Supplementary Materials for

Title: A More Ammonium Solution for Nitrogen Pollution and to Boost Crop Yields

GV Subbarao and Timothy D. Searchinger

Correspondence to: tearchi@princeton.edu, subbarao@jircas.affrc.go.jp

This PDF file includes:

Materials & Methods
Supplementary Text
Figs. S1 to S2
Tables S1 to S2

Materials and Methods

Additional experiments

As shown in Figure S1, wheat *cv*. 'Munal' and Sorghum genotypes, 296B, IS20205 were grown for 60 days in nutrient solution culture at 1 mM N, but varying ratios of NH_4^+ (NH_4Cl) and NO_3^- (KNO_3) (ranging from 0% NH_4^+ to 100% NH_4^+ as N source; K levels in all the N-treatments were kept constant; total N concentration was maintained at 1 mM in all these N-treatment solutions) in a temperature-humidity controlled growth chamber (25/22°C; 14/10 h for wheat; 30/28°C; 14/10h for sorghum) for 60 d. Nutrient solutions were changed once in 15 d and pH of the nutrient solutions were adjusted to 6.5 at the beginning of solution change, but allowed to fluctuate during the 15 d period. Plants were harvested at 60 days after planting and root and shoot dry weights were recorded. For sorghum, the data shown is the mean of two genotypes. Both average sorghum response and wheat showed stimulatory effect on plant growth up to 20% of N as NH_4^+ (i.e. 20% NH_4^+: 80% NO_3^-). When NH_4^+ levels in nutrient solutions increased to 40% (40:60 NH_4^+ : NO_3^-) it had a negative effect on growth of sorghum, but for wheat, the negative effect was observed only from 60% (60:40 NH_4^+ : NO_3^-) and above, compared to 100% NO_3^- control. This result suggests that the crops differ in their stimulatory response to presence of NH_4^+ and also to the extent of tolerance to NH_4^+ levels in nutrient solutions.

As shown in Figure S2, we grew two sorghum genotypes, which differ in BNI-capacity, including production of sorgoleone, a BNI exudate. One was 296B, a low-sorgoleone producing line and low hydrophobic-BNI capacity genetic stock; the other was IS20205, about two to three-fold higher sorgoleone production from roots compared to 296B and high-hydrophobic-BNI capacity genetic stock. Each of these two varieties were grown for 60 days in nutrient solution culture at 1mM N, but varying ratios of NH_4^+ (NH_4Cl) and NO_3^- (KNO_3) (ranging from 0% NH_4^+ to 100% NH_4^+ as N source) in a temperature-humidity controlled growth chamber (30/28°C; 14/10h). Nutrient solutions were changed once in 15 days and pH of the nutrient solutions were adjusted to 6.5 at the beginning of solution change but allowed to fluctuate during the 15-day period. Plants were harvested and root and shoot dry weights were recorded. High-sorgoleone producing genotype responded positively to the presence of NH_4^+ up to 20% of N (i.e. 20% NH_4^+: 80% NO_3^- in nutrient solutions) and showed a 60% stimulation on growth and biomass production. There were no negative effects on growth up to 70% share of NH_4^+ of total inorganic nitrogen compared to 100% NO_3^- control. However, the low-sorgoleone producing genetic stock (296B) showed no such stimulation to presence of NH_4^+ at 20% (i.e. 20% of total N as ammonium and 80% is nitrate), and beyond that showed a negative effect on growth. These results show genetic variation to NH_4^+ tolerance in sorghum and suggests the potential to breed for sorghum cultivars that can benefit from NH_4^+ presence and tolerate high levels of NH_4^+ in the future.

Supplementary Text

Global nitrogen crisis

Papers describing the global nitrogen crisis include Stevens (2019) (S1), Schlesinger (2009) (S2) & Gilbert (2011) (S3).

Global NUE and future nitrogen projections.

NUE as used in the text is the N removed in harvested crops divided by the N in total inputs, including fertilizer, manure, nitrogen fixation and deposition. The numbers presented in text are from Lassalletta *et al.* (2014) (S4), and Zhang *et al*. (2015) (2), with estimates of recent NUE at 42-47%. Conant *et al.* (*2013*) (S5) estimates NUE at ~40%.

