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# Nitroplast : A New Eukaryotic Organelle that Holds Great Promise for Sustainable Agriculture

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## ABSTRACT

**Nitroplast is a recently discovered eukaryotic organelle that functions in nitrogen-fixation. The first report of nitroplast was from the marine algae *Braarudosphaera bigelowii*, in which the endosymbiotic relationship of a nitrogen-fixing cyanobacteria evolved into an organelle. Like other organelles, during the course of evolution, the genome of the endosymbiotic organism lost many important genes and hence it cannot exist independently. As the *B. bigelowii* genome encodes most of the proteins required for the nitroplast, it is now regarded as an organelle. Engineering nitroplast enables autotrophic nitrogen fixation in crop plants, which in turn reduces the usage of chemical fertilizers, thereby leading to sustainable agricultural practices.**

## INTRODUCTION

Nitrogen is a crucial element for crop growth and productivity, but it is also one of the most expensive plant nutrients to provide. Plants uptake inorganic Nitrogen sources such as ammonium or nitrates, and organic sources such as ammino acids. The nitrate is reduced to ammonia, and later assimilated into amino acids (Masclaux-Daubresse et al., 2010). Plants that thrive in low pH and reducing soils typically absorb ammonium or amino acids, while plants in aerobic soils with higher pH prefer nitrate (Masclaux-Daubresse et al., 2010). Crop plants rely heavily on ammonia as an organic nitrogen source. In nature, the reduction of atmospheric nitrogen (N2) to ammonia (NH3) occurs through symbiotic Nitrogen Fixation by aerobic microorganisms such as Rhizobium, in which the nitrogenase enzyme plays an important role (Rong et al., 2024). This process involves incorporating dinitrogen gas from the atmosphere into biomass through symbiotic or free-living diazotrophic bacteria that possess *nif* genes encoding the nitrogenase enzyme in their genome. Many photosynthetic plants form symbiotic relationships with nitrogen-fixing organisms to utilize ammonia, such as azolla water ferns, leguminous plants, and diatoms (Johnston, 2024). The biological nitrogen fixation remains as the major source of Nitrogen until the appearance of synthetic Nitrogen Fertilizers (Quemada et al., 2024). However, the chemical synthesis of ammonia through the Haber-Bosch process consumes a significant amount of energy and releases carbon dioxide into the environment, that accounts about 1 percent of the total carbon emissions in the world (Ye and Tsang, 2023). Excessive nitrogen fertilization can alter the physio-chemical properties of the soil.

The Rhizobia:legume symbiosis is highly specific; and each Rhizobia species infects a specific legume plant. While the mutualistic relationship between nitrogen-fixing Rhizobia and leguminous plants enriches soil nitrogen content and promotes sustainability, engineering non-host plants for nitrogen-fixing abilities remains a significant challenge.

## NITROPLAST : ENDOSYMBIONT TO ORGANELLE

Plant cells contain two crucial organelles; mitochondria and chloroplasts, which play vital roles in cellular functions such as respiration and photosynthesis. These organelles evolved through the integration and evolution of endosymbionts into plant cells, resulting in the reduction of the endosymbiont's genome as genes were lost or transferred to the host nucleus (Johnston et al., 2024). However, a specific set of genes was retained in the organelle. A recent discovery revealed that the unicellular marine microalgae *Braarudosphaera bigelowii* possesses a nitrogen-fixing organelle; known as nitroplast formed through the integration of an endosymbiont, similar to mitochondria and chloroplasts (Massana, 2024).

Although the discovery of nitroplast (Figure 1) was reported in 2024, the pioneering works on it began in the 1990s; when scientists observed the presence of the DNA sequence of unknown nitrogen-fixing cyanobacteria (UCYN-A) in sea water. Recently, Coale and his collaborators studied the marine alga *Braarudosphaera bigelowii*, which has the ability to fix nitrogen through its association with endosymbiont UCYN-A, also known as *Candidatus Atelocyanobacterium Thalassa*. Despite being closely related to cyanobacteria and having *nif* genes for nitrogen fixation, the endosymbiont UCYN-A now lacks genes for oxygenic photosynthesis, carbon fixation and tricarboxylic acid cycle (Liu et al., 2024) and therefore relies on the host cell.

Furthermore, the endosymbiont UCYN-A now has a reduced genome size compared to that of cyanobacteria.

