Modeling the pathway of nitrogen release from the symbiosome

1. The symbiotic nitrogen fixation organelle: "The Symbiosome"

Under conditions of limiting soil nitrogen, legumes attract soil bacteria of the Rhizobiaceae family and establish a nitrogen-fixing symbiosis. This process involves the induction of a novel symbiotic organ on the plant roots, known as the nodule. The nitrogen-fixing rhizobia bacteroids are "endosymbionts" which enter and live inside enlarged plant cells within the core of the nodule. The endosymbiotic bacteroids are enclosed within a specialized nitrogen-fixing organelle known as the "symbiosome" [1] (Fig. 1). In mature nodules the host infected cells are occupied by thousands of symbiosomes, which constitute the major organelle within this cell type. The symbiosome membrane (SM) is the outer boundary of this organelle which controls the transport of metabolites between the bacteroid symbiont and the plant host [2]. The metabolic exchange (**Fig. 1**) that is the heart of the symbiosis is: A. The uptake of a carbon energy source (malate) provided by the plant and utilized by the bacteroid to produced

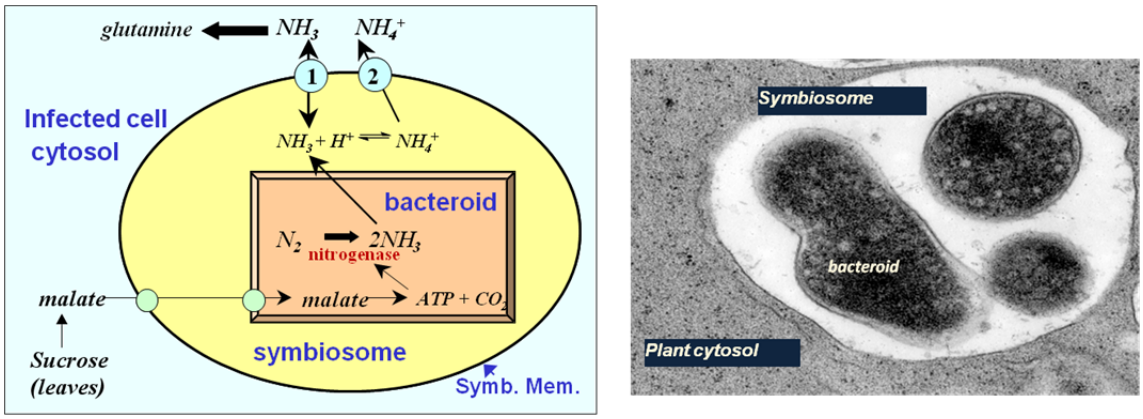

Fig 1 Carbon and nitrogen exchange between legumes and endosymbiotic rhizobia bacteroids
Right, Electron micrograph of a soybean root nodule symbiosome with rhizobia bacteroids enclosed by the symbiosome membrane, *Left*, Model for metabolic flux of carbon and fixed nitrogen within nitrogen fixing symbiosomes.

ATP for nitrogen fixation; and B. The release of reduced product of nitrogen fixation as either ammonia (NH_3) or ammonium ion (NH_4^+). This reduced product is assimilated into an organic form (glutamine) by an enzyme, glutamine synthetase, which is located in the plant cell cytosol. Glutamine is then transported as a useable form for nitrogen to other plant tissues. Nitrogen fixation in general is an energetically expensive process for the plant and it is tightly regulated.

2. Multiple transport pathways through the symbiosome membrane.

The process of fixed NH_3 and NH_4^+ release from the symbiosome to the plant cytosol for assimilation is complex and is postulated to occur by two pathways, one favoring uncharged NH_3 and the other favoring charged NH_4^+ cations :

Pathway 1: Facilitated transport of NH_3 via the nodulin 26 channel Nodulin 26 is a major channel protein specific to the SM where it composes over 10% of all membrane protein. It is a member of the aquaporin superfamily of membrane channel proteins and has been shown to transport NH_3 (as well as water) in a bidirectional fashion (Fig. 2B). The direction of transport depends on the concentration gradient of NH_3. [3,4].

