Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Nitrogen Deficiency Stimulates Sorgoleone Production in Sorghum Roots and lower Soil-nitrification and N_2O Emissions

Xiang Gao
Kenichi Uno
Damaris A Odeny
Nithya Rajan
Tadashi Yoshihashi
Subbarao Venkata Guntur
gunturs0148@jircas.go.jp

JIRCAS: Kokusai Norin Suisangyo Kenkyu Center

Research Article

Keywords: Biological nitrification inhibition, Sorgoleone, Nitrogen deficiency, Soil nitrification, Root morphology, Nitrous oxide emissions

Posted Date: May 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9712580/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background and aims

Plant-derived biological nitrification inhibitors (BNIs) offer an ecological strategy to suppress soil nitrification and improve nitrogen-use-efficiency (NUE) in agricultural systems. Sorghum (*Sorghum bicolor* L.) roots release several BNIs, with sorgoleone identified as the primary hydrophobic-BNI compound. However, the influence of nitrogen (N) fertilization on root morphology and sorgoleone release remains insufficiently understood.

Methods

Two sorghum genetic-stocks differing in sorgoleone production capacity, high-sorgoleone (HS) and low-sorgoleone (LS) were evaluated using paper culture experiments under contrasting N-forms and levels, followed by two consecutive years of field validation. The inhibitory effects of sorgoleone on soil nitrification were further verified through exogenous applications in a soil microcosm study. Potential nitrification, abundances of ammonia-oxidizing archaea (AOA) and -bacteria (AOB), and N_2O emissions were quantified.

Results

Nitrogen deficiency significantly enhanced sorgoleone production in both genetic-stocks regardless of N-form in both paper culture and field trials. This increase in sorgoleone release was strongly associated with greater root length and surface area. Exogenous application of sorgoleone markedly reduced soil nitrification, AOA and AOB populations, and N_2O emissions in soil incubation experiments. In the field, HS consistently exhibited higher sorgoleone production, lower nitrification, reduced nitrifier abundance compared with LS, particularly under high-N conditions.

Conclusions

These findings demonstrate that N deficiency stimulates sorgoleone production through root morphological adaptation and that sorgoleone suppresses nitrification by inhibiting ammonia oxidizers. The study provides mechanistic and field-based evidence supporting the role of hydrophobic-BNI in regulating soil N transformations and mitigating N losses in agroecosystems.

Introduction

Biological nitrification inhibition (BNI) refers to plant-mediated compounds that inhibit soil nitrification, serving as a cost-effective ecological strategy to limit nitrogen (N) losses and synergize N fertilizer use

in farmlands (Subbarao et al. 2015, 2021a; O'Sullivan et al. 2016; Weckwerth et al. 2025; Buss et al. 2025; Garba et al. 2025). The role of BNIs has gained increasing attention within the framework of sustainable agriculture, which emphasizes environmentally friendly practices to reduce fertilizer derived pollution (Subbarao et al. 2015; Coskun et al. 2017; Otaka et al. 2022; 2023; Buss et al. 2025). The primary function of BNIs is to maintain relatively high soil-NH_4^+ levels while delaying the formation of mobile soil-NO_3^-, thereby suppressing subsequent N_2O emissions (Subbarao et al. 2009; Byrnes et al. 2017; Ghosh et al. 2024; Karwat et al. 2025).

Sorghum (*Sorghum bicolor* L.) is known for its strong BNI capacity, releasing several inhibitory compounds from its roots (Subbarao et al. 2007; 2013; Zakir et al. 2008; Sarr et al. 2020; Bozal-Leorri et al. 2023). The major BNIs identified in sorghum include methyl 3-(4 hydroxyphenyl) propionate, sorgoleone, and sakuranetin (Subbarao et al. 2013; Zhang et al. 2023; Okumoto et al. 2025). Among these, sorgoleone is the major potent hydrophobic-BNI compound released from living root hairs (Yang et al. 2004; Dayan et al. 2010). It suppresses *Nitrosomonas* by inhibiting ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) activities (Subbarao et al. 2013; Sarr et al. 2020). Because of its hydrophobic nature, sorgoleone tends to remain near the root surfaces, binding to soil mineral and organic particles, which enhances its persistence and efficacy in the rhizosphere (Dayan et al. 2010; Subbarao et al. 2013; Gao et al. 2022). Consequently, sorghum is considered a promising BNI crop capable of alleviating N losses through biological suppression of nitrification.

Nitrogen is one of the most critical nutrients limiting plant growth in terrestrial ecosystems, with plants typically acquiring it as ammonium and/or nitrate from the soil (Subbarao et al. 2015; 2021a; Kiba and Krapp 2016; Lu et al. 2024). Root systems exhibit remarkable plasticity in nutrient uptake, with variations in N availability profoundly influence root system architecture (RSA), including modifications in root number, root-hair formation, root-length and root-diameter (Fang et al. 2011; Lynch 2013; Giehl et al. 2014; Liu et al. 2025). Several studies have demonstrated that root-traits dynamically respond to spatial and temporal variations in soil N availability (Lynch et al. 2023). For instance, some maize genotypes develop fewer but longer nodal roots with greater lateral elongation under low-N conditions (Gaudin et al. 2011; Guo and York 2019), resulting in deeper rooting profiles (Trachsel et al. 2013). Similarly, Arabidopsis exhibited a 48% increase in total root-length under moderate N deficiency (Gruber et al. 2013), and crop species generally show greater root-length per unit shoot biomass and higher root-to-shoot ratio under N-limited conditions (Lopez et al. 2023). These morphological changes improve N acquisition efficiency by enhancing the soil-nutrient foraging capacity of roots (Lynch 2013; Nacry et al. 2013; Kiba and Krapp 2016). Moreover, some of the high-BNI capacity tropical grasses identified so far are known to have evolved and adapted to low-N production environments (Subbarao et al. 2009). However, the response of sorghum root morphology and sorgoleone release to varying external nutrient supplies remains unclear. In particular, the relationship between N availability, root morphological plasticity, and hydrophobic BNI release has not been thoroughly investigated.

BNIs are released into the rhizosphere primarily through root exudation, mediating interactions between rhizosphere microbes and roots (Subbarao et al. 2017; Afzal et al. 2020; Ghatak et al. 2022). While

hydrophilic-BNIs are mainly stimulated by ammonium presence, the regulatory effects of different N forms on the hydrophobic BNI sorgoleone has not been fully elucidated. Therefore, the present study is aimed at evaluating the impact of various N combination treatments on root morphology and sorgoleone production using paper culture experiments. Additionally, we aim to verify the influence of N fertilization on sorgoleone production capacity through two consecutive years of field experiments. A microcosm study is also conducted to assess the effects of exogenously applied sorgoleone on soil nitrification. We hypothesized that: (1) different N forms and doses will modulate sorghum root morphology and consequently alter sorgoleone production; (2) exogenous application of sorgoleone will inhibit soil nitrification and suppress N_2O emissions; (3) high sorgoleone (HS) genetic-stocks will exhibit lower soil nitrification and N_2O emissions than low sorgoleone (LS) genetic-stocks under field conditions.

Materials and methods

Experiment 1

Influence of nitrogen-forms in nutrient solutions on root morphology and its impact on sorgoleone production in paper culture experiments.

Two sorghum genetic-stocks previously characterized for their differential sorgoleone production capacity were selected for the paper culture experiment (Gao et al. 2022). The high-sorgoleone (HS, EC670311) and low-sorgoleone (LS, EC670350) genetic stocks were selected. Seven treatments of different nitrogen forms included: No nitrogen (N0); 0.36 mM NH_4^+-N; 1.80 mM NH_4^+-N; 0.72 mM NO_3^--N; 3.60 mM NO_3^--N; 0.18 mM NH_4^+-N + 0.36 mM NO_3^--N; 0.90 mM NH_4^+- N + 1.80 mM NO_3^--N. The seeds were germinated on folded filter paper (MN 710, MACHEREY-NAGEL GmbH & Co. KG, Germany) supported by a rigid board with one end touching the bottom of the seedling growth box. A 25% strength modified Hoagland nutrient solution (Subbarao et al. 2013) was provided in a plant growth chamber (25°C, 14/10 h light/dark period, average photosynthetic flux of 300 mmol m^{-2} s^{-1}). One liter of nutrient solution with the respective N treatments was supplied at the start of the experiment, and an additional 200 mL was added every other day to maintain sufficient nutrient availability and promote normal sorghum growth. At 14 days after sowing (DAS), the roots from ten seedlings per treatment were separated, washed with acidified DCM for sorgoleone determination as described earlier (Subbarao et al. 2013) and dry weights are recorded. Also, 3 seedlings were sampled to determine root length and root surface area from scanned root images using (WinRHIZO, Regent Inc. Canada) with three replicates.

Experiment 2

Exogenous application of sorgoleone on soil nitrification in a soil-microcosm study.

Volcanic ash soil (Typic Hapludands) sourced from the Japan International Research Center for Agricultural Sciences (JIRCAS) experimental station was used in this soil-microcosm study. Three treatments were implemented: addition of 100 mg sorgoleone kg^{-1} soil; 20 mg Dicyandiamide (DCD)

kg^{-1} soil; and a control group. Three grams of air-dried sieved soil (2 mm mesh) were placed in a 10 mL glass bottle, and 200 mg N kg^{-1} soil in the form of $(NH_4)_2SO_4$ was added for incubation experiment. The samples were incubated at 20°C, and relative humidity was maintained at 80%, and soil water content held at 60% water filled pore space (WFPS) with three replicates. The sorgoleone concentration was selected based on previous BNI incubation studies and to simulate localized rhizosphere conditions. The incubation conditions and methodology were adapted from Subbarao et al. (2013). The soil nitrification rate, abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) *amo*A genes were determined (Gao et al. 2022) after a 10-day incubation period.

