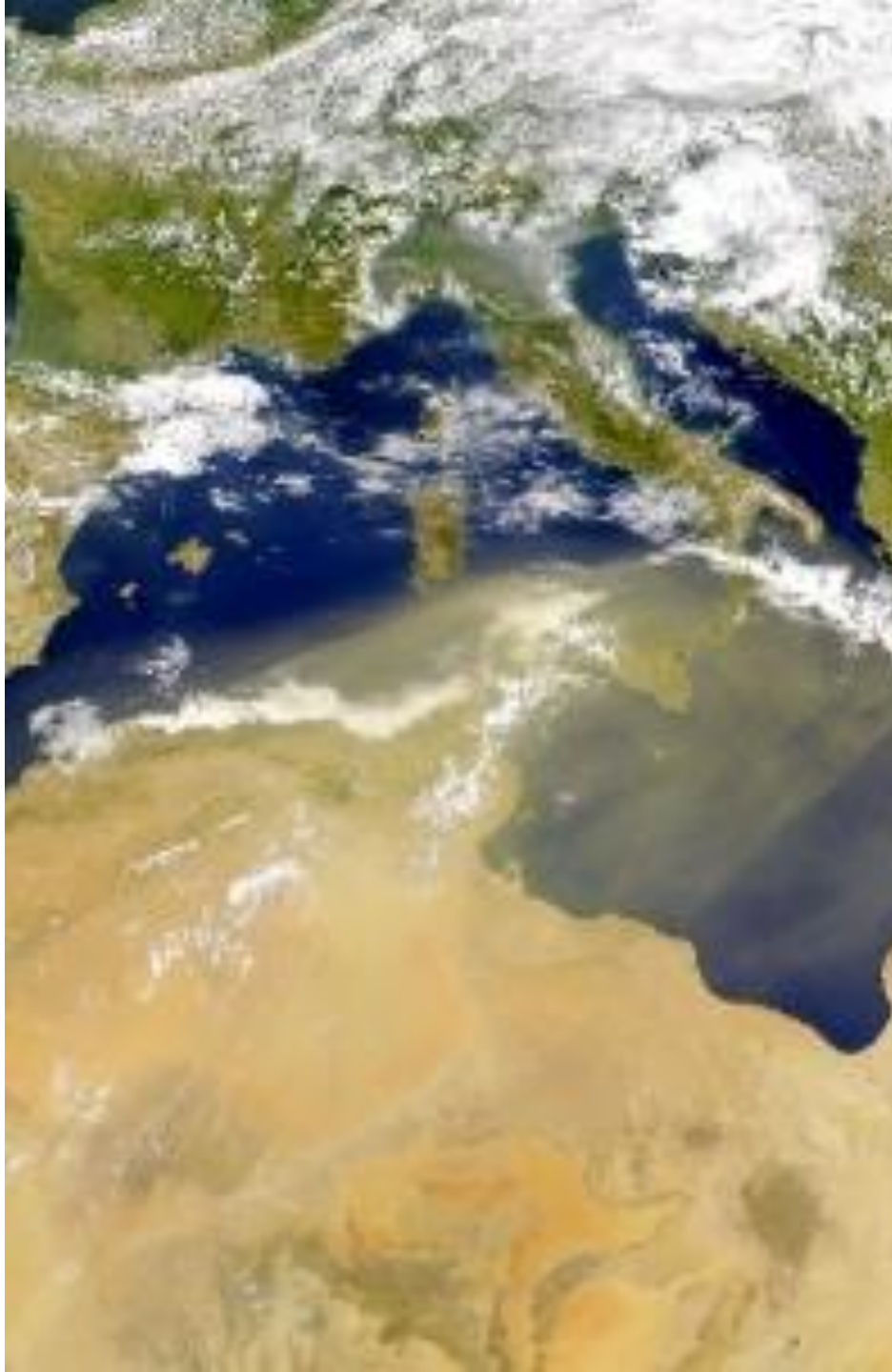
Nitrogen Inhibitors

Precision fertilisation

Cover crops, catch crops and green manures

Diverse/Multi-species pastures

Companion and intercropping with legumes



Why Foliar Feed?

- Foliar fertilisation strategies can achieve:
 - Higher nutrient use efficiency (& economics)
 - Improve yield, quality and metabolism of crops
 - Reduce the negative impact on the environment
 - Potentially enhance consumer/livestock health benefits



Nutrient	Efficiency (%)
Nitrogen	30–50
Phosphorus	15–20
Potassium	50–60
Sulphur	8–12
Zinc	2–5
Iron	1–2
Copper	1–2
Manganese	1–2
Boron	2–3
Molybdenum	2–5

Plant response depends on...