Projections of future nitrogen use in 2050 range from annual increases of 25%, Bodirsky *et al*. (2014) (S6), 54% by Alexandratos & Burinsma (2012) (S7), and 75% in Searchinger *et al*. (2019) (3), but Bodirsky's estimate already assumes large gains in NUE. The middle estimate was based on a population projection of roughly 9 billion in 2050 rather than more recent estimates of 10 billion, and the last estimate was based on projections that existing NUE by region would remain unchanged in 2050 due to lack of any clear statistical demonstration of increasing global NUE trend lines.

Shares of N field losses from ammonia v. nitrification pathways

Estimates for the pathways of N losses are only for cropland and do not include losses of N from managed manure before manure is applied to farmlands. For global estimates, we use recommended new tier one volatilization loss rates to fertilizer and applied manure from 2019 refinements to IPCC reporting guidelines (IPCC 2019) (S8), which are 11% for synthetic fertilizer N (up from 10% in the 1996 guidelines), 21% for manure and other organic N application rates, and 1% for N deposition. We apply these to the quantities of global N applied to cropland estimated for 2010 by Lassellata et al. (2014) (S4) of 95 Tg N from synthetic fertilizer and 30 Tg N from manure and 10 Tg N for nitrogen deposition, which generates 15.8 Tg N in ammonia. Lassellata et al. (2014) also estimates 160 Tg N of total applied N to croplands, including 30 Tg N from nitrogen fixation, and a surplus of N not absorbed by crops of 85 Tg N. (The IPCC does not provide a Tier One ammonia emission factor for N fixation but it is likely to be modest.) This calculation results in an estimate of 18.6% of total N loses in N, which we round to 20% to account for the broad nature of this calculation and some likely ammonia volatilization from residues from nitrogen fixation. A similar estimate is available for the U.S. and Europe from Table 3 of van Grisvenen et al. (2015) (6).

Effects on NUE from improvements in crop varieties and management in U.S.

Papers finding an important role for improvements in crop varieties in increasing NUE in the U.S. include Mueller *et al*. (2019) (S9) and DeBruin *et al.* (2017) (S10), Ciampitti *et al*. (2011) (S11) describes the role of increased planting density in increasing NUE in the U.S.

Rapid Nitrification

Papers finding rapid nitrification in different soils include Sahrawat (1982) (S12), Fortuna *et al.* (2003) (S13) and Norton *et al.* (2019) (S14).

Reasons mineralized N causes N losses even if fertilizer application is efficient.

Much of the N that runs off results form mineralized N. Papers describing the incorporation into soil organic matter and subsequent release of inorganic nitrogen include Dourado-Neto *et al*. (2010) (S15) and Ladha *et al*. (2005) (S16), and Zhao *et al*. (2016) (S160. Papers showing the role of the release of mineralized N in ammonia losses and leaching of nitrogen include Radersma et. al. (2011) (10), and De Notaris et al. (2018) (11).

Why delayed nitrification should limit N losses

One reason keeping N as ammonium longer is likely to reduce N losses even if remaining soil ammonium after crop harvests is nitrified is the preference microbes have for ammonium, which is well known Tietema and Wessel, 1992 (12); Accoe et al. 2004 (S16), Dannenmann et al. 2006 (S17), Rennenberg et al. 2009 (S18), Stockdale et al. 2002 (S19).

Inhibition of nitrate absorption by higher CO_2

Papers finding that elevated CO_2 inhibits nitrate assimilation in at least some crops include Bloom *et al*. (2014) (S20) and Pleijel & Uddling (2012) (S21).

Nitrate assimilation is metabolically more expensive than ammonium assimilation

Papers exploring these metabolic costs include L. Salsac *et al*. (1987) (17) and S. Guo *et al*. (2007) (S22). A summary of how plants can avoid some of these metabolic costs using light in chloroplasts to reduce nitrate is provided in Hageman (1984) (S23).

Interactions in absorption and use of nitrate and ammonium in plants

The complex ways in which availability and absorption by plants of nitrate and ammonium interact use of other are reviewed in Hachiya & Sakakibaraa (2017) (S24).

Meta-analyses of nitrification inhibitors

Meta-analyses of nitrification inhibitors showing yield gains and N_2O emissions reductions include Abalos *et al*. (2016) (S25); Qiao *et al*. (2015) (S26) and Feng et al. (2016) (S27) others summarized in Kanter & Searchinger (20).