Additionally, a parallel soil-incubation experiment was established to measure N_2O emissions using five grams of air-dried soil samples under identical incubation conditions, following the protocol described earlier (Subbarao et al. 2021b; Gao et al. 2022). Briefly, 5 g soil was placed in a 100 mL glass vial (height 12.0 cm; diameter 3.5 cm) and treated with 200 mg N kg^{-1} soil, supplied as $(NH_4)_2SO_4$. The experiment was arranged in a randomized block design with three replications. The soil moisture was maintained at 70% water-filled pore space throughout the 21-day incubation period. The glass vials containing the treatment soils were incubated in the dark at 20°C with 80% relative humidity. During the incubation, the vials were covered with parafilm. For gas sampling, the parafilm was removed 24 hours before sampling, and the vials were sealed with butyl rubber stopper and melamine white screw cap. After 24 hours of closure, 30 mL gas was sampled from each vial using a 50 mL syringe at designated intervals (0–21 days of incubation) and transferred into 10 mL pre-vacuum vial for storage. N_2O concentrations were analyzed using a gas chromatograph (GC-14B, Shimadzu, Japan). Following each gas sampling, the rubber stoppers were removed, and the vials were ventilated with an aeration pump to re-establish normal air levels. The cumulative N_2O emissions were calculated by summing the N_2O values measured at each sampling point (Subbarao et al. 2021b).

Experiment 3

Field validation of high and low sorgoleone-releasing genetic stocks under different nitrogen fertilization applications.

Two consecutive years of field trials were conducted from June to October 2021 and August to October 2022, respectively, at JIRCAS experimental station in Tsukuba, Japan (36.05 N 140.08 E). The upper horizon of the soil was characterized as volcanic ash Typic Hapludands, with the following properties in 2021: pH (H_2O) 5.6, clay 54.8%, silt 26.3%, sand 18.9%, total carbon 28.8 mg C g^{-1} soil; NH_4^+-N, 2.8 mg kg^{-1}; NO_3^--N, 3.6 mg kg^{-1}. The physical and chemical properties of the soil remained similar in 2022.

The experimental design was split-plot, with nitrogen treatments as main-plot and sorghum genetic stocks as sub-plot, replicated three times. Two sorghum genetic-stocks with high and low sorgoleone-releasing capacities (HS and LS) were used. Potassium (K) and phosphorus (P) were applied at rates of 100 kg K_2O ha^{-1} and 100 kg P_2O_5 ha^{-1} as basal fertilization using KCl and triple superphosphate (TSP).

Each experimental plot comprised of four seedbeds, each measuring 1.5 m in length and 3 m in width. Two rows of sorghum were planted in each seedbed with row spacing of 10 cm and plant spacing of 20 cm. After 20 days, the plants thinned to one seedling per hole. Two nitrogen levels were applied: with no N (LN) and 100 kg N application (HN). During the 2021 growing season, the sorghum genetic-stocks HS (EC670311) and LS (EC670350) were selected. For high-N treatment, $(NH_4)_2SO_4$ was applied at 100 kg N ha^{-1}, in three split-applications; 20 kg N applied at 30 DAS, 40 kg N applied at 50 DAS and 40 kg N applied at 80 DAS (days after sowing). Nitrogen fertilizer was solubilized in water and applied uniformly for the experimental plot. For the no-N treatment, equal amount of deionized water was applied. In the 2022 growing season, the same sorghum genetic-stocks were used, with N fertilizer applied at a rate of 100 kg N ha^{-1} at 25, 48 and 63 days after sowing.

Sorghum roots samples for sorgoleone content analysis were collected at 50, 80 and 110 DAS in 2021, and at 48, 63 and 76 DAS in 2022. Four plants and their corresponding roots were collected from each plot. The soil adhering to the roots was gently shaken off (considered as rhizosphere soil), and then the roots were cut into approximately 1 cm pieces. Two subsamples of 2 g root material were taken from each treatment for determination of sorgoleone content.

At 80 DAS in 2021 and at 63 DAS in 2022, four plants along with rhizosphere soil were collected at a depth of 0–20 cm from each plot. These samples were analyzed for potential nitrification (PN), abundance of AOA and AOB *amo*A genes, and N_2O emissions.

Analysis of soil and plant samples

Root-DCM wash and determination of sorgoleone concentration

Two grams of sorghum roots from the field were excised and immersed in 20 mL of an acidified dichloromethane (DCM) solution (1% v/v DCM: acetic acid) for 1 min to extract hydrophobic compounds from the root surface. The DCM-root wash was then filtered and evaporated to dryness at 40°C. The residue obtained was subsequently extracted with 20 mL of methanol, evaporated and re-extracted with 200 ul of DMSO. The processed samples were analyzed for sorgoleone concentration using HPLC analysis (Shimadzu, Kyoto, Japan), following the protocol described by Subbarao et al. (2013). The total amount of sorgoleone released was calculated as specific sorgoleone concentration × roots dry weight.

Potential nitrification and nitrification rate in soil samples

The potential nitrification (PN) and nitrification rate (NR) of soil samples were determined following the protocol by Subbarao et al. (2021b). Briefly, 10 g of air-dried rhizosphere soil was placed into a 250 ml Erlenmeyer flask containing 100 ml of N-potential solution (1 mM KH_2PO_4, 1 mM $(NH_4)_2SO_4$, 10 mM $NaClO_3$, and pH adjusted to 7.0). The flask was covered with a breathable sponge-cap and incubated at 20°C in a temperature-controlled roto-mixer set to 250 rpm. A 10 mL sample of soil slurry was taken from each flask every 24 h for 10 days. After centrifugation, the supernatant was analyzed for NO_3^- -N

using an autoanalyzer (BL-TEC K.K., Tokyo, Japan). The NR was calculated as soil NO_3^-/ (NO_3^- + NH_4^+) ×100%.

Determination of soil nitrifier activity

Soil DNA was extracted from 0.4 g of rhizosphere soil using Fast DNA Spin Kit for soil (Mo-Bio, Carlsbad, CA, USA), following the manufacturer's instructions with slight modifications. The concentration of extracted DNA was quantified using the Qubit Quantification Platform with the dsDNA HS Assay kit (Invitrogen, Carlsbad, CA, USA). Quantitative PCR (qPCR) was performed to quantify the abundance of the *amoA* genes from both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). The primer sequences used were as follows: *amo*A-AOA, GenAOAF: ATAGAGCCTCAAGTAGGAAAGTTCTA, GenAOAR: CCAAGCGGCCATCCAGCTGTATGTCC. *amo*A-AOB, amoA-1Fomd: CTGGGGTTCTACTGGTGGTC, GenAOBR: GCAGTGATCATCCAGTTGCG. The qPCR protocol was performed as described in Sarr et al. (2020) and Gao et al. (2022).

Statistical analysis

Data were analyzed using split-plot ANOVA in SAS 9.1 (SAS Institute, Cary, NC, USA), with nitrogen treatment as the main-plot factor and genotype as the sub-plot factor. Year, nitrogen treatment, genotype, and their interactions were treated as fixed effects, while replication was treated as a random effect. Mean comparisons were performed using Tukey's HSD test at $p < 0.05$.

Results

Sorgoleone production in paper culture

Sorghum exhibited significantly higher levels of sorgoleone production in the absence of nitrogen supplementation (No Nitrogen) compared to the treatment where N was supplied in various N forms, regardless of genetic stocks, i.e. whether high-sorgoleone (HS) or low-sorgoleone (LS) genetic-stocks were used (Fig. 1). For instance, sorgoleone release increased by 216% and 206% in the HS under nitrogen-deficient conditions compared to the treatments receiving 3.60 mmol L^{-1} NO_3^--N and 1.80 mmol L^{-1} NH_4^+-N, respectively. Simultaneously, under nitrogen-deficient conditions, sorgoleone released in the LS increased by 32% and 236% compared to the 3.60 mmol L^{-1} NO_3^--N and 1.80 mmol L^{-1} NH_4^+-N treatments, respectively. Additionally, HS genetic-stocks consistently released higher amount of sorgoleone compared to LS, regardless of the N treatments. The total sorgoleone production followed a similar trend to the specific release capacity (Fig. 1b), with the high-BNI genetic stocks consistently outperforming the low-BNI genetic stocks regardless of the nitrogen addition.

Total root length and surface area in paper culture

The trends in total root length and root surface area were consistent with the patterns of sorgoleone release across the various N treatments, with the highest value observed under No-N treatment,

regardless of genetic-stocks (Fig. 2; Fig. S1). For example, the total root length of HS increased by 27% and 345% under treatments with 3.60 NO_3^--N mmol L^{-1} and 1.80 NH_4^+-N mmol L^{-1}, respectively. Similarly, the LS exhibited an improvement of 69% and 61% respectively, under the same mentioned treatments. In addition, HS genetic-stocks consistently showed higher total root length and root surface area than LS genetic-stocks, across all N addition treatments in the paper culture system.

Correlation between sorgoleone release and root morphology

The release of sorgoleone was significantly correlated with root morphology (Fig. 3). Specifically, increases in total root length and root surface area in sorghum roots were associated with improved sorgoleone released in the paper culture system, with correlation coefficients reaching 0.71 and 0.69, respectively. Notably, the high-BNI genetic stock exhibited larger root morphology, resulting in greater sorgoleone release capacity.

Exogenous addition of sorgoleone or DCD in a soil incubation experiment.

As depicted in Table 1, the exogenous addition of sorgoleone or DCD significantly reduced the soil nitrification rate, with reductions of 42% and 45% compared to the control, respectively. Simultaneously, sorgoleone and DCD treatments resulted in reductions of N_2O emissions by 42% and 91%, and reduced the AOB abundance by 71% and 72% relative to the control, respectively. Notably, only sorgoleone significantly reduced the abundance of AOA, while no remarkable difference was observed between control and DCD treatments in the soil-microcosm study.

