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Nitrogen transformation and growth-yield-quality responses of rice in the neem leaf extract applied and water-lowered soils

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Abstract

Neem leaf extract applied to lower soil moisture could inhibit nitrification, increase nitrogen (N) availability, and improve rice yield. The study aimed to evaluate the effects of neem leaf extract on soil N transformation, rice growth, yield, and quality in different soil moisture contents. Two factors were studied under a greenhouse pot experiment: soil moisture contents (field capacity, saturated, and flooded) and nitrification inhibitors (none, nitrapyrin, and neem leaf extract at 0.2, 0.4, and 0.8 g dry neem leaf basis 100 g^{-1} soil), with a total of fifteen treatment combination. Lowering soil moisture increased urea hydrolysis and nitrification rates and NH_4^+-N and NO_3^--N concentrations, promoting rice vegetative growth and delaying harvest dates (138 and 151 days for saturated and field capacity soils, compared to 130 days for flooded soil). Yet grain yield (in g pot^{-1}) decreased (from 34.3–40.3 in flooded soil to 33.3–37.2 in saturated soil and 15.0–24.9 in field capacity soil). Neem leaf extract generally depressed grain yield, specifically at higher rates in lower soil moisture. In flooded soil, neem extract maintained grain yield (35.9–40.3 vs. 35.9 without it), while in saturated soil, it decreased (33.3–37.2 vs. 36.8 without extract). In field capacity soil, neem extract decreased yield (18.9–21.4 vs. 24.9 without it). The grain yield depression was linked to high soil NH_4^+-N due to enhanced urea hydrolysis and inhibited nitrification by neem. Rice quality remained unaffected by soil moisture and neem extract, including grain shape, elongation, amylose, and amylopectin contents.

1. Introduction

Rice serves as a primary food source, supplying crucial carbohydrates to over 50% of the global population (Silalertruksa et al., 2017). Rice cultivation, exemplified by the Chao Phraya and Mun River basins in Thailand, requires substantial water resources, i.e., 7,300 m^3 ha^{-1} and 10,344 m^3 ha^{-1}, respectively (Silalertruksa et al., 2017). Climate change-induced droughts have significantly impacted rice production (Chen et al., 2023), and urban expansion and industrial pollution worsen water scarcity (Bouman and Tuong, 2001). Gleick (1993) reported a 40–60% decrease in freshwater availability from 1955 to 1990, with a projected further decrease of 15–54% by 2025.

Efficient water use in rice production not only mitigates droughts but also decreases potent greenhouse gas emissions. Methane and N_2O, with global warming potentials up to 21 and 310 times greater than CO_2, are generated in flooded soil with low O_2 levels (Mosier et al., 2004). Yao et al. (2017) found that decreasing water use in rice production could cut CH_4 and N_2O emissions by 54% and save up to 64% of water resources.

Yan et al. (2010) found that rice produced with saturated soils yielded 3,600 to 4,531 kg ha^{-1}, comparable to the flooded counterparts of 3,600 to 5,100 kg ha^{-1}, while Dou et al. (2016) observed similar results with soil moisture near field capacity (40 kPa) and 3–5 cm water depths. However, Bouman and Tuong (2001) reported that 92% of the studies related to water-saving rice production showed an average 6% decrease in yields. Brouwer and Heibloem (1986) suggested that rice water requirements were similar to sweet corn, peanuts, and soybeans. Lowered water use affects rice yields due to efficient soil N management, like in coarse-textured soils in Northeast Thailand, where N deficiency is common (Vityakon et al., 2000).

