The roles of nitrogen in a complex world: Assembling a mosaic

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Biological N-fixation is confined to bacteria and archaea. In both natural and agricultural systems, symbiotic associations with N-fixing bacteria play a very prominent role. In many cases, the amounts of fixed N provided to the host are comparable to the amounts of industrial fertilizer applied to crops. Mycorrhizal fungi form symbiotic associations with the majority of plants that provide nitrate, ammonia, and, in some cases, organic nitrogen compounds to the plant. Extensive networks of hyphae formed by the mycorrhizal fungi efficiently take up mineral nutrients (nitrate and phosphate) from soil. Many mycorrhizal fungi also secrete hydrolytic enzymes that degrade proteinaceous material in the soil, and provide amino acids to the plant, thus contributing to the recycling of organic nitrogen in the biosphere. In some ecosystems, mycorrhizal fungi provide well over half of the fixed N needed by the host. In both the bacterial and fungal symbioses, the host contributes by providing carbohydrate produced by photosynthesis to the symbiont. The transfer of compounds between the partners in such symbioses is extremely efficient.

Nitrogen fertilizer applications to the Earth's land surface have approximately doubled the amount of nitrogen fixed annually. Industrial nitrogen fixation and fossil fuel combustion generate ca. 150 TgN/yr, an amount roughly equal to that provided by biological N-fixation. The huge increase in fixed nitrogen availability has resulted in adverse outcomes that include increased NH_3, and NO_3^- deposition, eutrophication of freshwater lakes, hypoxic zones in the coastal waters, rising concentrations of NH_3, NO, NO_2, and N_2O (a potent greenhouse gas) in the atmosphere, decrease in tropospheric ozone, and contamination of drinking water supplies with NO_3^- (1, 2).

Agriculture is the largest consumer of excess fixed nitrogen and its needs are expected to grow substantially. Many on-going efforts aim at decreasing the agricultural use of industrial fertilizers in agriculture. Various approaches have proved to be effective. The challenge is to tailor solutions that address the particular conditions that farmers face in different parts of the world — such as variations in soil fertility, water availability, and climate. Anticipated climate change poses a serious challenge, as illustrated by a quote from a recent document published by The International Maize and Wheat Improvement Center (CIMMYT) "*...as a result of possible climate shifts, by 2050 as much as 51% of the Indo-Gangetic Plains—currently part of the high-potential, irrigated, low-rainfall global mega-environment that accounts for 15% of the world's wheat production—might be reclassified as a heat stressed, irrigated, short-season mega-environment. This shift would significantly reduce wheat yields, unless appropriate cultivars and crop management practices were offered to and adopted by South Asian farmers*" (3).

The degree to which crops utilize supplied fixed nitrogen is called nitrogen use efficiency (NUE), and can be measured as crop yield per unit of applied nitrogen. Important crop plants, rice, and maize, and wheat, account for about a half of the industrial fertilizer use. For these crops, the NUE is typically below 50%. For example, a third of the world's nitrogen fertilizer is applied to wheat crops with an NUE of only about 33%. Some of the currently available approaches to reducing nitrogen fertilizer use, without a corresponding decrease in crop yield, are noted below (4).

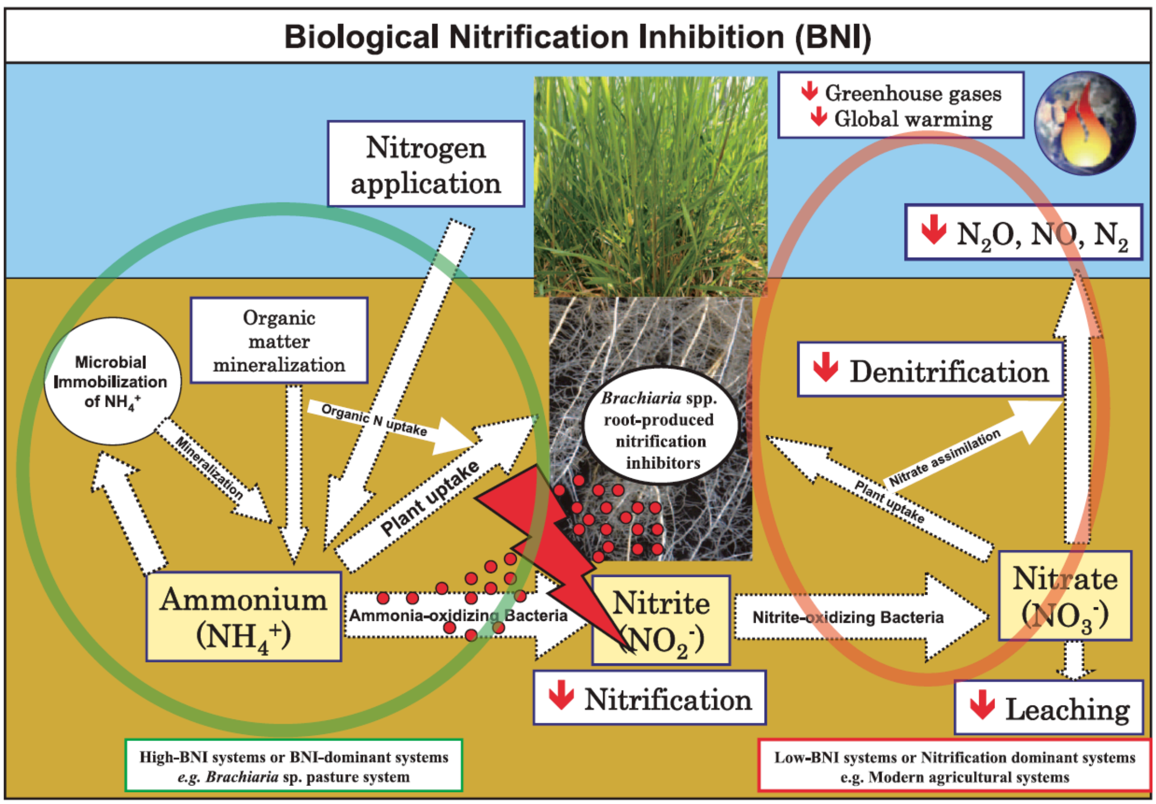
● Precision Agriculture. Results show that losses of applied fertilizer can be halved in irrigated wheat crops with no loss of yield by using infrared sensors and a normalized differential vegetative index to determine the right times and correct amounts of fertilizer to apply (5, 6).