Formulation



Application



Crop/Species

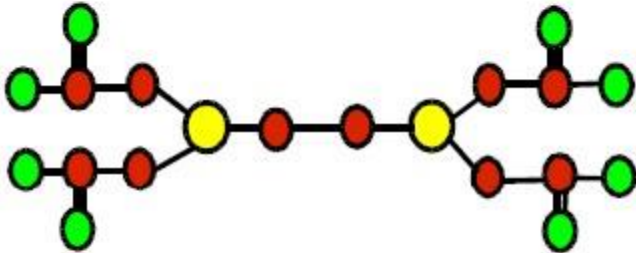
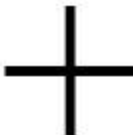


Environment

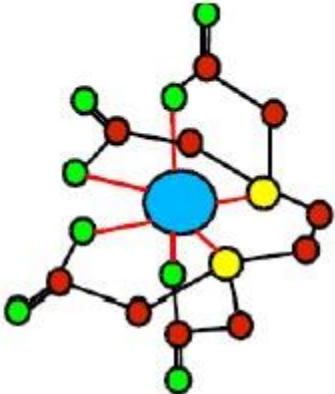
C-Based Inputs



Nutrient

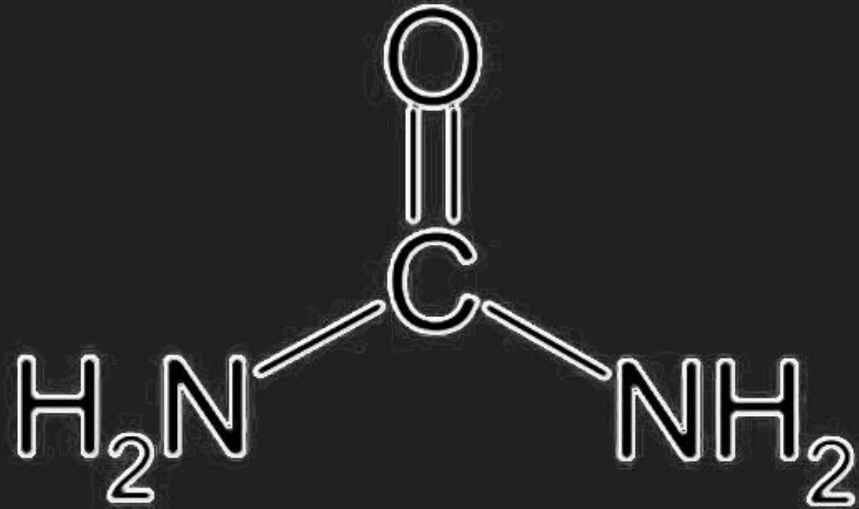