Table 1
Exogenous addition of sorgoleone and DCD on N_2O emissions and nitrifier populations in a soil-microcosm study

Treatments	**NR (%)**	**N_2O emissions (N_2O mg kg^{-1} soil)**	**AOA (copies g^{-1} soil)**	**AOB (copies g^{-1} soil)**
Control	92.1 ± 2.5a	9.1 ± 0.3a	230725 ± 6720a	66583 ± 1393a
Sorgoleone	50.6 ± 3.6b	5.3 ± 0.3b	173549 ± 5326b	19022 ± 1290b
DCD	47.2 ± 3.4b	0.5 ± 0.0c	219772 ± 4215a	18339 ± 976b

All the data are the mean of three replicates ± SE; the different letters after numbers in the same column for the same trait indicates significant difference at $p < 0.05$.

Sorgoleone production in field trials

In two consecutive years of field trials, the release of hydrophobic BNI-sorgoleone from sorghum roots was found to be influenced by the level of N fertilizer application, the growth stage, and the genetic stock (Fig. 4). For instance, in the 2022 results, the application of low nitrogen (LN) treatment significantly

increased the release of sorgoleone in both genetic populations. After 63 days after sowing (DAS63), sorgoleone levels in the HS and LS under LN treatment were notably increased by 264% and 173% (Fig. 4e), respectively, compared to the HN treatment. This trend was also shown on 48 and 76 days (Fig. 4d and 4f). In the 2021 results, except for DAS110 when sorghum was heading, all four treatments exhibited significant differences (Fig. 4). The findings from the other two sampling points were consistent with those of 2022. Furthermore, the sorgoleone levels in HS were consistently higher than those in LS. For instance, on DAS 63 in 2022, the sorgoleone release capacity of HS under both LN and HN treatments was 49% and 99% higher, respectively, compared to LS (Fig. 4e).

Nitrification

Nitrogen fertilization significantly increased potential nitrification and nitrification rate in rhizosphere soils of both genetic-stocks during two consecutive years of field trials. However, notable differences between the genetic-stocks were only shown at high nitrogen levels (Fig. 5). In 2021, the potential and nitrification rate in HS were significantly reduced by 24% and 13% compared to LS. Similarly, in 2022, reductions of 32% and 23% were recorded under HN conditions. In contrast, no significant differences between the two genetic-stocks were observed under LN treatment.

AOA and AOB abundance

Soil nitrifier populations (AOA and AOB) were significantly influenced by the sorgoleone production capacity of genetic-stocks and N fertilization treatments over two consecutive years of field trials (Fig. 6). The HS genetic-stock suppressed soil-AOA populations more effectively than the LS genetic-stock regardless of low or high N treatment (Figs. 6a and b). For instance, soil-AOA populations in HS were significantly lower by 36% and 47% compared to LS under HN conditions in 2021 and 2022, respectively, with similar trends observed under LN treatment. AOB exhibited significant inhibitory effects under HN conditions, with HS being 33% and 32% lower than LS in 2021 and 2022, respectively (Figs. 6c and d). Notably, while AOB populations were 13% lower than LS in 2021, no significant difference was shown in 2022.

N_2O emissions

Sorghum genetic-stocks (LS *vs* HS) exhibited significant differences in soil-N_2O emissions under HN treatments during two consecutive years of soil assay (Figs. 7a and b). However, no significant differences were observed under LN treatment (Figs. 7c and d). Cumulative soil-N_2O emissions were 12% and 16% lower in HS genetic-stocks compared to LS genetic-stocks in 2021 and 2022, respectively.

Discussion

Sorgoleone is a hydrophobic compound released from sorghum roots, which plays a pivotal role in Biological Nitrification Inhibition (BNI) (Subbarao et al. 2013; Tesfamariam et al. 2014; Sarr et al. 2020; Okumoto et al. 2025). While previous BNI studies have primarily focused on its inhibitory effects under

controlled conditions, its regulation under field relevant N environment, particularly the role of root morphological adaptation in modulating its release, remains less well understood. By integrating controlled experiments with two consecutive years of field observations, this study provides evidence that N deficiency enhances sorgoleone release, largely through root morphological responses rather than differences in N form. The increased release of sorgoleone was associated with reduced nitrifier abundance and lower N_2O emissions, supporting its functional role in regulating soil nitrification under field conditions.

Nitrogen deficiency stimulates sorgoleone release through root morphological plasticity

This study demonstrates that N deficiency consistently enhances sorgoleone production in sorghum across both controlled and field conditions. The increase in sorgoleone release under low-N conditions was strongly associated with changes in root morphology, particularly increases in total root length and root surface area (Figs. 1 and 2). These traits were positively correlated with sorgoleone release, suggesting that root system plasticity contributes to enhanced exudation capacity. Because sorgoleone is predominantly produced and released from living root hairs, expansion of root surface area likely increases the interface for exudation into the rhizosphere (Yang et al. 2004; Dayan 2010).

Root morphological plasticity under N limitation is a well-established adaptive strategy that enhances soil exploration and nutrient acquisition (Postma et al. 2014; Liu et al. 2025). Increased lateral root proliferation and elongation can substantially increase rhizosphere contact (Ma et al. 2014; Shao et al. 2017), whereas high N availability can suppress lateral root formation by limiting meristem activation (Nacry et al. 2013). Our findings extend this framework by linking morphological responses to functional changes in rhizosphere chemistry, specifically the release of hydrophobic BNI compounds.

Importantly, sorgoleone production was not strongly dependent on N form, in contrast to hydrophilic BNIs that are stimulated by ammonium supply (Subbarao et al. 2007b, 2013; Zhu et al. 2012). This suggests that hydrophobic BNI release is primarily regulated by plant developmental responses to N availability rather than by immediate rhizosphere N chemistry. The results also support a conceptual distinction between BNI strategies under contrasting N environments. Under low-N input production systems, enhanced root growth and increased sorgoleone release suggest a great role for hydrophobic-BNI, whereas under high-N input environments, hydrophilic-BNI stimulated by ammonium availability may contribute more strongly to nitrification suppression. Thus, depending on the type of production environment, the relative importance/contribution of hydrophobic-BNI-capacity *vs* hydrophilic-BNI-capacity in improving adaptation and productivity of sorghum genotypes can change.

Sorgoleone mediated suppression of soil nitrification

The inhibitory effect of sorgoleone on soil nitrification was confirmed through both soil incubation experiments and field observations. Exogenous application of sorgoleone significantly reduced

nitrification rates, AOB and AOA abundance, and N_2O emissions, demonstrating its direct inhibitory effect on ammonia-oxidizing microorganisms. These findings are consistent with previous studies showing that sorgoleone inhibits key enzymatic processes involved in ammonia oxidation, including ammonia monooxygenase activity (Subbarao et al. 2013; Di et al. 2018; Sarr et al. 2020).

Field results further support the ecological relevance of this mechanism. Sorghum genotypes with high sorgoleone production consistently exhibited lower potential nitrification rates and reduced nitrifier abundance compared with low-sorgoleone genotypes, particularly under high N conditions (Figs. 4 and 5). This indicates that genotypic variation in BNI capacity translates into measurable differences in soil N transformation processes under field conditions. Notably, the magnitude of inhibition suppression was more pronounced under high N inputs, where nitrifier activity is typically elevated. Under low N conditions, differences between genotypes were less evident, likely reflecting lower baseline nitrification potential. These results suggest that BNI effects are context-dependent and most effective under conditions that favor nitrifier activity.

Inhibition of nitrifiers and N_2O emissions

Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) were suppressed by high-BNI genotypes, although their responses varied with N availability (Fig. 6). AOB abundance was more strongly reduced under high N conditions, consistent with their dominance in environments with elevated ammonium availability. In contrast, AOA showed more consistent suppression across both low and high N conditions, suggesting broader sensitivity to BNI compounds or differences in ecological niche specialization. These patterns align with current understanding that AOB typically dominate in fertilized agricultural soils, whereas AOA are more competitive under low substrate conditions (Hink et al. 2018; Gubry-Rangin et al. 2020; Kaur-Bhambra et al. 2021). The observed reduction in both groups indicates that sorgoleone exerts a broad inhibitory effect on ammonia oxidizers, although the relative contribution of each group to nitrification and N_2O production cannot be resolved from abundance data alone. Linking functional gene expression or isotopic tracing approaches would help clarify their respective roles.

Reductions in nitrifier abundance were accompanied by lower N_2O emissions in both microcosm and field conditions, particularly under high N inputs. This supports the role of nitrification as a major pathway for N_2O production in these systems and highlights the potential of BNI to mitigate greenhouse gas emissions. Given that AOB-associated nitrification is often linked to higher N_2O yields (Hink et al. 2018; Prosser et al. 2020; Yao et al. 2020), the stronger suppression of AOB likely contributed disproportionately to the observed reduction in emissions. In addition to mitigating N_2O emissions, suppression of nitrification is expected to enhance soil NH_4^+ retention and reduce NO_3^- formation, thereby lowering the risk of N leaching losses.

Conclusion

These findings demonstrate that N deficiency enhances sorgoleone production in sorghum, primarily through increased total root length and root surface area. Sorgoleone release was not strongly influenced by nitrogen form, indicating that its regulation is largely driven by root morphological responses to N availability. In soil incubation experiments, sorgoleone significantly inhibited both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), resulting in reduced soil nitrate formation and lower N_2O emissions. Furthermore, field observations over two consecutive years further confirmed that genetic-stocks with high-sorgoleone (HS) consistently exhibited a greater ability to release sorgoleone and showed lower nitrification, reduced nitrifier abundance, and lower N_2O emissions than LS.