● Conservation Agriculture and Appropriate Cultivars. To address the anticipated future challenges confronting a farmer in the Indo-Gangetic Plains (see quote above), CIMMYT researchers and partners are developing heat-tolerant wheat germplasm and cultivars for conservation agriculture (for the principles of conservation agriculture, see ref. 7).

● Crop Breeding. Current programs leading to cultivars with improved NUE exploit either traditional breeding or attempt to produce varieties genetically engineered (GE) to that end. Such GE crops are still in the development phase (3, 4).

● Nitrogen-fixing Cover Crops and Intercrops. For example, legume cropping in maize-based systems through systematic rotations and intercrops can supply double the level of nitrogen that is typically provided by the usual level of application of synthetic fertilizers (3).

● Biological Nitrification Inhibition (BNI). Many plants have the ability to produce and deliver by roots compounds that inhibit nitrification in the soil (8, 9). Nearly 90% of worldwide N-fertilizer is applied in the form of NH_4^+. Chemolithotrophic bacteria



Schematic representation of the nitrogen cycle showing the steps where biological nitrification inhibitors (BNI) produced by the roots of certain plants inhibit the enzymes that convert NH_3 to NO_3^- (red lightning bolt). In ecosystems with large amounts of BNI, such as *Brachiaria* pastures, the flow of nitrogen from ammonia to nitrate is restricted and NH_4^+ builds up in the root soil system. In systems with little or no BNI, such as modern agricultural systems, nitrification tends to occur at a rapid rate converting NH_4^+ to the highly mobile NO_3^- that tends to be rapidly lost from the soil root system [From Subbarao, G.V. et al. (2009) Biological nitrification inhibition – Is there potential for genetic intervention in the Triticaceae? *Breeding Science* 59: 529-545]

(*Nitrosomonas* and *Nitrobacter* spp.), through nitrification (see figure, above), convert NH_4^+ within days or weeks to NO_3^-, a highly mobile anion. NH_4^+ remains tightly bound to negatively charged constituents of soil. Nitrification is the most important process in the N cycle that leads directly to N losses. Under anaerobic, or partially anaerobic conditions, a number of heterotrophic bacteria denitrify NO_3^- with the formation N_2O, NO, NO_2. The potential of high BNI production capacity exists in wild wheat (3). It appears likely that it will be possible, through traditional breeding techniques, to introduce and/or improve the ability of economically important varieties of wheat, barley, and rye to produce BNI.

● Establishment of new partnerships between wheat, maize, and rice, and endophytic nitrogen-fixing bacteria. Examples are provided by the intracellular colonization of crop plants by *Gluconacetobacter diazotrophicus,* and the successful inoculation of wheat with the nitrogen-fixing bacterium *Klebsiella pneumoniae* 342 leading to an endophytic association (10-12). In both instances, the endophytic bacteria fix nitrogen within the host and share it with the host. The quantitative aspects of the levels of nitrogen fixation reached within these partnerships have yet to be described.

● *De novo* genetic engineering of new symbioses. In his 1970 Nobel Peace Prize lecture, Norman Borlaug said "*In my dream I see green, vigorous, high-yielding fields of wheat, rice, maize, sorghums, and millets, which are obtaining, free of expense, 100 kilograms of nitrogen per hectare from nodule-forming, nitrogen-fixing bacteria. ………Then I wake up and become disillusioned to find that mutation genetics programs are still engaged mostly in such minutiae as putting beards on wheat plants and taking off the hairs*" (13). Forty years later, Borlaug's dream remains unrealized.

The levels of nitrogen fixation in the symbioses between rhizobia and legumes are very high and there is substantial detailed information on the host-symbiont interactions that underlie these highly successful partnerships (14). The attempt to engineer *de novo* endosymbiotic interactions of nitrogen-fixing bacteria and wheat, maize, and rice that would achieve the high levels of nitrogen fixation exhibited by the partnerships between rhizobia and legumes may still be overly ambitious, but stepwise efforts towards that goal would be desirable.

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