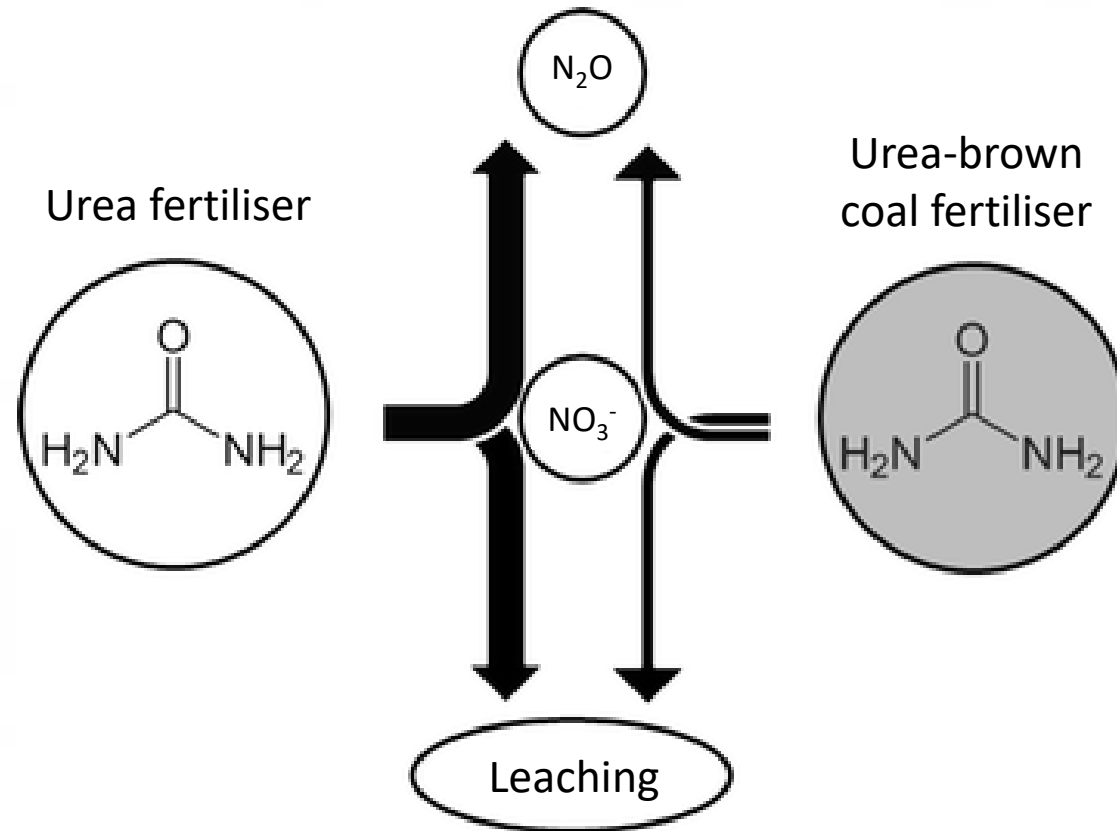
Carbon



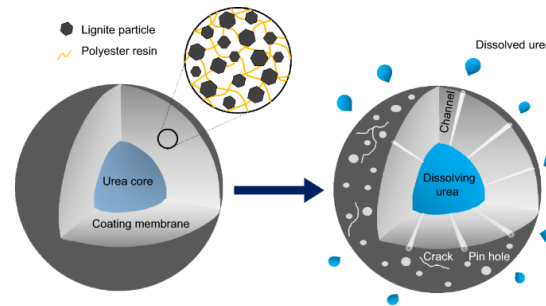
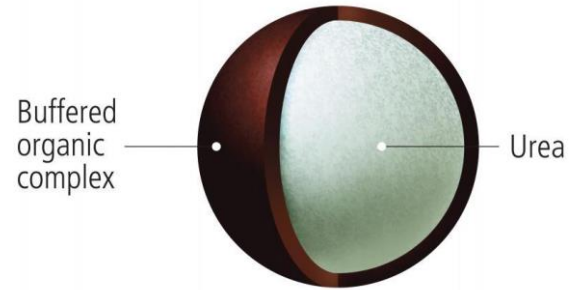
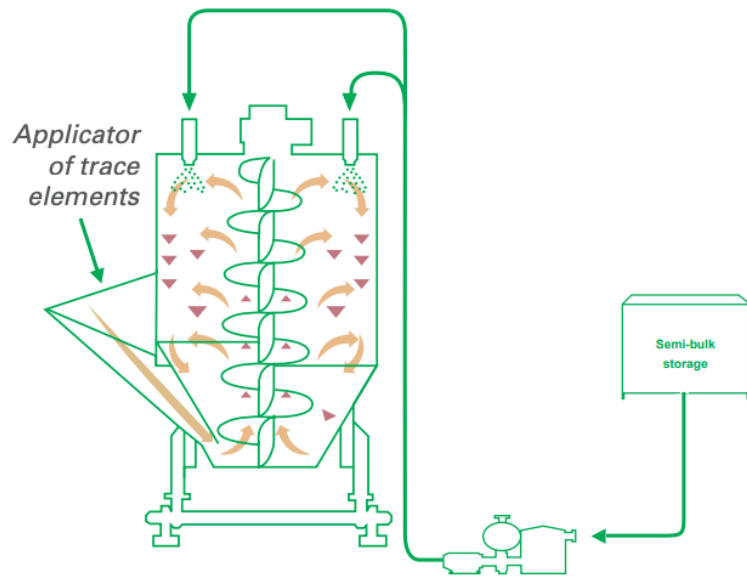
Complex

Urea: C-based N





Granule Treatment



6-8 L/T of seed [1.5-2 gal/T]