Pathway 2: Directional NH_4^+ release via a cation channel A second pathway for NH_4^+ movement from the symbiosome space to the cytosol occurs via a nonselective cation channel (NSCC) on the symbiosome membrane [5,6]. This channel is tightly regulated and opens only in response to a voltage gradient ($\Delta\psi$) across the membrane. The $\Delta\psi$ is

established by a proton pumping ATPase (Fig 2A) which also is found on the symbiosome membrane. The ATPase hydrolyzes ATP and simultaneously transports a proton from the plant cytosol into the symbiosome. This establishes a "proton motive force" which consists of two components: an electrical component (the voltage potential Δψ) and a chemical component, which is the pH gradient (ΔpH) caused by a higher H^+ inside the symbiosome. NH_4^+ is transported unidirectionally towards the cytosolic compartment by the NSCC with no significant backwards flux.

The relative contribution of these pathways to the overall process of fixed nitrogen efflux and assimilation is unknown. It is postulated to depend upon several factors: 1. The concentration gradient of NH_4^+ and NH_3 between the symbiosome space and the cytosol; 2. The ΔpH between the symbiosome space and cytosolic compartments; and 3. The resting voltage potential (Δψ) of the symbiosome membrane. These parameters are likely controlled by the activity of the symbiosome membrane H^+-pumping ATPase. For example, when a highly active H^+-ATPase hyperpolarizes (high Δψ) and acidifies the symbiosome space (elevated ΔpH) this would favor efflux of fixed nitrogen in the form of charged NH_4^+ by voltage-dependent activation of the NSCC (Fig. 2A). In contrast, low ATPase activity would result in reduced SM potential (low Δψ) and a decreased ΔpH, which would lead to a higher NH_3/NH_4^+ concentration ratio and a closed NSCC which would favor NH_3 efflux via nodulin 26 across the symbiosome membrane (Fig. 2B).

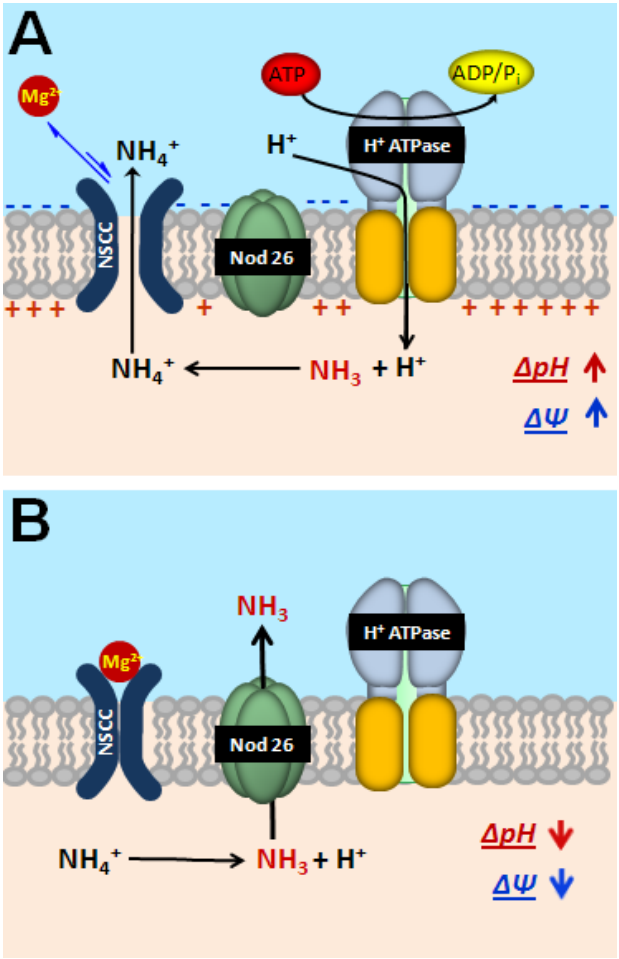

Fig. 2 Two pathways for fixed nitrogen efflux. A. Voltage dependent efflux of ammonium ion through a non selective cation channel. This pathway is favored under **high ATPase** activity which leads to a proton motive force across the SM. **B.** Facilitated diffusion and uncharged ammonia through the nodulin 26 aquaporin-like channel. This pathway is favored under low ATPase activity.