Collectively, these results provide mechanistic and field-based evidence that sorgoleone plays a central role in sorghum BNI capacity. Sorgoleone production is likely to be the main driver for BNI-capacity under low-N input conditions often is the case in African Production environments, thus developing high-sorgoleone producing sorghum varieties will improve adaptation to low-N input production systems of Africa. Whereas, hydrophilic-BNI function drives the BNI-capacity of sorghum under high-N input conditions of Asia and Americas to improve NUE, productivity and lower N_2O emissions from agricultural systems.

Declarations

Declaration of Competing Interest

The authors declare no competing interests.

Funding

This work was supported by Japan International Research Center for Agricultural Sciences (JIRCAS) Visiting Research Fellowship Program.

Author contributions

XG, OD, GVS, TY and NR designed the experiments. XG, KU and GVS conducted experiments. GVS performed biological studies. KU and XG performed N_2O emission measurements. XG, NR and GVS wrote the manuscript. All authors have contributed to the writing of the manuscript.

Acknowledgements

We would like to appreciate the JIRCAS Visiting Research Fellowship Program, and thank Makoto Yamamoto, Hiroko Aoki, Yoko Koizumi, Sanae Suzuki, Masami Aoyama for their help with the laboratory

and field experiments.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

References

1. Afzal MR, Zhang M, Jin H, Wang G, Zhang M, Ding M, Raza S, Hu J, Zeng H, Gao X, Subbarao GV, Zhu Y (2020) Post-translation regulation of plasma membrane H^+-ATPase is involved in the release of biological nitrification inhibitors from sorghum roots. Plant Soil 450:357–372. https://doi.org/10.1007/s11104-020-04511-6
2. Bozal-Leorri A, Arregui L, Torralbo F, González-Moro M, Gonzalez-Murua C, Aparicio-Tejo P (2023) Soil moisture modulates biological nitrification inhibitors release in sorghum plants. Plant Soil. https://doi.org/10.1007/s11104-023-05913-y
3. Buss W, Verburg K, Garba II, O'Sullivan CA, Wang E, Gupta VV, Bentley AR (2025) Harnessing biological nitrification inhibition to reduce soil nitrogen losses–Systematic quantification of plant and soil factors to maximise field-scale benefits. Agr Ecosyst Environ 396:110002. https://doi.org/10.1016/j.agee.2025.110002
4. Byrnes RC, Nuñez J, Arenas L, Rao I, Trujillo C, Alvarez C, Arango J, Rasche F, Chirinda N (2017) Biological nitrification inhibition by *Brachiaria* grasses mitigated soil nitrous emissions from bovine urine patches. Soil Biol Biochem 177:156–163. https://doi.org/10.1016/j.soilbio.2016.12.029
5. Coskun D, Britto DT, Shi W, Kronzucker HJ (2017) Nitrogen transformations in modern agriculture and role of biological nitrification inhibition. Nat Plants 3:17074. https://doi.org/10.1038/nplants.2017.74
6. Dayan FE, Rimando AM, Pan Z, Baerson SR, Gimsing AL, Duke SO (2010) Sorgoleone Phytochem 10:1032–1039. https://doi.org/10.1016/j.phytochem.2010.03.011
7. Di T, Afzal MR, Yoshihashi T, Deshpande S, Zhu Y, Subbarao GV (2018) Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots. Plant Soil 423:99–110. https://doi.org/10.1007/s11104-017-3505-5
8. Fang S, Gao X, Deng Y, Chen X, Liao H (2011) Crop root behavior coordinates phosphorus status and neighbors: from field studies to three-dimensional in situ reconstruction of root system architecture. Plant Physiol 155:1277–1285. https://doi.org/10.1104/pp.110.167304
9. Garba II, Buss W, Wang E, O'Sullivan CA, Gupta VVSR, Bentley AR, Verburg K (2025) A modelling framework for assessing the plausible impacts of biological nitrification inhibition in cropping systems. Agric Sys 232:104569. https://doi.org/10.1016/j.agsy.2025.104569

10. Gao X, Uno K, Sarr P, Yoshihashi T, Zhu Y, Subbarao GV (2022) High-sorgoleone producing sorghum genetic stocks suppress soil nitrification and N_2O emissions better than low-sorgoleone producing sorghum genetic stocks. Plant Soil 477:793–805. https://doi.org/10.1007/s11104-022-05474-6
11. Gaudin A, McClymont S, Holmes B, Lyons E, Raizada M (2011) Novel temporal, fine-scale and growth variation phenotypes in roots of adult-stage maize (*Zea mays* L.) in response to low nitrogen stress. Plant Cell Environ 34:2122–2137. https://doi.org/10.1111/j.1365-3040.2011.02409.x
12. Ghatak A, Chaturvedi P, Waldherr S, Subbarao GV, Weckwerth W (2022) Panomics at the interface of root-soil microbiome and BNI. https://doi.org/10.1016/j.tplants.2022.08.016. Trends Plant Sci
13. Ghosh E, Rajan N, Phuyal D, Subramanian N, Bagavathiannan M (2024) High rhizospheric ammonium levels in *Sorghum halepense* (johnsongrass) suggests nitrification inhibition potential. Agr Environ Let 9:e20137. https://doi.org/10.1002/ael2.20137
14. Giehl R, Gruber B, von Wiren N (2014) It's time to make changes: modulation of root system architecture by nutrient signals. J Exp Bot 65:769–778. https://doi.org/10.1093/jxb/ert421
15. Gruber B, Giehl R, Friedel S, von Wiren N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. Plant Physiol 163:161–179. https://doi.org/10.1104/pp.113.218453
16. Gubry-Rangin C, Nicol G, Prosser JI (2020) Archaea rather than bacteria control nitrification in two agricultural acidic soils. FEMS Microbiol Ecol 74:566–574. https://doi.org/10.1111/j.1574-6941.2010.00971.x
17. Guo H, York L (2019) Maize with fewer nodal roots allocates mass to more lateral and deep roots that improve nitrogen uptake and shoot growth. J Exp Bot 70:5299–5309. https://doi.org/10.1093/jxb/erz258
18. Hink L, Gubry-Rangin, Nicol GW, Prosser JI (2018) The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. ISME J 12:1084–1093. https://doi.org/10.1038/s41396-017-0025-5
19. Karwat H, Kishii M, Cárdenas-Castañeda ME, Ibba MI, Kommerell V, Bentley AR, Braun HJ, Ortiz-Monasterio I (2025) Nitrogen dynamics and yield performance of an elite bread wheat line with BNI capacity expressed in an alkaline soil. Field Crops Res 334:110172. https://doi.org/10.1016/j.fcr.2025.110172
20. Kaur-Bhambra J, Wardak D, Prosser J, Gubry-Rangin C (2021) Revisiting plant biological nitrification inhibition efficiency using multiple archaeal and bacterial ammonia-oxidising cultures. Biol Fertil Soils. https://doi.org/10.1007/s00374-020-01533-1
21. Kiba T, Krapp A (2016) Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant Cell Physiol 57:707–714. https://doi.org/10.1093/pcp/pcw052
22. Liu X, Huang K, Chu C (2025) The genetic basis of nitrogen-dependent root system architecture in plants. Front Agr Sci Eng 12: 3–15. https://doi.10.15302/J-FASE-2024587
23. Lopez G, Ahmadi S, Amelung U et al (2023) Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. Front Plant Sci 13:1067498. https://doi:10.3389/fpls.2022.1067498

24. Lu Y, Kronzucker H, Yu M, Shabala S, Shi W (2024) Nitrogen-loss and carbon-footprint reduction by plant-rhizosphere exudates. Trends Plant Sci 29:469–481. https://DOI:10.1016/j.tplants.2023.09.007
25. Lynch J (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357. https://doi.org/10.1093/aob/mcs293
26. Lynch J, Galindo-Castaneda T, Schneider H, Sidhu J, Rangarajan H, York L (2023) Root phenotypes for improved nitrogen capture. Plant Soil. https://doi.org/10.1007/s11104-023-06301-2
27. Ma W, Li J, Qu B, He X, Zhao X, Li B, Fu X, Tong Y (2014) Auxin biosynthetic gene *TAR*2 is involved in low nitrogen-mediated reprogramming of root architecture in *Arabidopsis*. Plant J 78:70–79
28. Nacry P, Bouguyon E, Gojon A (2013) Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370:1–29. https://doi.org/10.1007/s11104-013-1645-9
29. O'Sullivan CA, Fillery IRP, Poper MM, Richards RA (2016) Identification of several wheat land races with biological nitrification inhibition capacity Plant Soil 404:61–74. https://doi.org/10.1007/s11104-016-2822-4
30. Okumoto S, Maharjan B, Rajan N, Xi J, Baerson SR, Rooney WL et al (2025) Synthesis, function, and genetic variation of sorgoleone, the major biological nitrification inhibitor in sorghum. Crop Sci 65:e70066. https://doi.org/10.1002/csc2.70066
31. Otaka J, Subbarao GV, Ono H, Yoshihashi T (2022) Biological nitrification inhibition in maize-isolation and identification of hydrophobic inhibitors from root exudates. Biol Fertil Soils. https://doi.org/10.1007/s00374-021-01577-x
32. Otaka J, Subbarao GV, Li M, Ono J, H., Yoshihashi T (2023) Isolation and characterization of the hydrophilic BNI compound 6-methoxy-2(3H) benzoxazolone (MBOA), from maize roots. Plant Soil, 489(1–2), 341–359. https://doi.org/10.1007/s11104-023-06021-7
33. Parker J, Cavell A, Dolan L, Roberts K, Grierson C (2000) Genetic interactions during root hair morphogenesis in Arabidopsis. Plant Cell 12:1961–1974. https://doi.org/10.1105/tpc.12.10.1961
34. Postma J, Dathe A, Lynch J (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiol 166:590–602. https://doi.org/10.1104/pp.113.233916
35. Prosser JI, Hink L, Gubry-Rangin C, Nicol GW (2020) Nitrous oxide production by ammonia oxidizers: Physiological diversity, niche differentiation and potential mitigation strategies. Glob Chang Biol 26:103–118. https://doi.org/10.1111/gcb.14877
36. Sarr PS, Ando Y, Nakamura S, Deshpande S, Subbarao GV (2020) Sorgoleone release from sorghum roots shapes the composition of nitrifying populations, total bacteria, and archaea and determines the level of nitrification. Biol Fertil Soils 56:145–166. https://doi.org/10.1007/s00374-019-01405-3
37. Shao A, Ma W, Zhao X, Hu M, He X, Teng W, Li H, Tong Y (2017) The auxin biosynthetic *TRYPTOPHAN AMINOTRANSFERASE RELATED TaTAR2.1-3A* increases grain yield of wheat. Plant Physiol 174:2274–2288. https://doi.org/10.1104/pp.17.00094