Multiple sources of inhibition in natural ecosystems

Papers discussing how plants, soil bacteria and fungi all generate nitrification inhibitors in natural ecosystems include DeBoer *et al.* (1996) (S28), Paavolainen *et al*. (1998) (S29), and Subbarao *et al.* (2006) (S30).

Bacteria and archea reactions to inhibitors

Papers discussing how nitrifying bacteria and archaea react differently to different inhibitors include Hayatsu *et al*. (2008) (S31), Daims *et al.* (2015) (S32), and van Kessel *et al*. (2015) (S33).

Papers showing BNI in Native Tropical Grasslands

Papers showing BNI in native tropical grasslands include Lata JC et al. (1999) (S34), Lata JC et al. (2004) (S35).

Papers showing BNI *in situ* in *Brachiaria*

Papers showing BNI in brachiaria fields include Nunez et al. (2018) (S36), and Teutscherova N. et al. (2019) (S37).

Papers Showing BNI *in situ* in Sorghum

Papers showing BNI in sorghum include Subbarao et al. (2013) (S38), Teesfamariam et al. (2014) (S39), Hossain et al. (2008) (S40), Zhu et al. (2013), (S41), Di et al. (2018) (S42), Sarr et al. (2020) (S43).

Papers showing BNI function *in situ* in wheat and rice.

Papers showing BNI function in situ in wheat and rice include Sun et al. (2016) (S44), Subbarao et al. (2007) (S45), O'Sullivan et al. (2016) (S46).

Other nitrogen management practices

Techniques to control ammonia emissions such as banding are discussed in Sommer et al. (2004) (S47). Papers discussing the effectiveness of cover crops on leaching include Abdalla et al. (2019) (S48), and challenges to adoption are discussed in Kladivko et al. (2014) (S49).

Supplemental References

S1. Stevens CJ (2019) Nitrogen in the environment. *Science* **363**:578-580 (2019).
S2. Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proc. Nat. Acad. Sci. USA*, **106**:203-208.
S3. Gilbert N (2011) Summit urged to clean up farming. *Nature* **479**:279.
S4. Lassellata L *et al* (2014) Garnier. 50-year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environ. Res. Lett.* **9**:105011.
S5. Conant R *et al.* (2013) Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture, *Glob. Biogeo. Cyc*. **27**:558-566.
S6. Bodirsky BL *et al.* (2014) Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nature Communications*. **5**:doi:10.1038/ncomms4858.
S7. Alexandratos N, Bruinsma J (2012) *World Agriculture Towards 2030/2050: The 2012 Revision*" (FAO, Rome).
S8. IPCC (Intergovernmental Panel on Climate Change), *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories Volume 4 Agriculture, Forestry and Other Land Use* (https://www.ipcc-nggip.iges.or.jp/public/2019rf/vol4.html).
S9. Mueller S *et al.* (2019) Simultaneous gains in grain yield and nitrogen efficiency over 70 years in maize genetic improvement. *Scientific Reports*. **9**:9095.
S10. DeBruin JL *et al* (2017) Grain Yield and Nitrogen Accumulation in Maize Hybrids Released during 1934 to 2013 in the US Midwest. *Crop Sci*. **57**:1431-1446.
S9. Ciampitti IA, Vyn TJ (2011) A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. *Field Crops Res.* **12:**2–18.
S10. Sahrawat KL (1982) Nitrification in some tropical soils. *Plant Soil* **65**:281-286.
S11. Fortuna A *et al* (2003) Seasonal changes in nitrification potential associated with application of N fertilizer and compost in maize systems of southwest Michigan. *Agric. Ecosys. Environ*. **97**:285-293.
S12. Norton J, Quyang Y (2019) Controls and adaptive management of nitrification in agricultural soils. *Frontiers Microbiol*. **10**:1-18.
S13. D. Dourado-Neto D *et al* (2010) Multi-season Recoveries of Organic and Inorganic Nitrogen-15 in Tropical Cropping Systems. *Soil Sci. Soc. America J.* **74**:139–152.
S14 Ladha J *et al* (2005) Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects. *Adv.Agron.* **87**:85–156
S15. Zhao X *et al.* (2016) Ue of nitrogen isotope to determine fertilizer- and soil-derived ammonia volatilization in a rice/wheat rotation system *J. Agric. Food Chem.* **64**:3017–3024.
S16. Accoe F *et al* (2004) Gross N transformation rates and net N mineralization rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. *Soil Biol. Biochem.* **36**:2075-2087.
S17. Dannenmann M *et al* (2006) Effects of forest management on soil N cycling in beech forests stocking on calcareous soils. *Plant Soil* **287**:279-300.