Screw Auger or Conveyor



Drum Granulator & Drier



Vertical Drum Blender



Ribbon Blender



Concrete Mixer



Horizontal Drum Blender

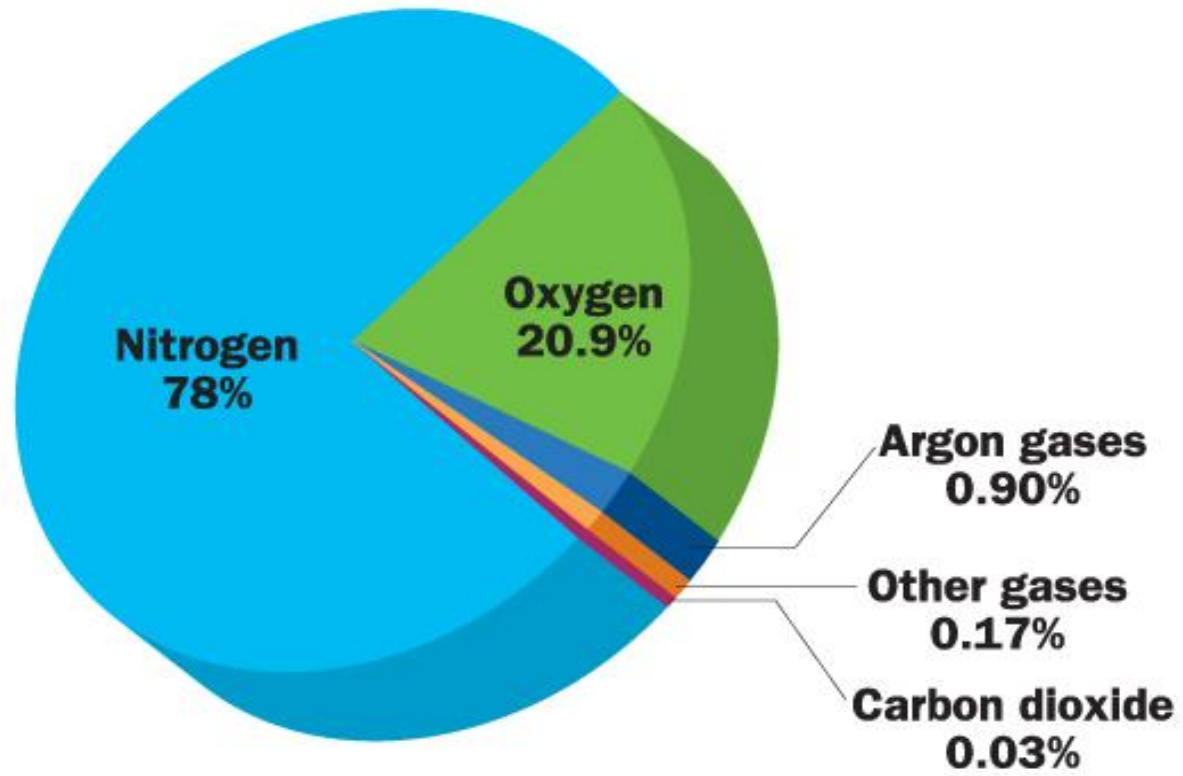
Carbon Sources

Liquid Carbon

Molasses & Sugars
Humic Acid
Fulvic Acids
Amino acids
Protein Hydrolysates
Seaweed/Kelp Extracts
Plant Extracts
Compost Extracts

Dry Carbon

Compost
Manures
Biochar
Raw Humates
Humic & Fulvic
granules/powder
Agricultural byproducts





**ROOT NODULE
SYMBIOTIC BACTERIA**



**ASSOCIATIVE
NITROGEN-FIXING BACTERIA**



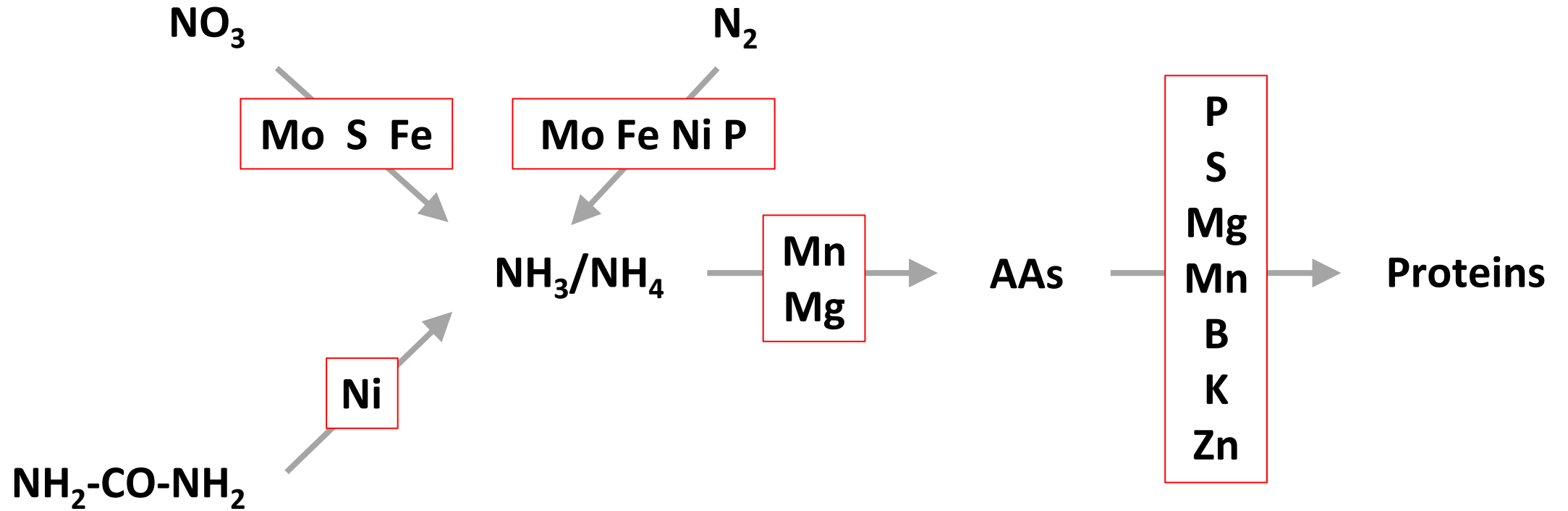
**FREE-LIVING
NITROGEN-FIXING BACTERIA**

Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota

Plants are associated with a complex microbiota that contributes to nutrient acquisition, plant growth, and plant defense. Nitrogen-fixing microbial associations are efficient and well characterized in legumes but are limited in cereals, including maize. We studied an indigenous landrace of maize grown in nitrogen-depleted soils in the Sierra Mixe region of Oaxaca, Mexico. This landrace is characterized by the extensive development of aerial roots that secrete a carbohydrate-rich mucilage. Analysis of the mucilage microbiota indicated that it was enriched in taxa for which many known species are diazotrophic, was enriched for homologs of genes encoding nitrogenase subunits, and harbored active nitrogenase activity as assessed by acetylene reduction and $^{15}\text{N}_2$ incorporation assays. Field experiments in Sierra Mixe using ^{15}N natural abundance or ^{15}N -enrichment assessments over 5 years indicated that atmospheric nitrogen fixation contributed 29%–82% of the nitrogen nutrition of Sierra Mixe maize.



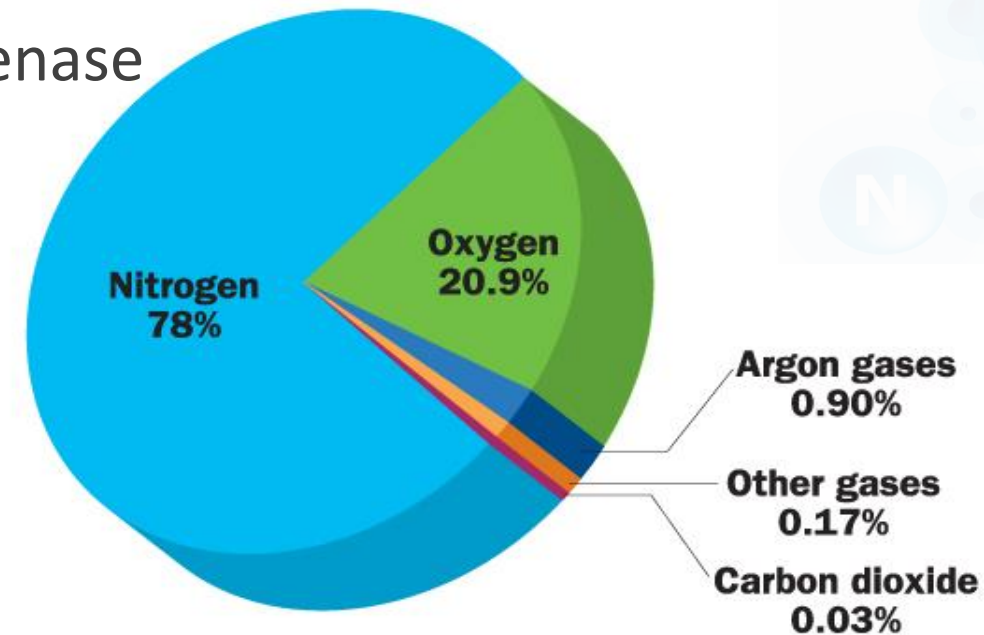
N fixation



Mineral Constraints to N Fixation

- Mineral constraints to Biological N Fixation?

