

# Plant vasculature's role in tackling N<sub>2</sub>O emissions

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## Abstract

Rising demand for protein-rich foods can impact N<sub>2</sub>O emissions from croplands. Recent research has pointed to the role of modified plant vasculature in grain protein increase. Here we highlight how discovering the mechanistic role of plant vasculature in protein improvement and nitrogen-use efficiency could reduce global N<sub>2</sub>O emissions.

Growth in the global economy and changes in dietary preferences, combined with an increase in the global population has led to an increased demand for protein-rich foods [1]. This has massively impacted global agricultural systems in terms of land use and farming practices, particularly regarding nitrogen (N) fertiliser use, which directly influences yield and protein production in crops. While the increased use of synthetic N fertiliser can boost yield and protein levels, it leads to not only soil degradation and toxicity but inadvertently increases emissions of N<sub>2</sub>O, which has 300 times more global warming potential than CO<sub>2</sub>. The Intergovernmental Panel on Climate Change (IPCC) holds global agriculture responsible for up to 12% greenhouse gas emissions, 50% of which are attributable to N<sub>2</sub>O emissions from croplands that have increased consistently in the last 30 years [2].

The rising demand of animal-based protein foods is already a challenge, with up to 50% increase estimated by 2050 [3], and its likely implications on global environment are widely debated. Thus, developing N-efficient crops becomes more important to ameliorate the environmental impacts of N fertilisers. The impacts of climate change are far reaching

with consistent episodes of heat and drought stress, rising sea levels, and altered ecosystems affecting crops globally. Addressing the issue of N<sub>2</sub>O emissions from croplands because of growing protein demands requires a multifaceted strategy encompassing the use of innovative technologies, sustainable agricultural practices, and exploitation of modern genetics.

### **The connection between N fertiliser and protein in crops**

Protein regulation in grain crops is strongly associated with soil N levels and the ability of a plant to uptake N and remobilise it, particularly from late vegetative to early reproductive stages [4]. N comprises a key component of amino acids and is required to synthesise protein. However, a balance of N application with plant demand is critical as applying more than a plant's capacity to uptake can add to nitrate leaching and N<sub>2</sub>O emissions [5]. Therefore, it is key to improve N-use efficiency of plants through genetic advancements and sustainable agriculture. The genetic regulation of protein is associated with post-anthesis N uptake and remobilisation from vegetative to reproductive parts during grain filling, and senescence plays an important role in determining low or high remobilisation [6]. A *No Apical Meristem (NAM)* gene was identified almost two decades ago to be involved in protein accumulation in grains, with its functional alleles accelerating senescence and advancing nitrogen remobilisation from vegetative parts to grain during grain filling to increase protein content [7]. The increase in grain protein content is inversely correlated with grain weight and yield, such that success in terms of increased protein levels in crops in nitrogen efficient environments has been limited.

### **The role of modified plant vasculature in reducing N<sub>2</sub>O emissions**

In vascular plants, the role of vascular bundles in N uptake is key to plant growth and development. Vascular bundles facilitate N uptake and transportation from root to shoot through xylem vessels, redistribution within the plant from mature to younger and stressed tissues or during photosynthesis from source (e.g., stem or leaves) to sink (e.g., storage organs or fruit) through phloem cells, and storage and retention, for example, in xylem parenchyma [8]. Therefore, increase in the number of vascular bundles can not only improve a plant's capacity to uptake N for photosynthesis and transport N from source to sink, but also enhance N-use efficiency under optimal and stress conditions. Furthermore, previous evidence in wheat indicates grain N accumulation is principally driven by the

availability of N from the sources [9], therefore increasing N supply to the grain through altered vascular architecture should enhance grain N content. As a result, the N fertiliser requirement can be minimised leading to reduced N<sub>2</sub>O emissions.