S18. Rennenberg H *et al* (2009) Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. *Plant Biol* **11**: 4-23.

S19. Stockdale FA *et al* (2002) Verifying the nitrification to immobilization ratio (N/I) as a key determinant of potential nitrate loss in grassland and arable soils. *Agronomie* **22**:831-838.

S20. M. Bloom, Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat, *Nature Climate Change* **4**, 477-480 (2014).

S21. Pleijel H, Uddling J (2012) Yield vs quality trade-offs for wheat in response to carbon dioxide and ozone. *Glob. Change Biol.* **18**:596–605.

S22. Guo S *et al* (2007) Effect of ammonium and nitrate nutrition on some physiological processes in higher plants-growth, photosynthesis, photorespiration and water relations, *Plant Biol*. **9**:21-9.

S23. Hageman RH (1984), Ammonium versus nitrate nutrition of higher plants, pp. 67-85 in Huack, R.D. (ed). *Nitrogen in Crop Production* (Amer. Soc. Agron. Madison, WI).

S24. Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation and signaling in plants. *J. Exp. Bot.* **68**: 2501-2512.

S25. Abalos D *et al* (2016) Improving fertilizer management in the US and Canada for N_2O mitigation: understanding potential positive and negative side-effects on corn yields. *Agr. Ecosyst.Environ.* **221:** 214–221.

S26. Qiao CL *et al* (2015) How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. *Global Change Biol.* **21**:1249–1257.

S27. Feng JF *et al* (2016) Integrated assessment of the impact of enhanced-efficiency nitrogen fertilizer on N_2O emission and crop yield. *Agr. Ecosyst. Environ*. **231**: 218–228.

S28. DeBoer W *et al* (1996) Variability of N mineralization and nitrification in a simple, simulated microbial forest soil community. *Soil Biol. Biochem*. **28**:203-211.

S29. Paavolainen L *et al* (1998) Inhibition of nitrification in forest soil by monoterpenes. *Plant Soil* **205**: 147-154.

S30. Subbarao G *et al.* (2006) Scope and strategies for regulation of nitrification in agricultural systems – challenges and opportunities. *Critic. Rev. Plant Sci*. **25**:303-335

S31. Hayatsu M *et al* (2008) Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Sci. Plant Nutr*. **54**:33-45.

S32. Daims H. *et al.* (2015) Complete nitrification by *Nitrospira* bacteria. *Nature* **528**:504-509.

S33. van Kessel M *et al* (2015) Complete nitrification by a single microorganism. *Nature* **528**:555-559.

S34. Lata JC *et al.* (1999) Stable coexistence of contrasted nitrification statuses in a wet tropical savanna ecosystem. *Funct. Ecol.* **13**:762-768.

S35. Lata JC *et al* (2004) Grass populations control nitrification in savanna soils. *Funct. Ecol*. **18**:605-611.

S36. Nunez J *et al* (2018) Biological nitrification inhibition activity in a soil-grown biparental population of the forage grass, *Brachiaria humidicola*. *Plant Soil* **426**:401-411.

S37. Teutscherova N *et al* (2019) Differences in arbuscular mycorrhizal colonization and P acquisition between gentoypes of the tropical *Brachiaria* grasses: Is there a relation with BNI activity? *Biology and Fertility of Soils* **55**:325-337.

S38. Subbarao GV *et al* (2013) Biological nitrification inhibition (BNI) activity in sorghum and its characterization. *Plant Soil* **366**:243-259.

S39. Tesfamariam T *et al* (2014) Biological nitrification inhibition in sorghum: the role of sorgoleone production. *Plant Soil* **379**:325-335.