38. Subbarao GV, Rondon M, Ito O, Ishikawa T, Rao IM, Nakahara K, Lascano C, Berry WL (2007a) Biological nitrification inhibition (BNI) – is it a widespread phenomenon? Plant Soil 294:5–18. https://doi.org/10.1270/jsbbs.59.529
39. Subbarao GV, Wang HY, Ito O, Nakahara K, Berry WL (2007b) NH_4^+ triggers the synthesis and release of biological nitrification inhibition compounds in *Brachiaria humidicola* roots. Plant Soil 290:245–257. https://doi.org/10.1007/s11104-006-9156-6
40. Subbarao GV, Nakahara K, Hurtodo MP, Ono H, Moreta DE, Salcedo AF, Yoshihashi T, Ishikawa T, Ishitani M, Ohnishi KM, Yoshida M, Rondon M, Rao IM, Lascano CE, Berry WL, Ito O (2009) Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proc Natl Acad Sci USA 106:17302–17307. https://doi.org/10.1073/pnas.0903694106
41. Subbarao GV, Sahrawat KL, Nakahara K, Ishikawa T, Kudo N, Kishii M, Rao IM, Hash CT, George TS, Srinivasa RP, Nardi P, Bonnett D, Berry WL, Suenage K, Ito O, Lata JC (2012) Biological nitrification inhibition (BNI) – a novel strategy to regulate nitrification in agricultural systems. Adv Agron 114:249–302. https://doi.org/10.1016/B978-0-12-394275-3.00001-8
42. Subbarao GV, Nakahara K, Ishikawa T, Ono H, Yoshida M, Yoshihashi T, Zhu Y, Zakir H, Deshpande SP, Hash CT, Sahrawat KL (2013) Biological nitrification inhibition (BNI) activity in sorghum and its characterization. Plant Soil 366:243–259. https://doi.org/10.1007/s11104-012-1419-9
43. Subbarao GV, Yoshihashi T, Worthington M, Nakahara K, Ando Y, Sahrawat KL, Rao IM, Lata JC, Kishii M, Braun HJ (2015) Suppression of soil nitrification by plants. Plant Sci 233:155–164. https://doi.org/10.1016/j.plantsci.2015.01.012
44. Subbarao GV, Arango J, Masahiro K, Hooper AM, Yoshihashi T, Ando Y, Nakahara K, Deshpande S, Ortiz-Monasterio I, Ishitani M, Peters M, Chirinda N, Wollenberg L, Lata JC, Gerard B, Tobita S, Rao IM, Braum HJ, Kommerell V, Tohme J, Iwanaga M (2017) Genetic mitigation strategies to tackle agricultural GHG emissions: The case for biological nitrification inhibition technology. Plant Sci 262:165–168. https://doi.org/10.1016/j.plantsci.2017.05.004
45. Subbarao GV, Searchinger TD (2021a) Opinion: A more ammonium solution to mitigate nitrogen pollution and boost crop yields. Proc Natl Acad Sci USA 118:e2107576118. https://doi.org/10.1073/pnas.2107576118
46. Subbarao GV, Kishii M, Bozal-Leorri A, Ortiz-Monasterio I, Gao X, Ibba MI, Karwat H, Gonzalez-Moro MB, Gonzalez-Murua C, Yoshihashi T, Tobita S, Kommerell V, Braun HJ, Iwanaga M (2021b) Enlisting wild-grass-genes to combat nitrification in wheat farming: a nature-based solution. Proc Natl Acad Sci USA 118:e2106595118. https://doi.org/10.1073/pnas.2106595118
47. Tesfamariam T, Yoshinaga H, Deshpande SP, Rao PS, Sahrawat KL, Ando Y, Nakahara K, Hash CT, Subbarao GV (2014) Biological nitrification inhibition in sorghum: the role of sorgoleone production. Plant Soil 379:325–335. https://doi.org/10.1007/s11104-014-2075-z
48. Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2013) Maize root growth angles become steeper under low N conditions. Field Crops Res 140:18–31. https://doi.org/10.1016/j.fcr.2012.09.010

49. Weckwerth W, Chaturvedi P, Ghatak A, Kerou M, Garg V, Bohra A, Subbarao GV, Stein L, Schleper C, Varshney RK, Snapp S (2025) Natural variation of the holobiont for sustainable agroecosystems. Trends Plant Sci 30:972–979. https://DOI:10.1016/j.tplants.2025.05.006
50. Yao Z, Pelster D, Liu C, Zheng X, Butterbach-Bahl K (2020) Soil N intensity as a measure to estimate annual N_2O and NO fluxes from natural and managed ecosystems. Curr Opin Environ Sustain 47:1–6. https://doi.org/10.1016/j.cosust.2020.03.008
51. Yang X, Owens T, Scheffler B, Weston L (2004) Manipulation of root hair development and sorgoleone production in sorghum seedlings. J Chem Ecol 30:199–213. https://doi.org/10.1023/B:JOEC.0000013191.35181.03
52. Zakir HAKM, Subbarao GV, Pearse SJ, Gopalakrishnan S, Ito O, Ishikawa T, Kawano N, Nakahara K, Yoshihashi T, Ono H, Yoshida M (2008) Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum Bicolor*). New Phytol 180:442–451. https://doi.org/10.1111/j.1469-8137.2008.02576.x
53. Zeng H, Di T, Zhu Y, Subbarao GV (2016) Transcriptional response of plasma membrane H^+ ATPase genes to ammonium nutrition and its functional link to the release of biological nitrification inhibitors from sorghum roots. Plant Soil 398:301–312. https://doi.org/10.1007/s11104-015-2675-2
54. Zhang M, Zeng H, Afzal MR, Gao X, Li Y, Subbarao GV, Zhu Y (2022) BNI-release mechanisms in plant root system: current status of understanding. Biol Fertil Soils. https://doi.org/10.1007/s00374-021-01568-y
55. Zhang M, Gao X, Chen G, Afzal MR, Wei T, Zeng H, Subbarao GV, Wei Z, Zhu Y (2023) Intercropping with BNI-sorghum benefits neighboring maize productivity and mitigates soil nitrification and N_2O emission. Agr Ecosyst Environ 352:108510. https://doi.org/10.1016/j.agee.2023.108510
56. Zhu Y, Zeng HQ, Shen QR, Ishikawa T, Subbarao GV (2012) 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. https://doi.org/10.1007/s11104-012-1151-5

