A "more ammonium solution" to mitigate nitrogen pollution and boost crop yields

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Two of the world's great agricultural challenges require bold new approaches and could share a solution. Nitrogen (N) pollution, affecting water, air, and the climate, presents one massive challenge. Ninety percent of increased reactive N originates as synthetic fertilizer applied to agricultural fields or N fixed in them (1). Because crops take up only 42 to 47% of the total applied N, more than half is lost to the environment in some way (2, 3). Despite some recent regional improvements in nitrogen use efficiency (NUE), global average NUE has not increased since 1980. Yet even if by 2050 the world increased NUE by 50% (to ~70%), likely 50%

We can address pollution and boost crop yields by exploiting new tools that keep a higher share of soil nitrogen as ammonium while selecting and breeding crops to exploit an ammonium/nitrate balance. Nitrification-inhibiting traits originally discovered in some tropical grasses can be enhanced in cereal crops too. Image credit: Flickr/CIAT.

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increases in food production would maintain N losses to the environment at roughly their present, unacceptable levels (3).

A second challenge is to increase crop yields at a more rapid (linear) rate in coming decades to meet rising food demands without clearing more forests and releasing their carbon (3). Just as yield growth has historically resulted from synergies of crop breeding and management changes, future gains must rely on doing both in even smarter ways. Large yield growth by adding fertilizer or doubling irrigation is no longer possible or environmentally acceptable in most of the world (3–5).

The scope of these challenges requires multiple new approaches, and here we make the case for a "more ammonium solution." With the exception of paddy rice, nitrate dominates the inorganic N in modern agricultural soils, leading to most N pollution problems. This solution would exploit new tools to keep a higher share of soil N as ammonium and select and breed crops to exploit an ammonium/nitrate balance.

First, it's important to ask how more ammonium (NH_4^+) and less nitrate (NO_3^-) would address nitrogen pollution. Although 20% of total N losses from field-applied N occurs through volatilization of ammonia (NH_3; *SI Appendix* and Table 3 in 6), the great majority of N losses occur after microbial reactions transform ammonium in soils into nitrate, typically in fewer than 10 days (7). As an anion, NO_3^- does not bind to soil (with limited exceptions) and thus easily leaches with water into groundwater and waterways. The formation and breakdown of NO_3^- by bacteria and archaea also release nitrous oxide, a powerful greenhouse gas, as well as more NO_x, which contributes to air pollution problems. Once N nitrifies into NO_3^-, unless crops or grasses quickly take it up, it has a good chance of polluting the environment.

Most promising strategies to reduce N pollution can only do so much because they focus on the "front end" by reducing fertilizer application. Strategies include educating farmers to restrict fertilizer to economically optimal rates, tools to help farmers apply most N during the growing season in amounts that account for N mineralized from soils (8), and possibly even bacteria that help cereals fix nitrogen (9). These measures could increase NUE, but substantial losses will continue in part because half of global, field-applied N occurs not in fertilizer but in manure, crop residues, air deposition, and irrigation water (2). More fundamentally, applying N more carefully cannot, by itself, prevent N from continuing to leak at the back end, which will continue so long as N turns into nitrate.

Significant back-end losses will continue because N continues to mineralize into nitrate from soil organic matter (SOM) even late in growing seasons—releasing N absorbed into soils in previous years (*SI Appendix*). Because this mineralization occurs when crops no longer take up N, it can easily turn into nitrate and escape. In some regions most N leaching occurs in winter when rains leach out this mineralized, inorganic N (10, 11). Cover crops provide one promising back-end strategy for annual cropping systems, but adoption rates are low and cover crops face various practical challenges (*SI Appendix*). To solve the N problem, agriculture needs additional tools to reduce this leakiness at the back end.