S40. Hossain AK *et al.* (2008) Detection, isolation and characterization of a root exuded compound, methyl 3-(4-hydroxyphenyl) propionate responsible for Biological Nitrification Inhibition by sorghum (*Sorghum bicolor* L.). *New Phytologist* **180**:442-451.

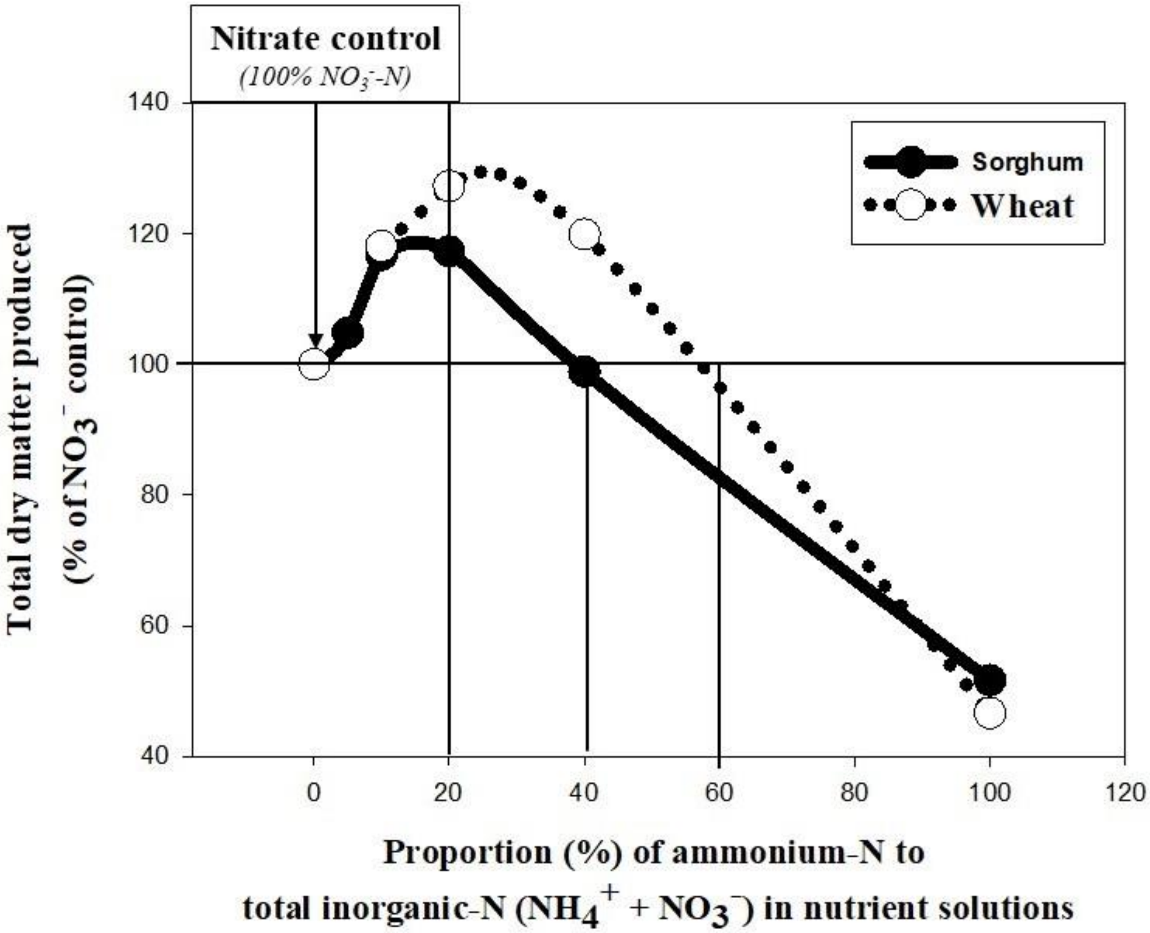
S41. Zhu Y *et al* (2013) Interplay among NH_4^+ uptake, rhizosphere pH and plasma membrane H^+ ATPase determine the release of BNIs in sorghum roots – possible mechanisms and underlying hypothesis. *Plant Soil* **358**:131-141.

S42. Di T *et al* (2018) Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots. *Plant Soil* **423**:99-110.