Figures

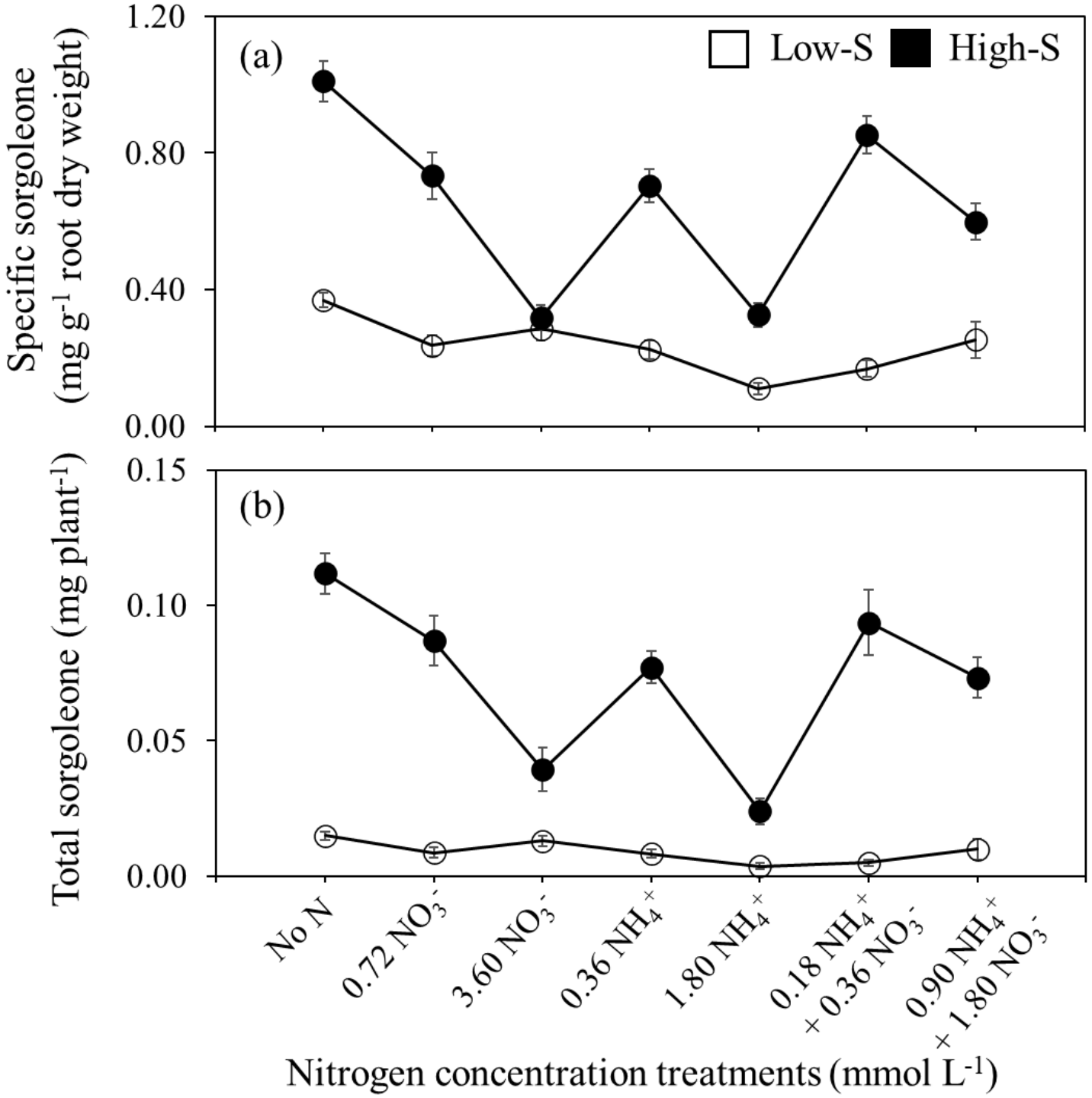


Figure 1

Sorgoleone production was compared among various forms of nitrogen (N) application using contrasting sorgoleone genetic-stocks of sorghum roots (LS and HS) in paper culture experiments. **(a)** Specific sorgoleone; **(b)** Total sorgoleone released. A total of seven different N forms: (1) no nitrogen; (2) 0.72 mM NO_3^--N; (3) 3.60 mM NO_3^--N; (4) 0.36 mM NH_4^+-N; (5) 1.80 mM NH_4^+-N; (6) 0.18 mM NH_4^+-N + 0.36 mM NO_3^--N; (7) 0.90 mM NH_4^+-N + 1.80 mM NO_3^--N. The low Sorgoleone genetic-stocks as

EC670350 (LS), while the high Sorgoleone genetic-stocks as EC670311 (HS). Each bar represents the mean ± SE of three replicates.

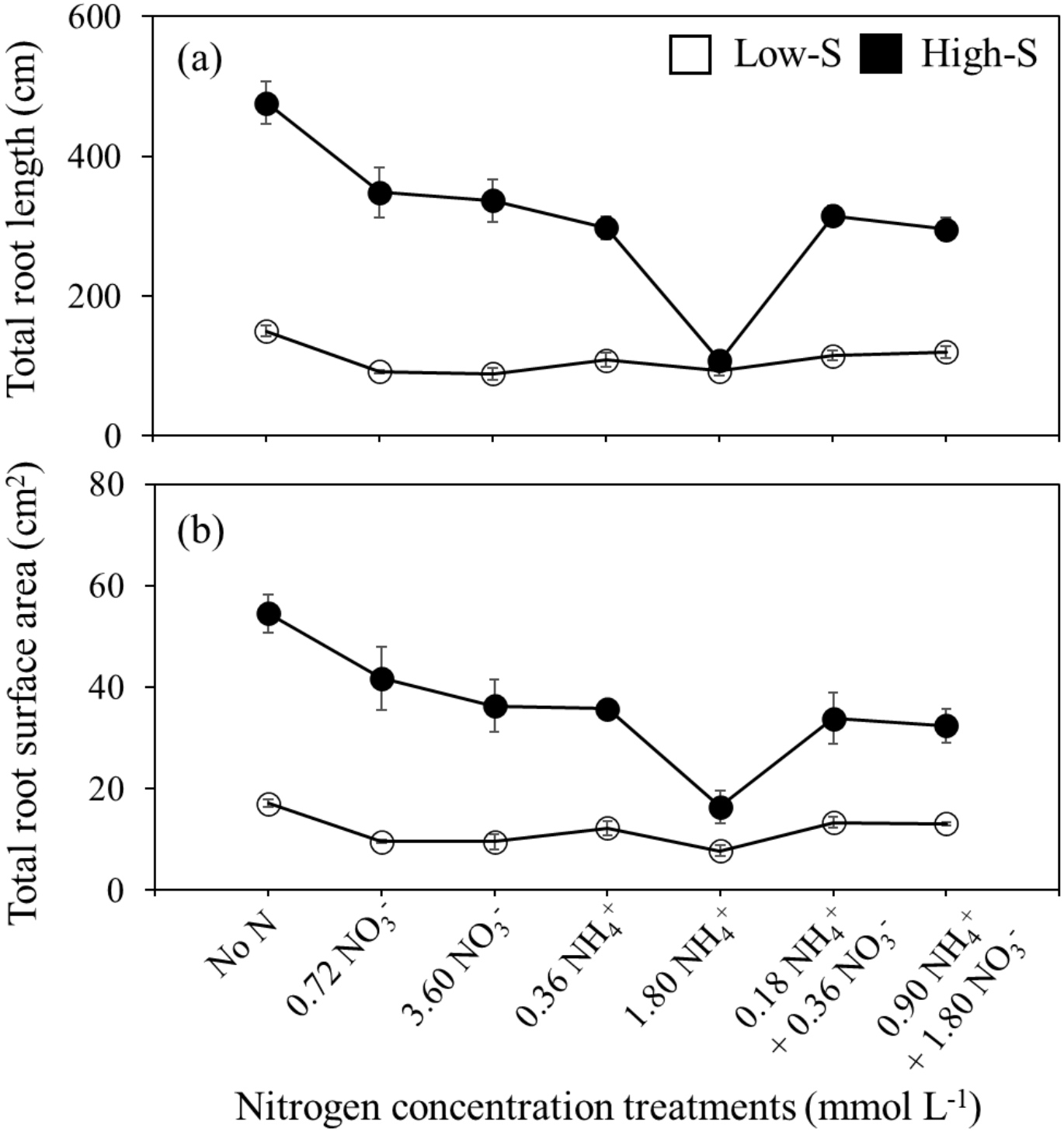


Figure 2

Root morphology was compared between contrasting sorgoleone genetic-stocks of sorghum roots (LS and HS) under various N forms in paper culture experiments. **(a)** Total root length **(b)** Root surface area.

A total of seven different N forms were applied: (1) no nitrogen; (2) 0.72 mM NO_3^--N; (3) 3.60 mM NO_3^--N; (4) 0.36 mM NH_4^+-N; (5) 1.80 mM NH_4^+-N; (6) 0.18 mM NH_4^+-N + 0.36 mM NO_3^--N; (7) 0.90 mM NH_4^+-N + 1.80 mM NO_3^--N. The low Sorgoleone genetic-stocks as EC670350 (LS), while the high Sorgoleone genetic-stocks as EC670311 (HS). Each bar represents the mean ± SE of three replicates.

