Evaluation of nitrate (NO_3^-) toxicity against *Pinus radiata* and its bioremediation potential under high levels of NO_3^-

:Literature review of nitrate impacts on the environment and plant biochemistry

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Abstract

Due to increasing intensive agricultural activities, the importance of water quality for drinking is increasing over time. Especially, high concentrations of nitrate (NO_3^-) can cause severe health problems such as cyanosis in infants. This study reviewed the general plant biochemical responses to NO_3^- contamination and examined case studies currently used for NO_3^- removal and phytoremediation of most common tree species and confirmed whether conifer tree species have been conducted for nitrogen pollution research and compiled currently available 117 publications in forest tree species in phytoremediation study. It was explored how intensive agricultural activity can cause nitrate pollution and documented that 89% of the New Zealand's plantation forests are *Pinus radiata* species, consisting of 1.63 million hectares and the N uptake potential by *P. radiata* ranged from 3 to 109 kg ha^{-1} y^{-1} based on literature review. Also, it was discussed that if it is necessary to pay attention to the role of *Pinus radiata* in farmlands and streams to develop novel ideas that can positively affect New Zealand's environmental policy in the post-Paris Agreement era. I propose to consider choosing and exploiting *Pinus radiata* for several reasons: (1) to foster *Pinus radiata* research in nitrogen pollution research, finding new biological functions including the benefits of NO_3^- removal with conifer tree species; (2) to attract the attention to conduct the study of NO_3^- toxicity to plants and (3) to improve the attention of agroecosystem resilience based on fast-growing conifer trees' regulating services. This study aims to re-evaluate our understanding of whether New Zealand's *Pinus radiata* can deal with environmental stress conditions similar to more commonly studied fast-growing broad-leaved tree species.

Introduction & Methodology

Nitrate (NO_3^-) is a common form of nitrogen fertilizer, its excess application combined with easy leaching from agricultural fields causes water and soil contamination, harms to human health, and eutrophication of aquatic ecosystems. Groundwater contributes a primary source of drinking water in many of countries worldwide. Due to increasing population, urbanization, industrialization, and intensive agricultural activities, the importance of the water quality for drinking is increasing over time. Nitrogen (N) in soil is mainly derived from animal urine and chemical fertilizer (e.g., urea fertilizer) due to high N loading rates and is vulnerable to losses from runoff, leaching, and volatilization. This is because it is not fully taken up by plants and/or im-mobilized in the soil organic pool. Ammonium (NH_4^+) is rapidly mineralized, unlike NO_3^-, which is basically water-soluble and not strongly held in the soil. Thus NO_3^- leaches freely from the soil matrix. Losses of N also contribute to greenhouse gas emission and cultural eutrophication (with phosphorus) of lakes/rivers water and oceanic environments.

In addition, high NO_3^- concentrations in pasture plants can have severe risks to animal health by causing NO_3^- poisoning and nitrous oxide (N_2O) emission. In humans' health, NO_3^- has not only been recognized as harmful but also detected as a luxurious level in drinking water, but also agricultural and industrial wastewater.

Especially, high concentrations of NO_3^- can cause severe problems such as cyanosis (methemoglobinemia) in infants, cancer of the alimentary canal, non-Hodgkins's lymphoma, and Parkinson's disease.