Optimizing N supply in the NH_4^+ form can help maintain or increase rice yields in water-saving practices (Chen et al., 1998). Only 30–40% of N fertilizer applied to rice fields is typically taken up by rice plants (Craswell and Vlek, 1983). The remainder

is lost through denitrification as gases (NO, N_2O, and N_2) and as NO_3^- via soil leaching and surface runoff, while the additional N portion is volatile as NH_3 (Prasad et al., 1971). This loss is mainly due to the rapid transformation of NH_4^+ to NO_3^- during nitrification (Mengel and Kirkby, 2001). Nitrification can even occur in the flooded soil near its surface due to the presence of O_2 (Singh and Singh, 2017). In addition, rice has a specialized tissue called aerenchyma, facilitating O_2 transport to the roots and creating an O_2-rich rhizosphere where nitrification occurs (Philippot et al., 2009). Nitrification, led by ammonia-oxidizing bacteria, occurs at 0–2 mm from the rice roots (Briones et al., 2002). This nitrification typically begins around seven days after flooding when the rhizosphere zone becomes O_2-rich (Chen et al., 1980).

Nitrification occurs faster in soils with lowered moisture content. To enhance nitrogen efficiency and rice yields, nitrification inhibitors like nitrapyrin, dicyandiamide, 2-amino-4-chloro-6-methylpyrimidine, sodium chlorate, sodium azide, and benzene hexachloride are used (Konwar et al., 2016). However, these inhibitors are costly and inaccessible to many farmers (Kumar et al., 2010).

Extracts from different parts of the neem tree (*Azadirachta indica*), such as leaves (Solomon et al., 2008; Alves et al., 2009; Mweetwa et al., 2016), seeds (Ruanpan and Mala, 2016), and bark (Solomon et al., 2008), have been reported to inhibit nitrification (Solomon et al., 2008) and enhance rice growth and yields (Vyas et al., 1991; Kumar et al., 2010). However, no study has been reported on using neem extract in combination with water-saving practices in rice production.

Using neem leaf extracts as a nitrification inhibitor would hold promise for preserving and enhancing rice yields under conditions characterized by limited water supply. The primary hypothesis underpinning this investigation postulated that neem leaf extracts can inhibit nitrification, and the inhibitory efficacy of the extract demonstrated an augmentative relationship with their concentration. Furthermore, it would be contended that applying neem leaf extract would result in heightened rice yields when even subjected to water-conserving conditions, encompassing soil moisture contents at field capacity and saturated conditions. Thus, the objectives of this study aimed to evaluate the effects of varying concentrations of neem leaf extracts on nitrification inhibition in rice production across diverse soil water contents at field capacity, saturated, and flooded soils. Our further objective was to evaluate the influence of neem leaf extract concentration on the growth and yield of rice within the context of these disparate water regimes.

2. Materials and methods

2.1. Soil and neem leaf extract

The soil used in this study was classified according to Soil Taxonomy as the isohyperthermic Aeric Kandiaquults, a common soil type in Northeast Thailand for rice cultivation. Soil samples were collected at 0–15 cm depth (17°11'10.1" N, 104°05'17.2" E), air-dried in the shade, and sifted through a 2-mm mesh for further use in the experiment. Some of the soil underwent initial property analysis (Table 1).

Table 1
Initial soil properties used in the current study

Soil property	Value
Particle size distribution	
Sand (%)	72.1
Silt (%)	20.1
Clay (%)	7.8
Soil texture	Sandy loam
Bulk density (g cm^{-3})	1.51
Water characteristics	
Water holding capacity (%)	25.0
Field capacity (%)	10.8
Permanent wilting point (%)	2.2
Ksat (cm sec^{-1})	$1.41x10^{-3}$
pH (soil:H_2O = 1:10)	5.88
Electrical conductivity (mS cm^{-1})	0.07
Organic C (g kg^{-1})	4.41
Total N (g kg^{-1})	0.27
NH_4^+-N (mg kg^{-1})	5.22
NO_3^--N (mg kg^{-1})	8.08
P (mg kg^{-1})	80.6
K (mg kg^{-1})	45.3
Ca (mg kg^{-1})	211.9
Mg (mg kg^{-1})	32.8
Fe (mg kg^{-1})	85.3
Al (mg kg^{-1})	10.3