- **Mo**: Mo-nitrogenase, nodule function
- **Fe**: Fe-nitrogenase, Leghemoglobin, Fe-hydrogenase
- **Ni**: Ni-hydrogenase
- **P**: ATP (high energy demand)
- **Ca**: low multiplication of rhizobia
- **B**: nodule development & maturation
- **Co**: nodule initiation
- **Cu**: N-fixing proteins in rhizobia

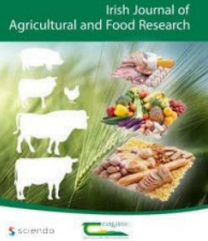


* O'hara *et al* (1988). doi.org/10.1007/BF02370104

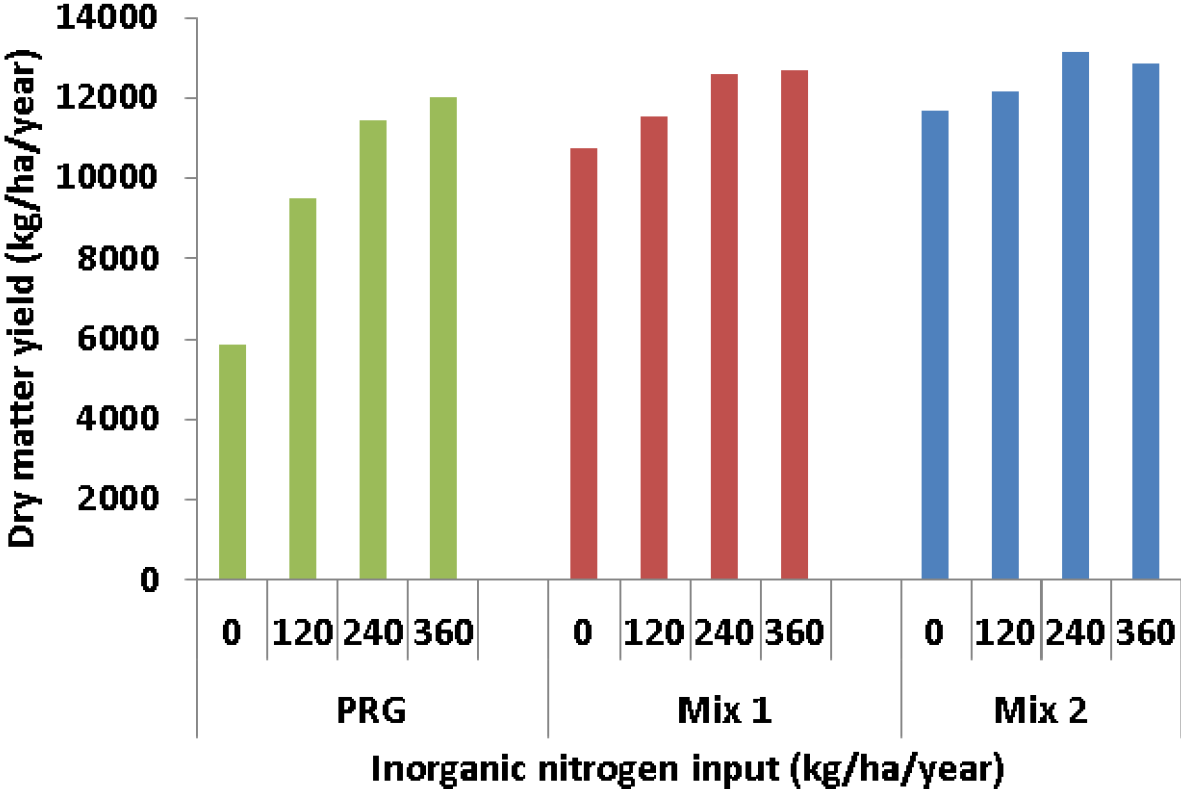
* Weisany, W., *et al* (2015).

Exploiting Biological Nitrogen Fixation: A Route Towards a Sustainable Agriculture

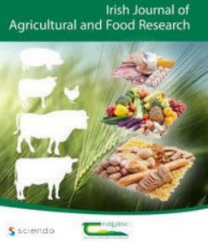
“ Biological nitrogen fixation is an energetically expensive process because 16 ATP molecules are needed to break down an N_2 molecule. Twelve additional ATP molecules are required for NH_4^+ assimilation and transport, totaling 28 ATP molecules. The nodulating plants must provide 12 g of glucose to their bacterial partners to benefit 1 g N in part. However, this process is still less energetically expensive than the Haber–Bosch process, developed in 1913. To produce the same amount of nitrogen, the Haber–Bosch process requires a temperature of 400–500 °C and a pressure of ~200–250 bars. ”