Enter Ammonium

That's where ammonium comes in. As a cation (NH_4^+), ammonium adheres to most soils, particularly to clays and SOM, and so escapes little with water. And so long as N remains as ammonium, it cannot generate nitrous oxide. Benefits will occur even if nitrification is not blocked year-round. Because N_2O emissions primarily occur in the spring in many climates (*SI Appendix*), even postponing nitrification for several weeks can reduce emissions as well as reduce fertilizer needs by reducing leaching losses. And the longer N stays in soils, particularly in the ammonium form preferred by microbes (12), the more opportunity for microorganisms to take it up, build soil organic carbon, and reduce leaching (*SI Appendix*).

More ammonium can also boost yields. Although too much ammonium is toxic to most crops, a fifty-year line of evidence has shown that a mixed share of NH_4^+ and NO_3^- can increase cereal crop yields compared with the near nitrate-only conditions that typically prevail in crop fields (13, 14). For example, in 1973, Cox and Reisenauer found that a mixture with 20% NH_4^+ increased wheat growth by 54% compared with all NO_3^- conditions (15), whereas Wang et al. in 2019 found >80% increases in maize growth with 25% NH_4^+ (14). As a 2002 review of ammonium toxicity wrote, "while toxicity is observed in many species when NH_4^+ is provided alone, . . .co-provision [with nitrate] induces a synergistic growth response that can surpass maximal growth rates on either N-source alone by as much as 40 to 70% in solution culture though by somewhat less in soil" (16).

The precise reasons are not fully known (16, 17), but the benefits make sense because nitrate and ammonium each have physiological advantages and disadvantages (13, 16–18). Plants also use mostly separate systems to absorb and use nitrate and ammonium, including different root exudates to facilitate root/soil interactions, different root absorption mechanisms, different transport pathways to leaves, and additional pathways for converting NO_3^- back to NH_4^+ so leaves can use the N. A mix of N forms may help plants just by allowing them to use each system. In one impressive study of maize, Loussaert et al. (13) found that for several weeks after silking, additional NO_3^- supply did not improve growth, but additional NH_4^+ supply boosted maize ear growth up to 50% (13). Saturation of the crop's pathway to reduce NO_3^- limited its assimilation, although the crop could still assimilate more NH_4^+ (13). Because rising atmospheric CO_2 likely inhibits plant assimilation of NO_3^- but not NH_4^+, NH_4^+ could become even more advantageous in the future.

Evidence that some varieties of the same crop respond better to ammonium than others shows genetic variation (19) and suggests that crop selection and breeding can enhance ammonium yield benefits. As one illustration, we found in bench experiments that increasing ammonium in hydroponic solution to 20 to 40% of total N boosted biomass production up to 60% in one variety of sorghum (compared with 100% nitrate control) but caused no gains in another (*SI Appendix*, Figs. S1 and S2).

Overall, inhibiting nitrification of ammonium into nitrate would not only reduce pollution but it might also enhance yield growth in three interrelated ways: 1) optimizing ammonium/nitrate ratios in soils, 2) limiting N losses, and 3) supporting crop varieties bred to exploit higher ammonium.

New Opportunities

Agronomists have paid only modest attention to these opportunities probably because any potential to limit nitrification has appeared modest and short-lived. In paddy rice and some ecosystems, soil saturation causes anaerobic conditions that limit nitrification, but most crops require aerobic conditions. Could nitrification be inhibited more and longer? In fact, many mature ecosystems suppress nitrification and N loss to low levels using chemicals produced by plants, soil bacteria, and fungi (7, 19). For agriculture, the opportunities include better development and use of synthetic nitrification inhibitors (SNIs) and biological nitrification inhibition (BNI).

Meta-analyses have found that existing SNIs increase NUE on average by 7 to 16% and reduce N_2O emissions on average 35 to 40% [with larger reductions often observed (20)]. SNIs also often lead to increases in yield by a few percent for some crops, enough to more than pay for their cost (20). Unfortunately, the effects are highly variable, which the literature seems to accept as a given.