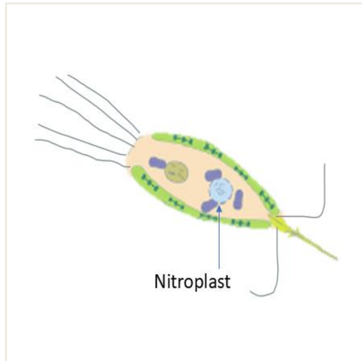


**Figure 1. *Braarudosphaera bigelowii,* the marine alga with nitroplast organelle (Image source: Rong *et al.,* 2024)**

The X-ray tomography images of the changes throughout the *B. bigelowii* cell cycle revealed precise and synchronized division of UCYN-A, coordinated with host cell growth and division. The control of UCYN-A division by the *B. bigelowii* host indicates that the host cell treats it like other organelles. Furthermore, proteome analysis revealed the absence of vital proteins in UCYN-A, whose functions are replaced by proteins translated from the algal nuclear DNA. These proteins were found to have long tails of peptides that aid in their transport to the organelle (Samuels, 2024). These experimental validations proved that UCYN-A evolved from a symbiont to an organelle in eukaryotic marine algae, and thus nitrogen fixation is no longer an exclusive feature of prokaryotes.

## ENGINEERING NITROPLAST: PROMISES AND CHALLENGES

Crop yield is strongly dependent on nitrogen, and the plant's ability to fix nitrogen on its own shows great potential for sustainable agriculture. The symbiotic relationship between rhizobia and legumes, Frankia and actinorhizal plants, and cyanobacteria in unicellular microalgae and angiosperm *Parasponia* species have natural nitrogen fixation processes that have evolved over time. Understanding the molecular mechanisms involved in the interaction of nitroplast in host cells (Figure 2) is crucial for developing artificial nitroplast in non-host plants. The adequate expression and cellular organization of the nitrogenase enzyme is critical, as it is easily degraded upon the exposure to Oxygen. A nano-sized artificial organelle that encapsulates the nitrogen-fixing machinery is a promising strategy. The cellular nitroplast organization has a similar architecture, where mitochondria surrounds the organelle membrane fix nitrogen through proper coordination with the host cell to prevent the oxidative damage of the nitrogenase enzyme (Rong *et al.,* 2024).

Nitroplast-like artificial nitrogen-fixing organelles can be developed through synthetic biology approaches. Engineering bacteroid as nitroplasts in non-nitrogen fixing crop plants enables the development of autotrophic nitrogen fixation (Rong et al., 2024). This allows the crop plants to obtain nitrogen (N2) from the air. Additionally, this advancement will decrease the need for chemical fertilizers and promote sustainable and environmentally- friendly agricultural practices.

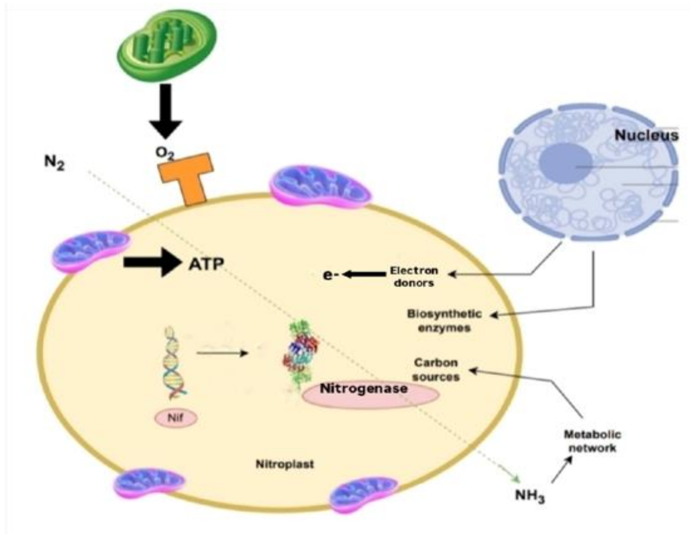


**Figure 2. Mechanism of nitrogen-fixation in the nitroplast**

Despite the specialized adaptive features of nitroplast such as reduced genome size and protein import; engineering the organelle in host plants presents numerous technical challenges. Ensuring compatibility between the engineered organelle and the host plant, inheritance to the next generation, minimizing energy expenditure, and optimizing protein targeting and translocation processes for symbiotic interactions are major hurdles in nitroplast engineering.

## CONCLUSION

The endosymbiont *Candidatus Atelocyanobacterium thalassa* (UCYN-A) in the marine microalga *Braarudosphaera bigelowii* was recently reported to have evolved into an organelle for nitrogen fixation. Nitrogen fixation was previously thought to be an exclusive process in prokaryotes. The discovery of the nitroplast from *Braarudosphaera bigelowii* was the first report of an organelle for nitrogen-fixation in eukaryotes. Creating an artificial organelle that encapsulates nitrogen-fixing machinery in crop plants through genetic engineering and synthetic biology approaches can make tremendous contribution to sustainable agricultural practices.

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