Recently, a *homeodomain leucine zipper transcription factor*, *Homeobox Domain-2 (HB-2)*, was reported to partake in grain protein changes through alterations in plant vascular architecture [10]. Wheat mutants with increased expression of *HB-2* had altered inflorescence architecture producing paired spikelets and grain with up to 25% more protein content, that include higher essential amino acids, i.e., methionine, leucine, and threonine, without any significant differences in yield or grain weight. The increased protein and free amino acid levels were attributed to higher number of vascular bundles in peduncles of mutant lines with increased *HB-2* expression, which resulted in greater hydraulic conductivity of peduncle and inflorescence tissue, leading to enhanced water and nutrient transport. While there has been limited research on the role of altered plant vasculature in efficient N use, alterations in small and large vascular bundles in the peduncle and cob of maize in response to N supply have also been linked to enhanced transport efficiency [11]. More generally there is evidence for a link between grain set per spike, the number of vascular bundles and thus total phloem surface cross-section area in wheat [12]. Quantitative trait loci associated with small and large vascular bundles located on different regions of the wheat genome have been reported previously [13]. Therefore, investigating the beneficial modifications in plant vasculature could help improve N uptake and use efficiency in plants, and consequently reduce fertiliser input. This will not only help growers in terms of production costs, but could significantly reduce N<sub>2</sub>O emissions from croplands, as illustrated in a hypothetical model (**Figure 1**). Moreover, recent studies have also highlighted the importance of investigating branching and connectivity of vascular bundles within the spike and spikelets and understanding the genetic regulation of spike vascular architecture to enhance the flow of assimilates within the spike to the rapidly growing florets [14]. Thus, a modified plant vasculature with increased vascular bundles in peduncles and inflorescence and enhanced branching of vascular bundles within the spike could have major benefits in reducing the use of N fertilisers without compromising yield and grain protein. Furthermore, recent advances in phenotyping will facilitate the high-throughput

study of anatomical traits such as vascular architecture, e.g., laser ablation tomography analysed via machine learning algorithms [15].

## Concluding remarks

Producing sustainable crops to meet rising demands for food without detrimental environmental impacts is a daunting challenge for plant scientists. Recent discoveries on the role of modified plant vasculature in improving protein levels that are directly linked to N fertiliser use could assist in this task. However, further research into how these modifications are distributed within the plant and affect overall plant architecture as well as yield and grain quality and their genetic regulation under different environments will be required to benefit from their full potential. Overall, this could aid the global sustainability effort and minimise N<sub>2</sub>O emissions from croplands.

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## Conflict of interests

The authors declare no conflicts.

## References

1. Ritchie, H. and Roser, M. (2017) Meat and Dairy Production. Our World in Data.
2. (!!! INVALID CITATION !!! [2]).
3. Henchion, M. et al. (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods* 6 (7), 53.
4. Foulkes, M.J. et al. (2009) Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research* 114 (3), 329-342.
5. Takeda, N. et al. (2021) Exponential response of nitrous oxide (N<sub>2</sub>O) emissions to increasing nitrogen fertiliser rates in a tropical sugarcane cropping system. *Agriculture, Ecosystems & Environment* 313, 107376.
6. Gaju, O. et al. (2011) Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crops Research* 123 (2), 139-152.
7. Uauy, C. et al. (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314 (5803), 1298-1301.
8. De Rybel, B. et al. (2016) Plant vascular development: from early specification to differentiation. *Nature reviews Molecular cell biology* 17 (1), 30-40.

9. Martre, P. et al. (2003) Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. *Plant Physiol* 133 (4), 1959-67.
10. Dixon, L.E. et al. (2022) MicroRNA-resistant alleles of HOMEODOMAIN-2 modify inflorescence branching and increase grain protein content of wheat. *Science advances* 8 (19), eabn5907.
11. Ren, H. et al. (2021) Nitrogen Supply Regulates Vascular Bundle Structure and Matter Transport Characteristics of Spring Maize Under High Plant Density. *Frontiers in Plant Science* 11.
12. Tcherkez, G. et al. Revisiting yield in terms of phloem transport to grains suggests phloem sap movement might be homeostatic. *Plant, Cell & Environment* n/a (n/a).
13. Sang, Y. et al. (2010) QTLs for the vascular bundle system of the uppermost internode using a doubled haploid population of two elite Chinese wheat cultivars. *Plant Breeding* 129 (6), 605-610.
14. Slafer, G.A. et al. (2022) A 'wiring diagram' for sink strength traits impacting wheat yield potential. *Journal of Experimental Botany*.
15. Vanhees, D.J. et al. (2022) Soil penetration by maize roots is negatively related to ethylene-induced thickening. *Plant, Cell & Environment* 45 (3), 789-804.

**Figure 1. The role of modified plant vasculature in reducing N<sub>2</sub>O emissions.** This illustrative model depicts how modifications in plant vascular architecture can improve N uptake, redistribution, and remobilisation leading to reduced fertiliser requirement, and as a result minimise N<sub>2</sub>O emissions. **(A)** A plant with lower number of vascular bundles and poor branching/connectivity within the spike unable to transport and redistribute N efficiently and hence requiring high input, compared to **(B)** A plant with higher number of vascular bundles and better branching/connectivity within the spike transporting and redistributing N efficiently and hence requiring low input, and therefore releasing lesser N<sub>2</sub>O to the atmosphere. (created with BioRender.com)