But the world does not need to settle for existing SNI formulations. Today, only three SNIs dominate agricultural use (nitrapyrin, DCD [dicyandiamide], and DMPP [3,4-dimethylpyrazole phosphate]) (21), and they have severe limits: They probably do not limit nitrification by archaea, and effects in bacteria only inhibit the first step of conversion toward nitrate. There is no reason in principle that additional SNIs could not inhibit these other processes. Even today, one option would combine a portion of SNIs with delayed-release compounds to extend the NI effect beyond a few weeks (20).

The world can also improve its use of existing SNIs. Varying performance reflects variability in weather but probably also differences in soils, microbial communities, and crops (22). By better understanding these conditions, it should be possible to improve performance by deploying SNIs for different crops and environmental conditions.

BNI provides a potentially better, and lower-cost alternative. As early as the 1960s, ecologists observed low nitrification rates in certain grassland and forest ecosystems attributable to phytochemicals exuded by plant roots that blocked nitrification (7, 19). Yet agricultural breeders only began to take interest in BNI chemicals with the discovery of low N_2O emission rates from tropical pastures of *Brachiaria humidicola*, which researchers traced to root exudation of *brachialactone* (7, 19). Subsequent work by a loose network of plant researchers (*SI Appendix*, Table S1) has been able to identify BNI traits in many staple crops that include sorghum, wheat, maize, and rice (7; *SI Appendix*).

These discoveries create potential for breeding to strengthen the BNI effect and to incorporate BNI traits into high yielding crop varieties (using classical and molecular breeding tools; Table 1). Wheat BNI research is most advanced. Without genetic engineering, researchers have successfully transferred the chromosome segment carrying BNI trait from a wild grass (*Leymus racemosus*) into cultivated wheat and are now

Table 1. BNI research status and anticipated improvements in the next 10 years

Crop/ Pasture sp.	BNI characterization status	Availability of high-BNI genetic stocks	Knowledge on BNI chemical identity and mode of inhibitory action	Possibility of introducing BNI trait into elite crop cultivars (10 years from now)	Expected level of inhibition in field (root zone)
Brachiaria sp. pasture grasses	Characterized	Yes	Yes brachialactone, linoleic acid, linolenic acid - block both AMO and HAO enzymatic pathways	Yes	40 to 50% inhibition
Wheat	Somewhat	Yes	No	Yes	30% inhibition from first generation BNI-enabled wheat cultivars
Sorghum	Characterized	Yes	Yes sorgoleone (AMO and HAO) MHPP (AMO)	Yes	30% inhibition
Maize	Work has started	Yes	Yes (not published yet)	Yes	20 to 30% inhibition
Rice*	Somewhat	Not known	Yes	Not known	Yet to be assessed

References to "AMO" (ammonia monooxygenase) or "HAO" (hydroxylamine oxidoreductase) refer to the enzyme involved in ntirification that is inhibited. "MHPP" refers to methyl 3 (4-hydroxyphenyl) propionate, exuded by BNI sorghum.

*Although nitrification in paddy rice fields is low, nitrification and N_2O emission rates may reach extreme levels where water levels fluctuate (24).

testing the first generation of high-yielding, BNI wheat varieties. For sorghum, research has identified the approximate chromosome region controlling sorgoleone production, the primary BNI exudate, in efforts to allow marker-assisted selection to develop high-yielding, BNI sorghum varieties. Development of BNI agropastoral systems is in progress by exploiting the high BNI capacity of *Brachiaria* pastures in rotation with maize.

Overcoming Limitations

BNI has potential to overcome many of the limitations of SNIs. Once bred into crops, farmers all over the world can adopt them without added expense or management. And unlike SNIs (22, 23), at least some BNI chemicals are likely effective against both nitrifying bacteria and archaea (7, 19).