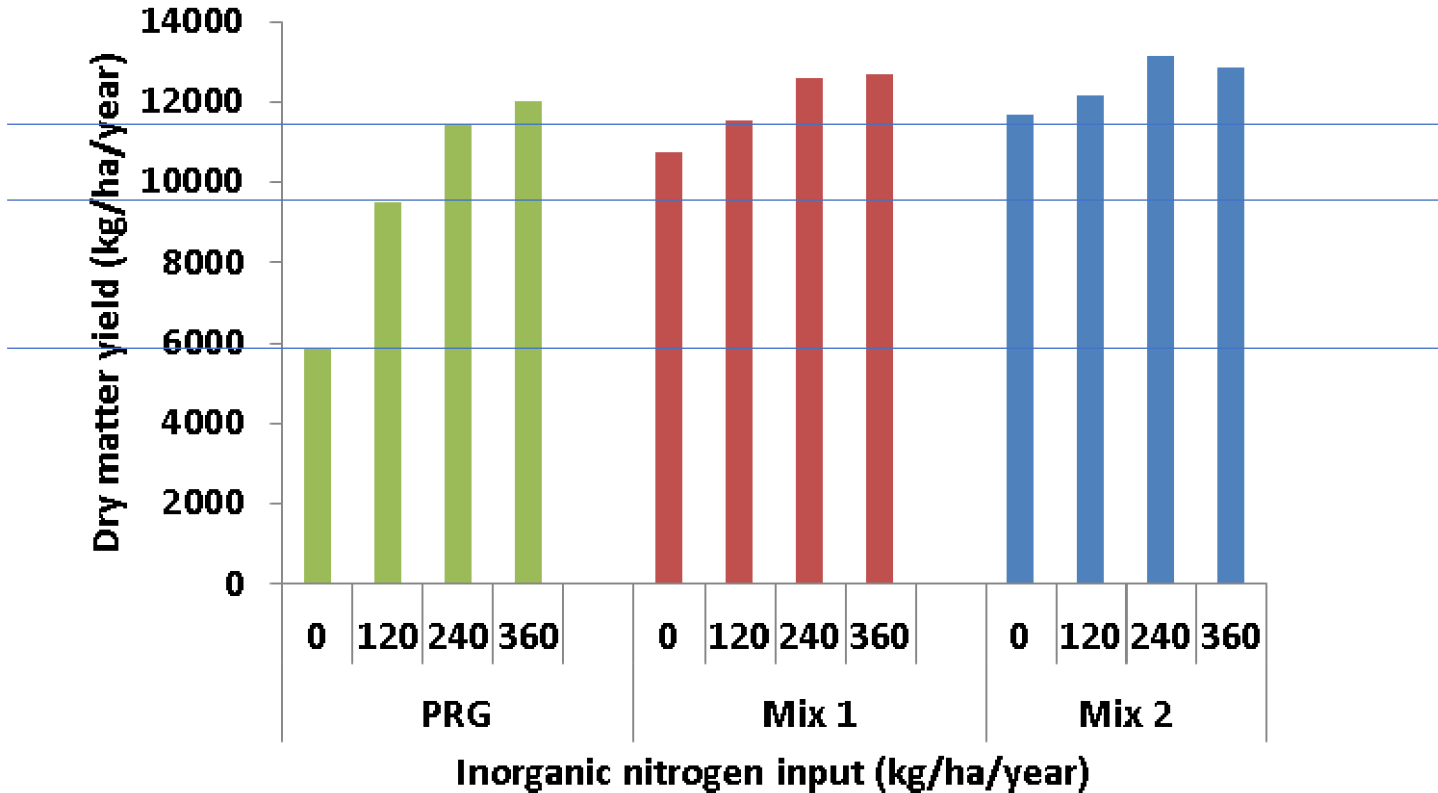
Yield of binary- and multi-species swards relative to single-species swards in intensive silage systems