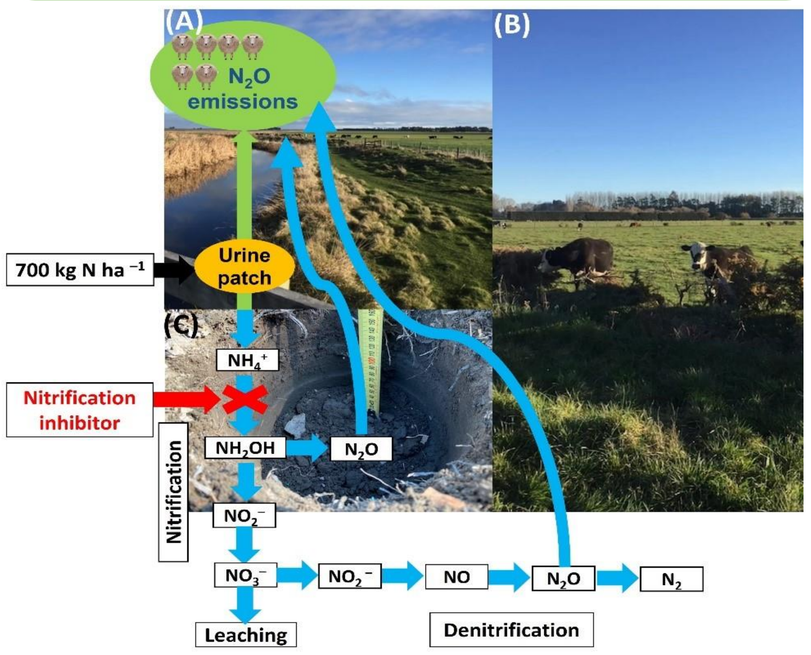

Fig. 1. Livestock urine-N as the main source for GHG (N_2O) emissions and NO_3^- leaching losses in a grassland of dairy farm in New Zealand

This study reviewed the general plant biochemical responses to NO_3^- contamination and examined case studies currently used for NO_3^- removal and phytoremediation of most common tree species and confirmed whether conifer tree species have been conducted for nitrogen pollution research and compiled currently accessible results in forest tree species in phytoremediation study.

It was compiled 117 publications that reported existing data, literature, and opinion on forestry, environmental sciences, and experimental botany.

Results & Discussion

In New Zealand, pastoral agriculture is the dominant land use, where animals graze outdoor pastures annually. New Zealand's one of national income is focusing on primary industries such as agriculture and horticulture. This comprises about 25% of its total income. More than half of the total exports from New Zealand are agriculture-based products. The total land area of New Zealand is 12.1 million hectares, of which about half (45.3%) consists of farming dominant pastoral land (7.8 million ha)

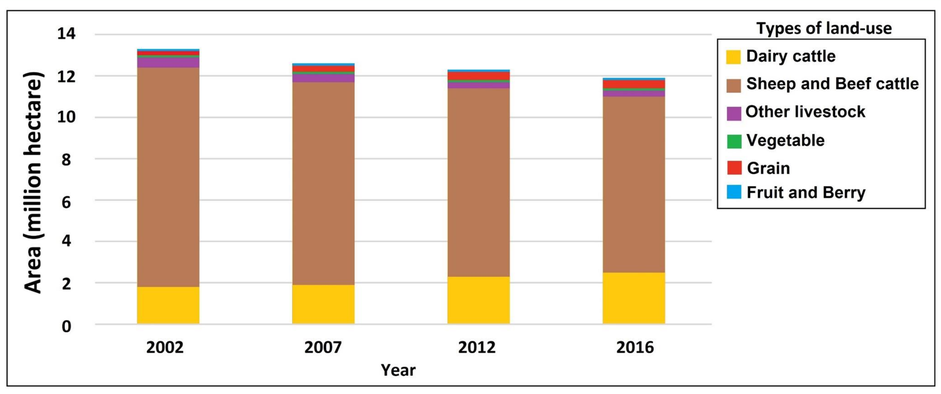

Fig. 2. Land-use types on main agricultural and horticultural sector in NZ during the period of 2002–2016

Glutamine synthetase (GS) plays a part in the intricate matrix of N metabolism of plants as the enzyme catalyzes and glutamate to form glutamine as a precursor of all nitrogenous compounds (e.g., nitrate) needed for plant growth and development. It is reported that enhanced GS expression in poplar trees can induce increased efficiency of N assimilation. In addition to this, it is reported GS transgenic hybrid poplar (*Populus tremula* × *P. alba*) can enhance N assimilation by maintaining C:N assimilation balances under high-level NO_3^- conditions. Nitrate reductase (NR) is a rate-limiting enzyme in leaf N metabolism, that reduces NO_3^- to NO_2^-; GS also plays a crucial role in leaf N metabolism, as it is linked with amino acid formation. GS not only plays the role of assimilation of inorganic N, but also participates in the photorespiration, therefore, the increase in

GS activity may be attributed to higher photorespiration rather than to stimulated N metabolism. In 2021, it is also reported photorespiration is up-regulated by NO_3^- rather than NH_4^+.