Although delivery of SNIs into nitrifying sites is challenging, plant root systems release BNI chemicals directly into soil microsites where ammonium is most present and where nitrifying bacteria populate (7, 23). At least some plants, including sorghum, can release both hydrophobic BNI chemicals, which remain confined to the rhizosphere, and hydrophilic BNI chemicals, which move with water flow to suppress nitrification elsewhere in soils.

Although SNIs can contribute to modest increases in ammonia losses from urea unless combined with urease inhibitors or fertilizer is placed in a band below the surface (20), BNI is unlikely to increase ammonia losses because they work overwhelmingly at least 10 centimeters underground and mostly in the rhizosphere. BNI can also result from a cocktail of phytochemicals inhibiting nitrifying bacteria in multiple ways (7, 19).

Finally, the BNI effect may be able to persist in soils and suppress nitrification year round. For example, the residual BNI effect from *Brachiaria* pastures has substantially reduced soil nitrification rates and improved maize grain yields for three subsequent years in a *Brachiaria*–maize rotation (7). Roots of other BNI crops are likely to have at least some persistent effect. Completely stopping nitrification is neither possible nor beneficial for crops, but a "more ammonium solution" has potential to boost yields and reduce nitrogen pollution.

Among challenges, the technological improvements required are by definition uncertain. Some may fear "chemical" approaches, although BNI is a natural plant phenomenon, and the rhizosphere is already a chemical battleground among plants and microbes. BNI is now most effective in acidic soils, which could limit their reach. BNI strength in legumes is also weak or nonexistent (7); at least absent genetic engineering, BNI is likely restricted to cereals and other grasses. Potential also exists for soil nitrifying organisms to become resistant to precise nitirification inhibiting proteins. But BNI has been proven persistent in natural, tropical ecosystems (7, 19). The combination of multiple compounds released, plus resetting of microbial communities as a result of breaks in BNI activity (and possibly breaks in use of BNI crops) should help to avoid build-up of resistance, although new breeding may also become necessary.

Pursuing this solution requires policy initiatives that help overcome two challenges simultaneously—improved inhibition and crop breeding to maximize yield benefits. Scientists have little reason to pursue either solution unless the other is also being pursued.

To start, governments should fund SNI development, which currently has virtually no public funding. Public funding should support precommercial efforts to develop compounds that work on both major steps in the enzymatic pathways of converting ammonium to nitrate, have multi-mode inhibition, and work on nitrifying archaea as well as bacteria. Publicly funded field testing of SNIs has been more common but too limited to differentiate how to use them. Governments should fund large-scale, coordinated testing networks for different SNIs, combinations, and controlled release formats on different crop varieties in different soils and agroecological zones.

For BNI, the biggest need is for breeding to develop varieties with higher BNI and that gain yield advantages from higher ammonium. The BNI consortium, founded in 2005 by Japan International Research Center for Agricultural Sciences and three institutes of the CGIAR, has grown into a 17-institute partnership to advance BNI research (*SI Appendix*, Table S1). Funding is still minimal. Private sector breeding efforts would also be valuable for crops, such as maize, in which that sector plays a prominent role.

To encourage these innovations, governments can shift farm or fertilizer subsidies to support the use of any form of NI. Governments could legally commit themselves to require or subsidize BNI use up to a modest cost premium once breeds achieve a specified level of effectiveness. More broadly, governments could vary subsidies to support any methods that reduce N losses based on the likely level of improvement. Fertilizer producers could also be required to sell increasing shares of N in combination with increasingly effective SNIs (20). To facilitate sales, companies would have incentives to develop both better SNIs and more information about how best to deploy existing SNIs.

The world will not be able to solve the nitrogen problem unless it can find ways to plug the nitrate leaks at the "back-end," for which there are limited tools. The potential emergence of BNI could represent one means of doing so, while allowing crop breeding to exploit potential yield gains from a balance of N forms. This combination of desirable results makes the case for advancing the "more ammonium solution."

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Data and Materials Availability. Further data regarding the new wheat and sorghum experimental results described in this study are available on request from G.V.S.

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