* Moloney *et al* (2020). doi.org/10.2478/ijafr-2020-0002



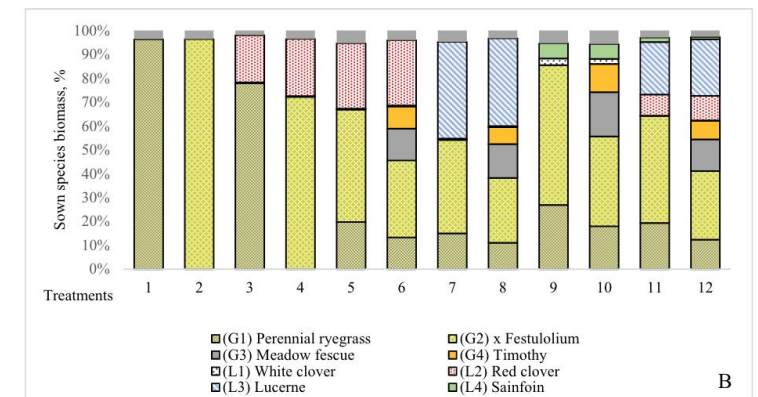
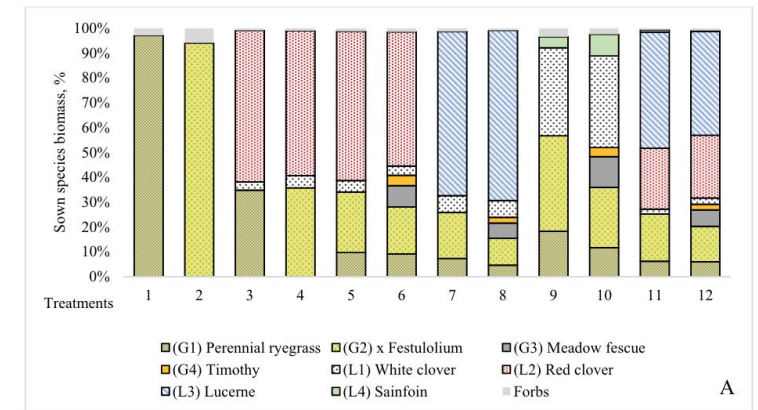
Yield of binary- and multi-species swards relative to single-species swards in intensive silage systems

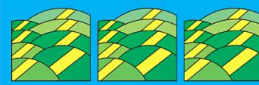


* Moloney *et al* (2020). doi.org/10.2478/ijafr-2020-0002

Plant Diversity, Functional Group Composition and Legumes Effects versus Fertilisation on the Yield and Forage Quality

Abstract: Elevating plant diversity and functional group composition amount in the swards may contribute to lower N fertiliser use. The excessive use of fertilisers in agriculture is one of the causes of environmental pollution issues. We investigated the effects of plant diversity, functional community composition, and fertilisation on the dry matter yield and its quality at the Lithuanian Research Centre for Agriculture and Forestry, Central Lithuania. The study aimed to determine the productivity potential of single-species and multi-species swards with three, four, six, and eight plant species in the mixtures including four grasses and four legumes. Two experimental backgrounds were used with N_0 and N_{150} $\text{kg ha}^{-1} \text{yr}^{-1}$ for all treatments. In the two-year experiment manipulating species richness and functional group diversity had a positive effect on the dry matter yield and produced better quality of the forage when compared with single-species swards. Crude protein in the forage of grass–legume mixtures was significantly greater than for grass monocultures. Investigating fertilisation background was a concern; it had a positive effect on the single-species sward yield but decreased the yield of multi-species swards.





Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources

Concerted use of legumes and of functional diversity in grassland forage systems can provide major contributions to the challenges of agricultural systems being productive yet environmental friendly. Acquisition and transformation of nitrogen (N) resources by legumes and grasses were studied in a temperate grassland experiment near Zurich (Switzerland) to investigate mechanisms driving effects of functional diversity in mixed swards and to optimise mixtures for efficient resource use.

Grass–legume interactions and N availability were varied by manipulating legume percentage of the sward (0–100%) and N fertiliser application (50, 150 or 450 kg of N ha⁻¹ year⁻¹). ¹⁵N technology quantified N derived from symbiotic (N_{sym}) and non-symbiotic (N_{nonsym}) sources.

Generally, acquisition of N_{sym} by the entire mixture was stimulated by grasses. As a result, strong overyielding of N_{sym} occurred (e.g. 75 and 114% for year 1 and 2 at N150) and mixtures with only 60% and 37% legumes (year 1 and 2) already attained the same N_{sym} yield as pure legume stands. Legumes stimulated N_{nonsym} acquisition by the entire mixture, largely via increased uptake by the grass component. Thus, overyielding of N_{nonsym} of 31% occurred in year 1 (N150).

Mutual grass–legume interactions stimulated acquisition of N_{sym}, acquisition of N_{nonsym} and efficient transformation of N into biomass compared to either monocultures. These effects of functional diversity can substantially contribute to productive and resource efficient agricultural grassland systems and were maximised in mixtures with 40–60% legumes.

- ▶ We describe how legume proportion modifies N acquisition from different sources.
- ▶ Symbiotic N₂ fixation was stimulated in mixtures compared to monocultures.
- ▶ Uptake of N from soil N pools was stimulated in mixtures compared to monocultures.
- ▶ The acquired N was used more efficiently by mixtures for biomass production.



In Closing

- N is very reactive/leaky – economic & environmental imperative.
- Plants can make use of many forms of N – org N is more efficient.
- N is not an island – manage other synergistic nutrients, ideally determined via plant analysis.
- Integrate many strategies to manage N – foliars, C-stabilisers etc.
- Diverse pastures with legumes

Questions & Discussion

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