Neem leaves were collected in Sakon Nakhon, Thailand, and systematically processed. After air drying under the shade for two weeks and thorough cleansing, neem leaves were dried at 60°C under a drying house for five days, ground to a size smaller than 1 mm, and mixed with 95% ethanol using 1:10 w/v. The mixture was filtered through a fine cloth and subsequently Whatman no.1, and the filtrate was evaporated using a rotary evaporator at 50°C till dry. The resulting neem leaf extract was stored in an amber bottle at –20°C for subsequent use in the experiment. Neem leaf yielded 2.1% extract that contained 0.393 mg azadirachtin mg^{-1} extract, 216 g C kg^{-1} extract, and 1012 mg N kg^{-1} extract.

2.2. Pot experiment

The experiment was conducted from June to November 2020 in a greenhouse equipped with an evaporative cooling system. The experiment employed a 3×5 factorial experiment

in a completely randomized design with three replications, resulting in 15 treatment combinations. There were, therefore, a total of 45 experimental units, each containing 4 pots, for a total of 180 pots. The experiment considered two factors: soil moisture contents, i.e., field capacity, saturated, flooded; and nitrification inhibitors, i.e., no inhibitor, nitrapyrin at 0.25% of urea-N, neem leaf extract at 0.2, 0.4, and 0.8 g of dry neem leaf basis per 100 g soil.

Cylindrical-shaped pots (25.5 cm top *d*, 17 cm bottom *d*, 21 cm *h*) with 6 kg of dry soil each were used. The greenhouse's mean air temperature and relative humidity throughout the experiment were 33.6°C and 78.1%, respectively.

Prior to rice transplanting for fourteen days, soil moisture contents were adjusted to the field capacity, saturation, and a 3-cm flood depth. In the flooded treatment, the soil was kept at a 3-cm depth till seven days before rice with their treatments was harvested.

In all treatments, urea fertilizer was applied at 180 kg N ha^{-1}, according to Sun et al. (2015). This was divided into three phases: 50% during rice transplanting and the remaining two 25% at 20 and 37 days after transplanting. Nitrification inhibitors, i.e., nitrapyrin and neem leaf extract, were combined with urea fertilizer inputs at the prescribed rates for each treatment. Additionally, phosphorus (60 kg P_2O_5 ha^{-1}) and potassium fertilizers (90 kg K_2O ha^{-1}) were applied during transplanting.

The non-photosensitive RD22 glutinous rice variety, suitable for year-round cultivation, was used. Seedlings were nursed for 30 days in trays, and healthy, uniform seedlings were transplanted into the pots. The transplanting involved two seedlings per pot.

The soil was sampled systematically from pots during rice planting, from transplanting to heading stages (0 to 70 days after transplanting). The samplings occurred weekly, with an additional sample taken on the day of the rice harvest. In essence, soil samples were taken when the rice plants were 0, 7, 14, 21, 28, 35, 42, 70, and 100 days after transplanting.

The rice crop's growth, i.e., tillering, height, and leaf chlorophyll contents, was monitored weekly. The latter parameter was measured using a SPAD chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Inc., Aurora, IL, USA). Shoot and root dry weights were determined at harvest after oven-drying at 65°C. Rice yield and its components, i.e., panicle number per pot, grain number per panicle, 1000-grain weight, and harvest index, were determined at the harvest.

2.3. Laboratory analysis

Soil texture was analyzed using the pipette method. Soil bulk density was assessed using the soil core method (Pansu and Gautheyrou, 2006). The soil moisture content at the field capacity and permanent wilting point was determined via the pressure plate method (Dane and Hopmans, 2002), and saturation water content was measured using the maximum water holding capacity method (Wilke, 2005), while the saturated hydraulic conductivity (K_s) was by falling head soil core method (Reynolds et al., 2002).