S43. Sarr P *et al* (2020) Sorgoleone release from sorghum roots shapes the composition of nitrifying populations, total bacteria, archae and determines the level of nitrification. *Biol. Fertil. Soils* 56:145-166.
S44. Sun L *et al* (2016) Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. *New Phytologist* **212**:646-656
S45. Subbarao GV et al. (2007) Can biological nitrification inhibition (BNI) genes from perennial *Leymus racemosus* (Triticeae) combat nitrification in wheat farming? *Plant Soil* **299**: 55-64.
S46. O'Sullivan C. *et al* (2016) Identification of several wheat landraces with biological nitrification inhibition capacity. *Plant Soil* **404**:61-74.
S47. Sommer et al (2004) Ammonia emission from mineral fertilizers and fertilized crops. *Adv. Agron*. **82**, 557-622.
S48. Abadalla M et al (2019) A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity, *Glob. Ch. Biol.* 25:2530–2543.
S49. Kladivko EJ et al (2014) Cover crops in the upper midwestern United States: Potential adoption and reduction of nitrate leaching in the Mississippi River Basin, *J. Soil Water Cons*. 69:279-291.

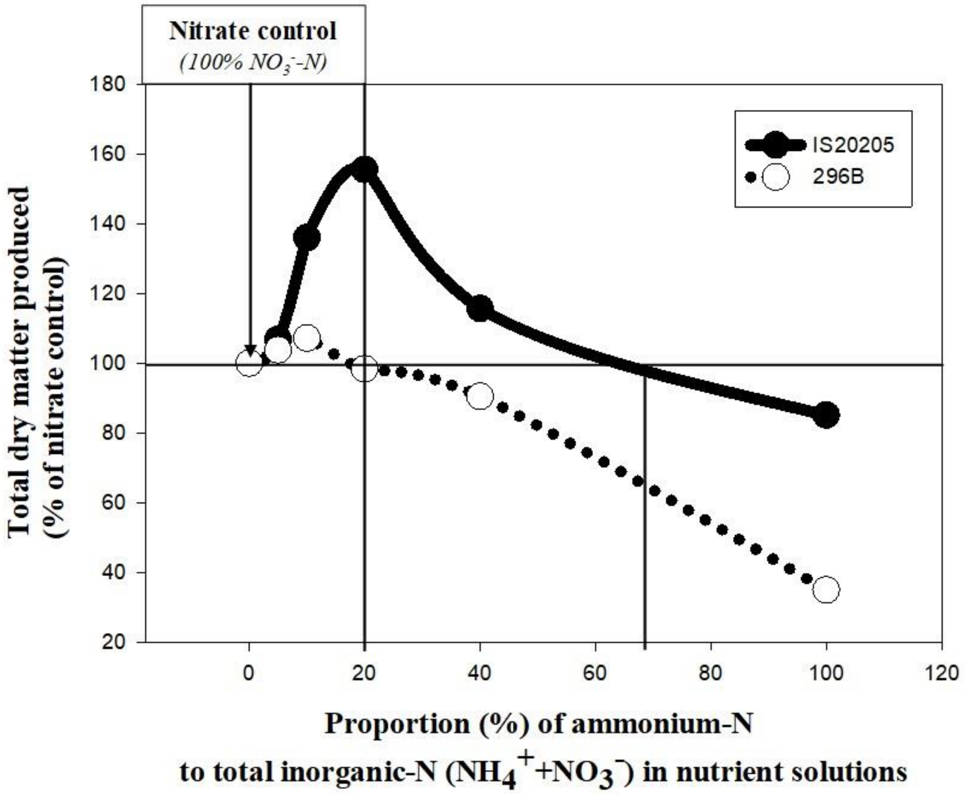
Supplemental Figures

Fig. S1. Wheat and sorghum growth response to share of NH_4^+-N in nutrient solutions



- Our recent research suggests that there is no negative effect on plant growth from NH_4-N in nutrient solutions up to 40% of the total-N.
- A stimulatory effect on plant growth was observed in both wheat and sorghum
- Some genetic stocks show more positive response to NH_4^+ in plant growth, which can vary up to 50% stimulation compared to nitrate (100%) control

Fig. S2. Differential response of two sorghum genotypes (differ in sorgeleone release from roots) to share of NH_4-N in nutrient solutions