3. Additional Complexity: The problem of ammonia toxicity and futile cycling.

One potential issue with having two pathways of NH_4^+/NH_3 movement across the SM is the issue of toxicity, which is a potential outcome of wasteful "ammonia futile cycling" [7] (Fig. 3). During active nitrogen fixation, charged NH_4^+ accumulates in the acidic symbiosome space. Movement of the NH_4^+ into the more alkaline cytosolic compartments, however causes deprotonation and generation of uncharged NH_3, a substrate which can reenter back to the symbiosome space via nodulin 26. As a result, such a repetitive pathway would result in futile cycling, waste ATP, and dissipate the SM proton motive. One potential mechanism to prevent futile cycling would be to facilitate rapid NH_3/NH_4^+ assimilation in the cytosol to block back diffusion of NH_3. The NH_3/NH_4^+ concentration gradient between the symbiosome and cytosol is likely controlled by the activity of the glutamine

synthetase in the plant cytosol which rapidly incorporates these toxic compounds into the amino acid glutamine (Fig. 1) [8]. Work by Streeter [9] has estimated the concentration of cytosolic levels of ammonia/ammonium ion to be 50-fold lower than the concentration in the symbiosome space.

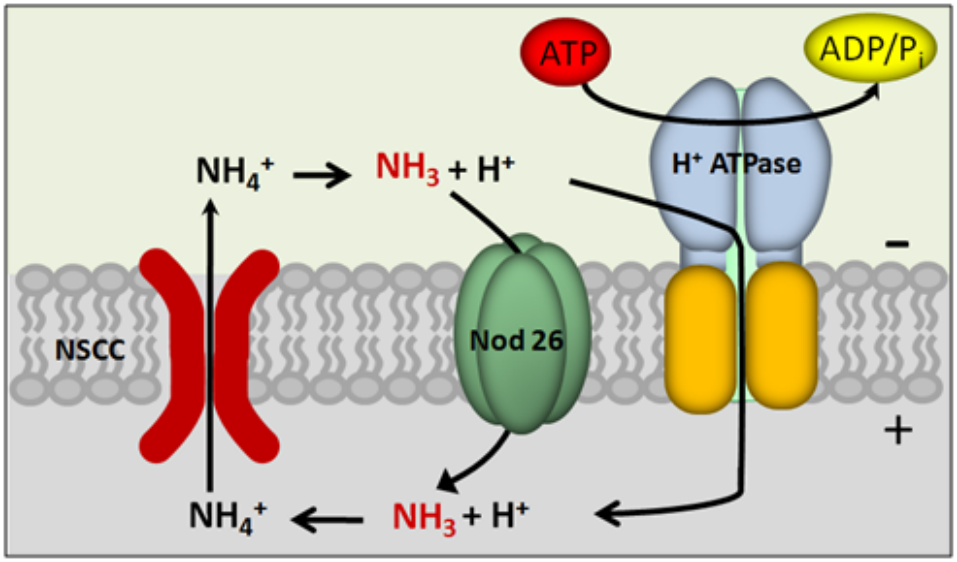

Fig. 3. Model for NH_4^+/NH_3 futile cycling on the SM. A potential mechanism for ammonia futile cycling through the symbiosome membrane is shown. The symbiosome membrane is energized by an H^+-ATPase which generates a proton gradient by pumping H^+ into the symbiosome space. When the symbiosome membrane is hyperpolarized, the NSCC is activated which directionally transports NH_4^+ into the cytosolic compartment. Since the cytosol is more alkaline than the symbiosome space, NH_4^+ can release a H^+ with NH_3 potentially reentering the symbiosome space through nodulin 26. Maintenance of cytosolic NH_4^+ levels at low concentrations by rapid assimilation via GS would be one approach to prevent this potential metabolite cycling.

4. Potential questions that could be addressed by modeling approaches

- *What is the major pathway of fixed nitrogen efflux through the symbiosome membrane?*
  This is the overarching question which hopefully mathematical modeling will help address.

- *Can we develop a quantitative model to predict the pathway and efficiency of nitrogen efflux based on parameters of pH, membrane potential, energy charge and other biochemical factors?*
  This is a complex process that involves multiple variables. We have biophysical quantitative information on the flux of ammonium through the NSCC and its dependence on voltage (e.g., [5], [6]) and the permeability data for ammonia through nodulin 26 [4], and estimates of steady state concentrations of ammonia and ammonium ion in the plant cytosol [9].

- *Can the potential process of futile cycling be modeled? Under what physiological conditions would futile cycling occur?*

- *What role would glutamine synthetase potentially play in preventing futile cycling? Could quantitative approaches provide experimentally testable predictions?*

- *Nodulin 26 possesses both aquaporin and ammoniaporin activities which are proposed to be regulated by phosphorylation. Can the effects of phosphorylation on the nitrogen efflux process be modeled?*
  Although not yet published, we have quantitative analyses of the effects of phosphorylation on nodulin 26 ammonia permeability that could be used as inputs to model this process.

5. References

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