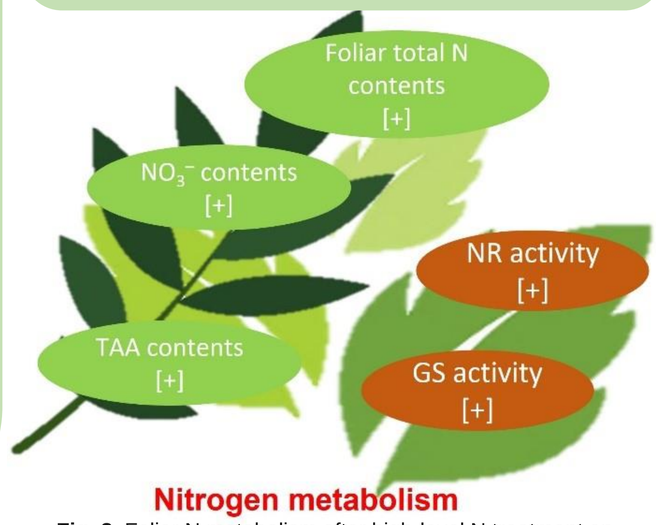

Fig. 3. Foliar N metabolism after high-level N treatment on absorbing through root systems. NR, nitrate reductase; GS, glutamine synthetase; TAA, total amino acid

Conclusion

The N metabolism process in plant growth involves nutrient uptake, transportation, assimilation, and utilization for amino acid biosynthesis.

Fig. 4 showed that conceptual model to address the N metabolism processes in response to N availability.

In the N absorption process of plants, the two main inorganic forms in the soil for plants N use by uptake processes are NO_3^- and NH_4^+.

They enter the cytosol via ammonium (AMTs), nitrate transporters (NRTs) and plasma membrane H+-ATPases (VHAs).

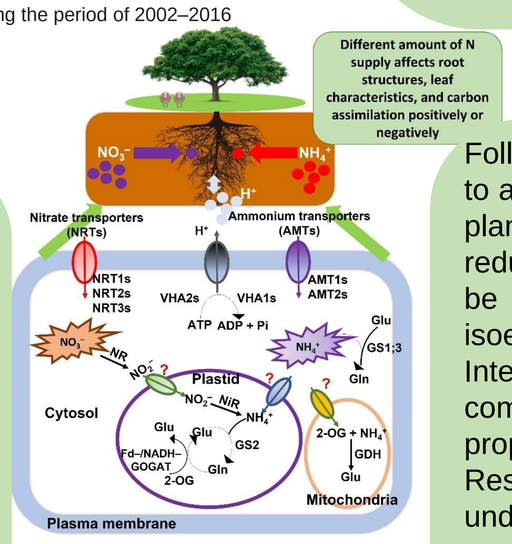

Fig. 4. A conceptual model of N metabolism in higher plants

Following the nutrient absorbing in root systems, N (i.e., NH_4^+ and NO_3^-) can be translocated to aboveground (i.e., leaves, needles, branches or stems). In the process of absorption of N in plants (i.e., assimilation process), NO_3^- is transformed to NH_4^+ by the cytosolic nitrate reductase (NR) and the plastidic/chloroplastic nitrite reductase (NiR). Subsequently, NH_4^+ can be assimilated to glutamine (Gln), which is catalyzed by glutamine synthetase (GS) isoenzymes, either in the plastid or the cytosol.

Intensive tree physiological and biochemical studies during high level of NO_3^- leaching and the combined effects of more than two factors on species tolerance to soil contamination will aid in proper tree species selection and environmental policy in New Zealand's agricultural area. Research on phytoremediation to NO_3^- polluted sites would improve the current poor understanding of conifer tree responses in the farmland of New Zealand. Therefore, it is necessary to pay attention to the role of *Pinus radiata* in farmlands and streams to develop novel ideas that can positively affect New Zealand's environmental policy in the post-Paris Agreement era.

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