- Nearly 60% stimulation in growth was observed in high-sorgoleone producing germplasm line IS20205 when 20% of nitrate-N is replaced with ammonium-N in nutrient solutions
- No such stimulation to ammonium-N in nutrient solutions were observed in low-sorgoleone producing sorghum germplasm lines 296B
- There was no negative effect on growth of IS20205 up to 70% of N as ammonium; but for 296B, the growth was severely affected with ammonium levels in nutrient solutions

Supplemental Table

Table S1. Participating institutions in BNI-research consortium

Serial No.	Institute	Priority issues/project ideas	Research category
1	**JIRCAS**	Act as a catalyst to develop BNI research in major field crops and pastures with several CG and non-CG partners	Physiology, nutrition, genetics, agronomy
2a	**CIMMYT • Wheat**	Field performance of Leymus-BNI translocations	Field testings
2b.	**CIMMYT • Wheat**	Finding new BNI sources and their combinations	Genetics, Pre-breeding
2c	**CIMMYT Maize**	Characterization of maize-BNI function and searching for high-BNI genetic sources in cultivated and wild maize germplasm	Genetics, Pre-breeding
2d	**CIMMYT • Wheat**	Isolation of BNI compound from *Leymus*	Chemical analysis
2e	**CIMMYT - SocioEconomics**	Ex-ante analysis to determine potential benefits from BNI-technologies, with JIRCAS	business case
3a	**CIAT . Tropical pastures**	Characterization of BNI function in field and development of BNI-enabled pasture systems	Field test
3b	**CIAT-Socio Economics**	Ex-ante analysis to determine potential benefits from BNI-technologies in pasture production systems	business case
3c	**CIAT . Tropical pastures breeding**	Identification of high-BNI Brachiaria genetic stocks and prebreeding	Breeding and Genetics
4a	**ICRISAT**	Marker development for BNI-trait in sorghum	Genetics
4b	**ICRISAT**	Ex-ante assessment of Sorghum BNI-technology (from deployment of BNI-trait in sorghum)	business case
4c	**ICRISAT**	Assessing the function of BNI in sorghum based systems	Field test
4d	**ICRISAT**	Assessing the function of BNI in sorghum based systems	Field test
5a	**iEES-Paris**	iEES-Paris	Nitrogen economy of Natural pastures
5b	**iEES-Paris**	Test of Maize-Hyparrhenia diplandra mix culture	Field test
5c	**iEES-Paris**	Modeling of BNI impact on natural ecosystems functioning	Modeling
5d	**iEES-Paris**	BNI in Barley	Field test
6	**ILRI**	Initiating BNI research in a tropical forages collection held at ILRI	Nitrogen economy of Natural pastures
7	**Inst. Soil Science, CAAS**	Rice BNI characterization	Plant Biology, Soil Science
8	**Nanjing Agric. University**	Mechanisms involved in the BNI release across the plasma membrane	Physiology and molecular biology
9a	**NARO-Tsukuba**	Mode of inhibitory action from BNIs	Biochemical assays; structure analysis
9b	**NARO-Hokkaido**	BNI in Japanese winter wheat	Wheat Breeding
10a	**Texas A&M**	Development of BNI-sorghum varieties for Texas Production systems	Breeding, Agronomy, Soil Science, Physiology
10b	**Texas A&M**	Plant, microbiome and environmental interactions of BNI in sorghum	Molecular biology and physiology
11	**University of Vienna**	Proteomics and Metabolomics tools for BNI research	Systems Biology, Modelling, plant-microbe interaction
12	**Uni Copenhagen**	T2R-N2O: Technologies to reduce N2O from agriculture	Agronomy, Soil Science, Fertilizers
13a	**Crea**	15N isotope-dilution techniques to study N transformations in the rhizosphere of BNI plants	Soil biology, soil chemistry
14	**SLU, Uppasala, Sweden**	Potential of plant chemical elicitors to induce BNI activity in wheat and oilseed rape via root exudate modification	Metabolomics and Biochemistry
15	**University of Firenze, Firenze Italy**	Proteomics tools in BNI and editorial activities	Soil Science, soil biochemistry
16	**IIMR, India**	BNI characterization in fingermillet	Physiology and breeding
17	**Agriculture Canada**	Exploitation of BNI function from sorghum to control nitrogen losses in winter-wheat production in Canada	Agronomy and cropping systems