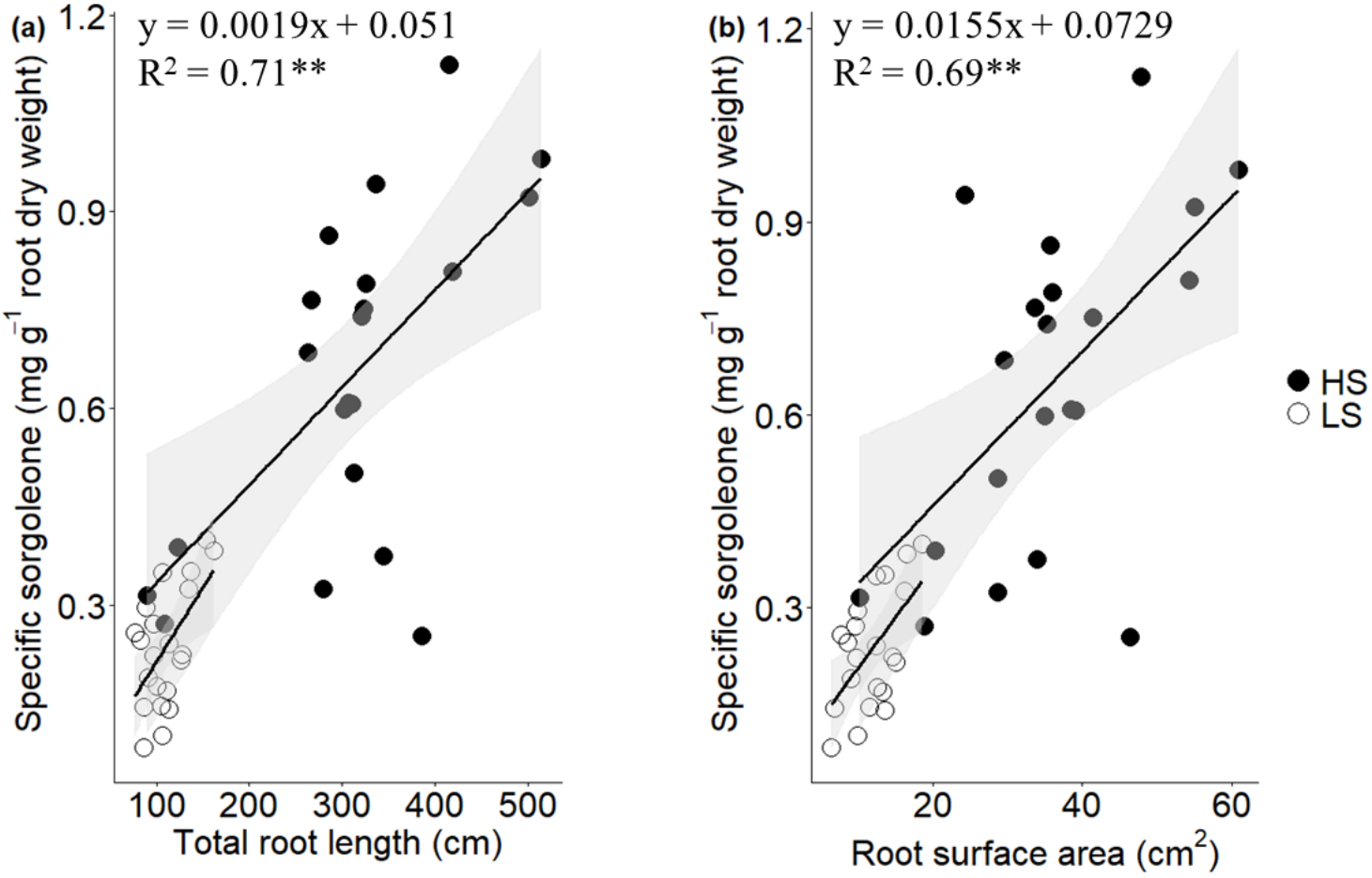


Figure 3

Relationship between specific sorgoleone release capacity with root morphology in differ genetic-stocks of sorghum under various N forms in paper culture experiments. **(a)** Relationship between specific sorgoleone and total root length; **(b)** Relationship between specific sorgoleone and root surface area. The low Sorgoleone genetic-stocks as EC670350 (LS), while the high Sorgoleone genetic-stocks as EC670311 (HS). For correlation analysis: R^2 value on asterisk * and ** denoted with $p < 0.05$ and $p < 0.01$, respectively.

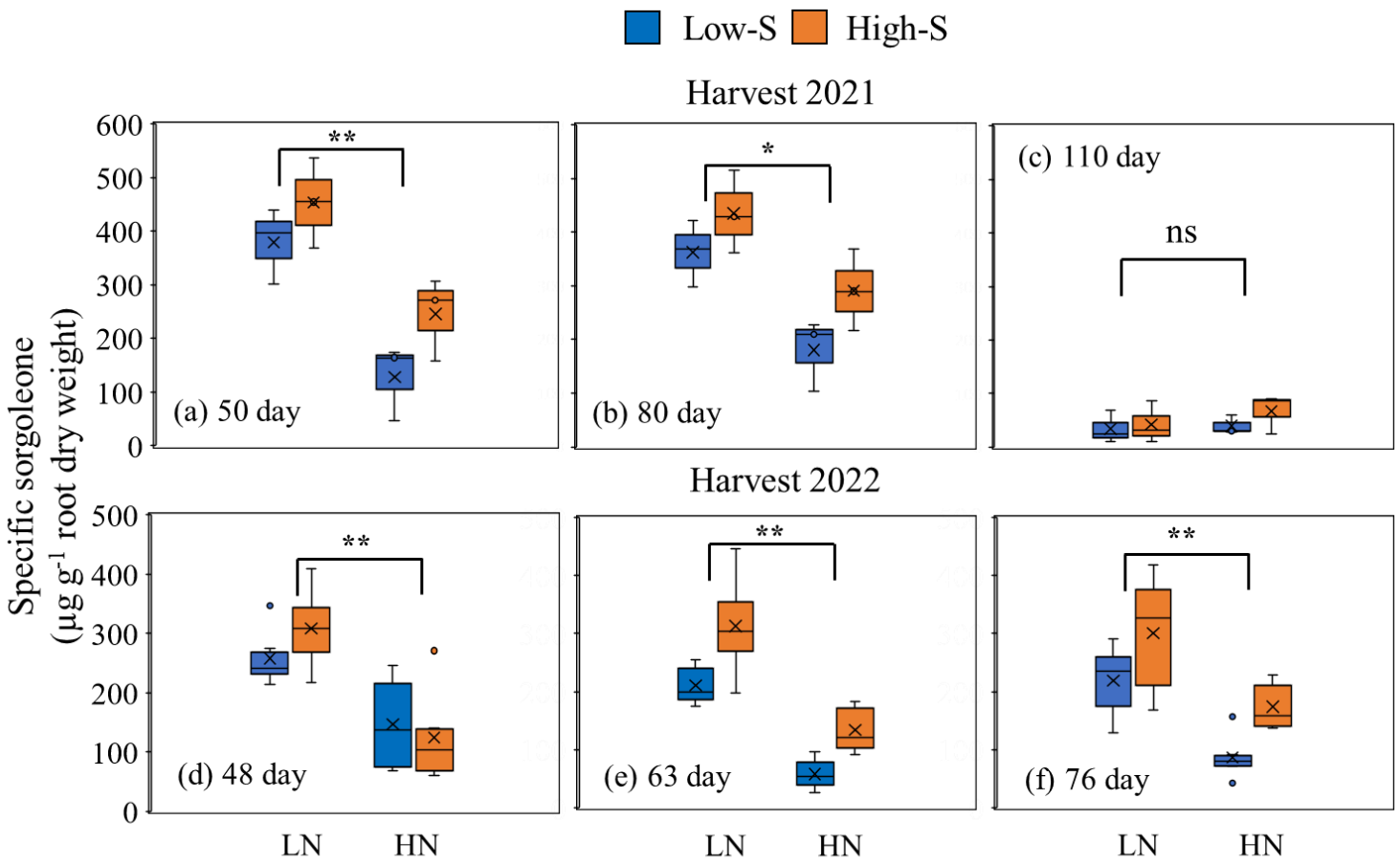


Figure 4

Specific sorgoleone released from contrasting BNI genetic-stocks of sorghum roots (LS and HS) with LN and HN treatment respectively in field trial in years 2021 and 2022. Low-S genetic-stock EC670350; High-S genetic-stock EC670311. HN treatment (100 kg N ha^{-1}); LN treatment (0 kg N ha^{-1}) in both 2021 and 2022. 50 day was the 50^{th} day after the sorghum was sown in field experiment, similar to 80, 48, 63, 76 and 110 days. Each boxplot shows the median, interquartile range, and whiskers representing the data distribution. Asterisks indicate significant differences among N treatments (* p<0.05, ** p<0.01).

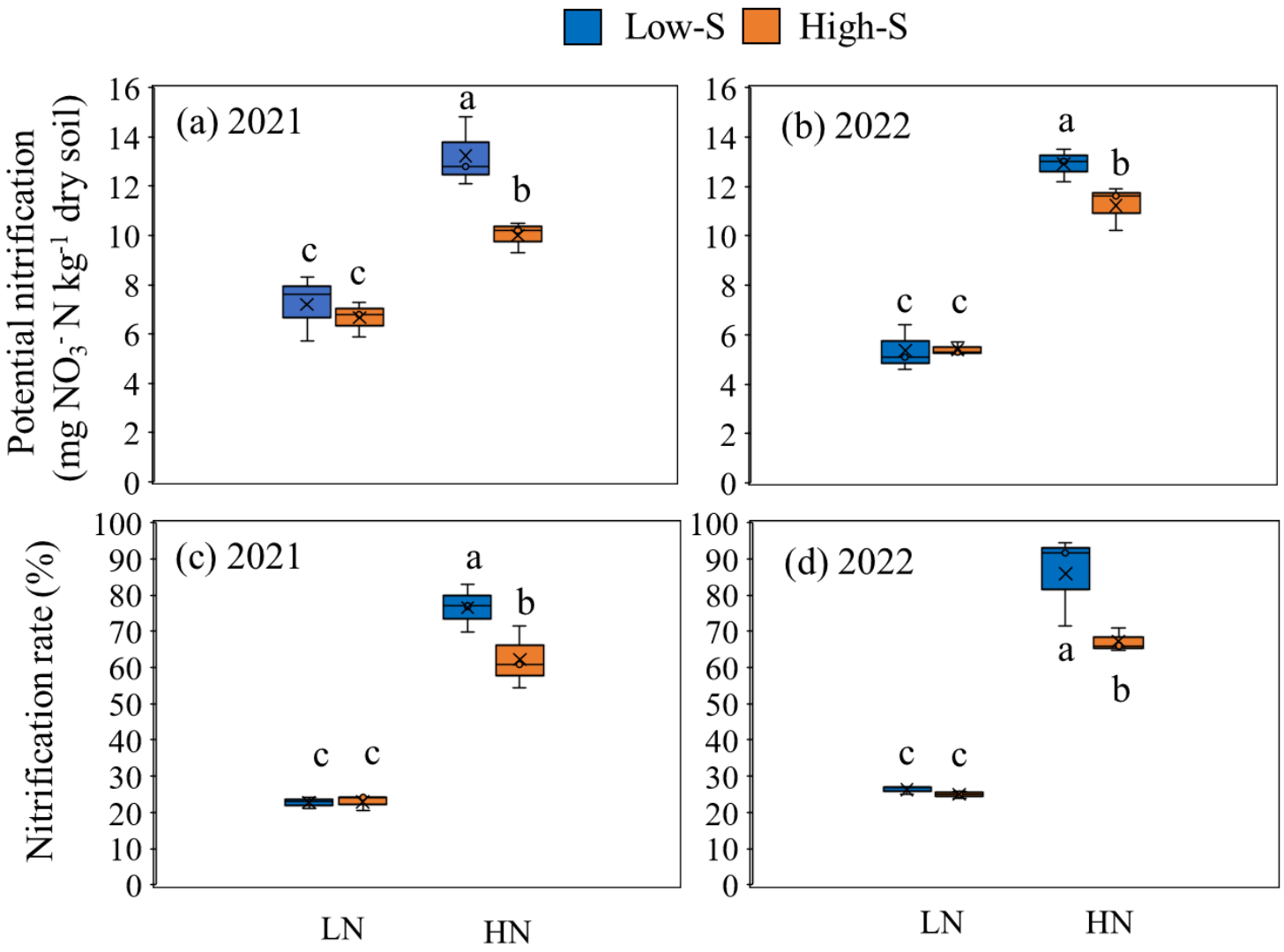


Figure 5

Potential nitrification and nitrification rate of contrasting sorgoleone genetic-stocks of sorghum (LS and HS) in two consecutive years of field trials. **(a)** PN in 2021; **(b)** PN in 2022; **(c)** NR in 2021; **(d)** NR in 2022. Low-S genetic-stock EC670350; and High-S genetic-stock EC670311. HN treatment (100 kg N ha^{-1}); LN treatment (0 kg N ha^{-1}) in both 2021 and 2022. Each boxplot shows the median, interquartile range, and whiskers representing the data distribution. Different letters indicate significant differences at $p<0.05$.

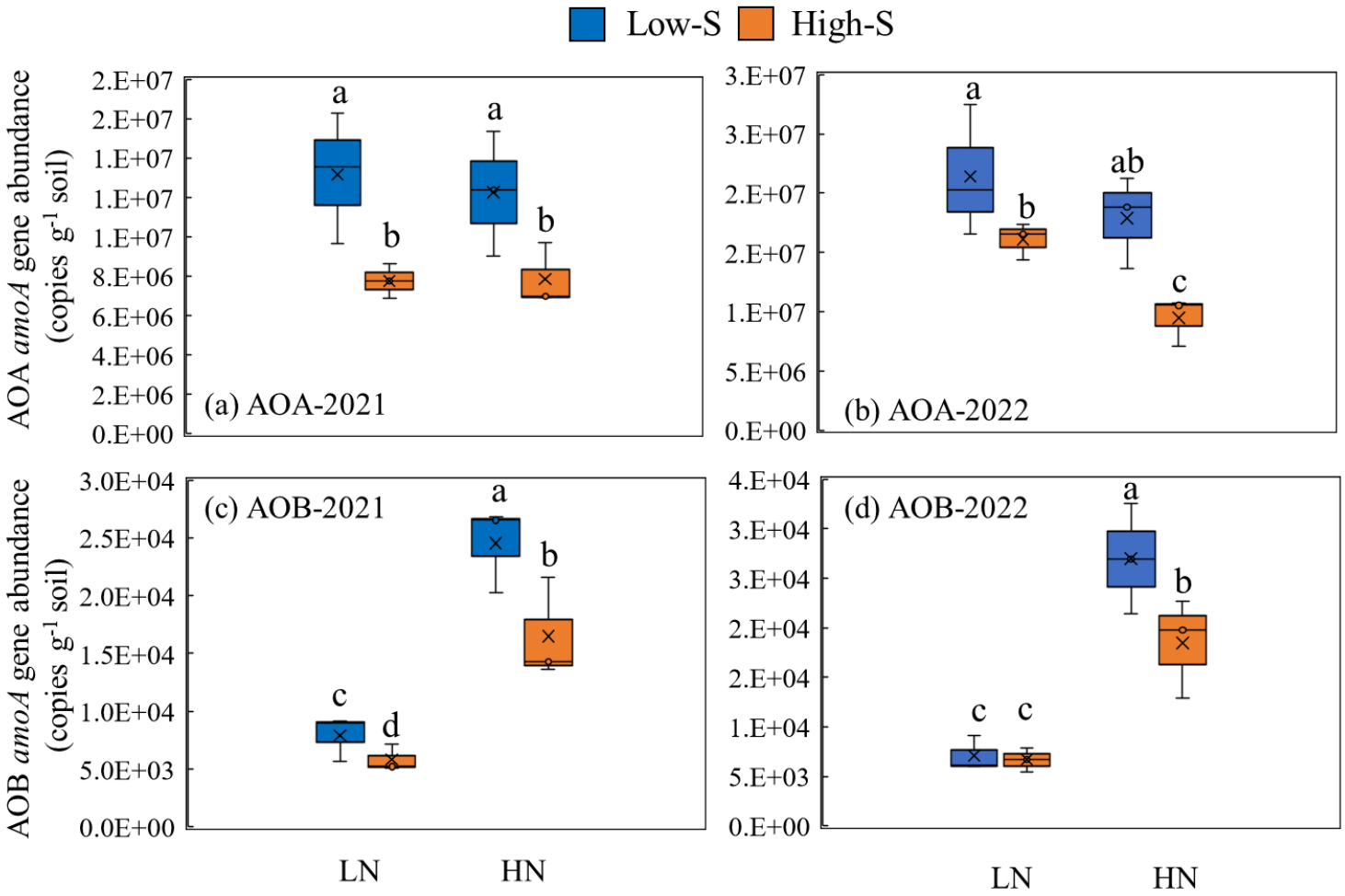


Figure 6

Abundance of AOA and AOB *amo*A genes of contrasting BNI genetic-stocks of sorghum (LS and HS) in two consecutive years of field trials. **(a)** AOA abundance in 2021; **(b)** AOA abundance in 2022. **(c)** AOB abundance in 2021; **(d)** AOB abundance in 2022. HN treatment (100 kg N ha^{-1}) in both 2021 and 2022; LN treatment (0 kg N ha^{-1}) in both 2021 and 2022. Low-S genetic-stock EC670350; and High-S genetic-stock EC670311. Each boxplot shows the median, interquartile range, and whiskers representing the data distribution. Different letters indicate significant differences at $p<0.05$.

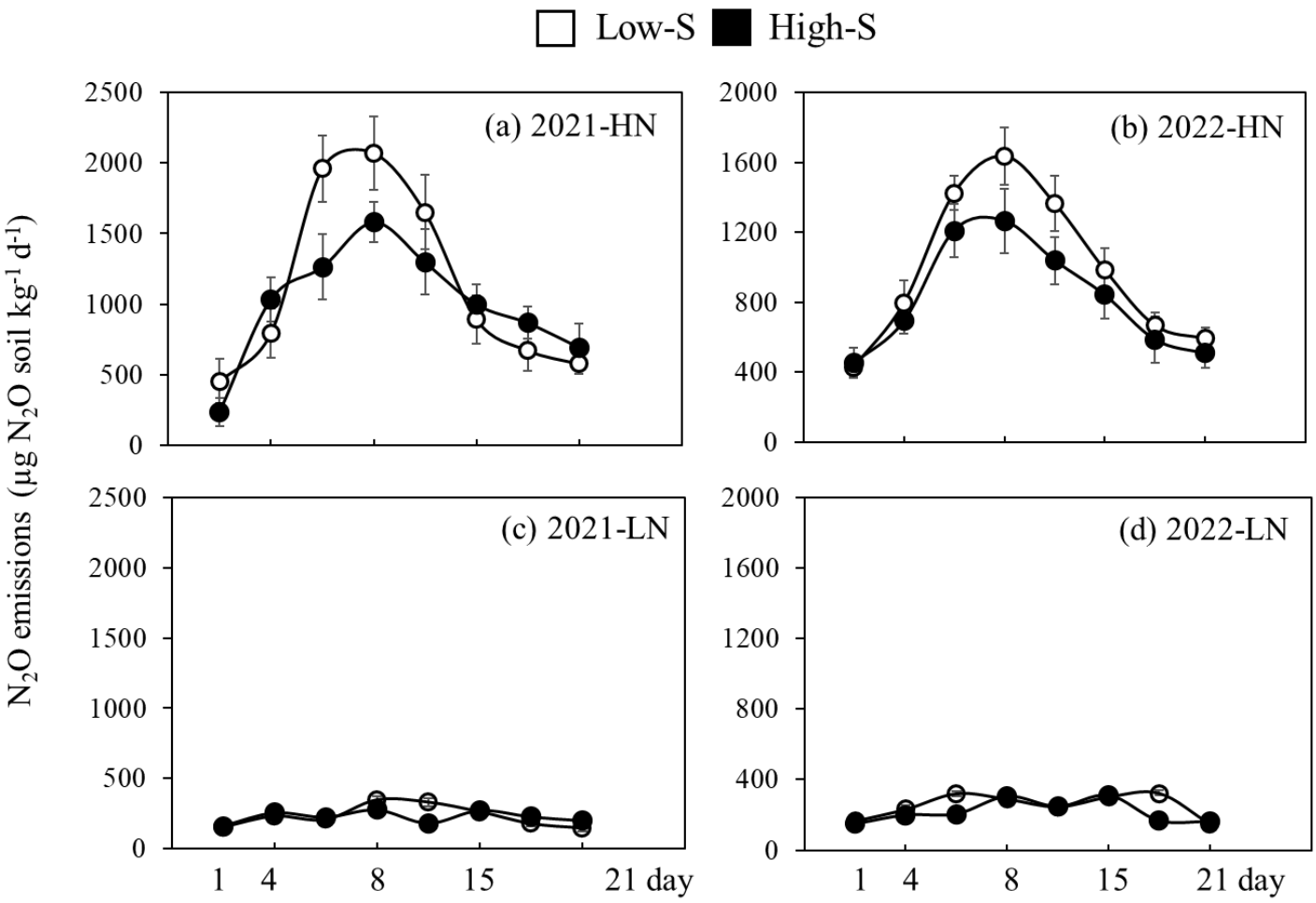


Figure 7

N_2O emissions from rhizosphere soils (from field grown plants) of contrasting BNI genetic-stocks of sorghum (LS and HS) in soil-microcosm study. **(a)** HN treatment (100 kg N ha^{-1}) in 2021; **(b)** HN treatment (100 kg N ha^{-1}) in 2022; **(c)** LN treatment (0 kg N ha^{-1}) in 2021; **(d)** LN treatment (0 kg N ha^{-1}) in 2022. Low-S genetic-stock EC670350; and High-S genetic-stock EC670311. Each bar represents the mean ± SE of three replicates.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1.docx