Soil pH and electrical conductivity were determined with a 1:10 soil-to-water ratio. Soil organic matter content was assessed using the wet oxidation method (Nelson and Sommers, 1982). Soil P concentration was measured with the Bray2 solution and quantified at 820 nm using a UV–Vis spectrophotometer (Specord250 plus, Analytik Jena, Germany) (Fixen and Grove, 1990). Soil cations (K, Ca, Mg, and Fe) were extracted with 1 *M* NH_4OAc, and their concentrations were determined through atomic absorption spectrophotometry (AAS) (novAA® 350, Analytik Jena, Germany) (Pansu and Gautheyrou, 2006). Soil Al concentration was extracted using 1 *M* KCl and quantified via titration (Bertsch and Bloom, 1996). Soil total N was measured using the steam distillation method on a micro-Kjeldahl apparatus (Pro–Nitro S 4002851, JP Selecta, Barcelona, Spain) (Bremner and Mulvaney, 1982), while NH_4^+ and NO_3^- were extracted with 2 *M* KCl and quantified using the same method (Stevenson, 1982).

Azadirachtin concentration in the soil and neem leaf extract was analyzed using the method modified from Stark and Walter (1995) on a high-performance liquid chromatography (HPLC) with a Shimadzu Prominence UFLC HPLC System equipped with an LC-20A pump, DGU20A degasser, SPD M-20A diode array detector, and CTO-20 A column oven. A reverse-phase Merck C-18 column (4.6×250 mm, 5 µm) and a mobile phase of methanol: water (60:40) with isocratic elution were used. The UV detector was set at 217 nm, operating at 25°C, and the elution flow rate was 1.0 ml min^{-1}.

The Gram-negative bacteria population involved in inorganic N transformation in the soil was determined using a modified plate count technique on MacConkey agar, following methods by Olsen and Bakken (1987).

The elemental contents of the tissue in rice shoots were extracted using the nitric-perchloric wet digestion method (Miller, 1998). Subsequently, tissue N content was determined by the steam distillation method (Horneck and Miller, 1998), while tissue P content was measured with a UV–Vis spectrophotometer. Tissue K, Ca, Mg, and Fe contents were determined using the AAS, while Al was on an inductively coupled plasma optical emission spectrometer (PlasmaQuant PQ9000, Analytik Jena, Germany).

Unmilled and milled rice grain qualities were assessed following Adair et al. (1966). Cooked grain elongation was measured as per Juliano and Perez (1984), and rice grain amylose content was analyzed according to Juliano (1971).

2.4. Data calculations and statistical analysis

Rice grain elongation rate and length index were calculated following Juliano and Perez (1984), the harvest index was computed following Fageria (2014), and amylopectin content was by Torruco-Uco et al. (2006). Net urea hydrolysis and net nitrification rates were calculated via the modified method of Bi et al. (2017), as follows:

$$\text{Elongation rate} = \frac{\text{Length of cooked grain}}{\text{Length of uncooked grain}} \quad \text{Eq. 1}$$

$$\text{Length index of cooked grain} = \frac{\text{Length/width of cooked grain}}{\text{Length/width of uncooked grain}} \quad \text{Eq. 2}$$

$$\text{Harvest index (\%)} = \frac{\text{Grain weight}}{\text{(Shoot dry weight+grain weight)}} \times 100 \quad \text{Eq. 3}$$

$$\text{Amylopectin (\%)} = 100 - \text{\% amylose} \quad \text{Eq. 4}$$

$$\text{Net urea hydrolysis rate (mg N kg}^{-1}\text{ soil day}^{-1}\text{)} = \frac{(NH_4^+\text{-}N_{t2})\text{-}(NH_4^+\text{-}N_{t1})}{t} \quad \text{Eq. 5}$$

$$\text{Net nitrification rate (mg N kg}^{-1}\text{ soil day}^{-1}\text{)} = \frac{(NO_3^-\text{-}N_{t2})\text{-}(NO_3^-\text{-}N_{t1})}{t} \quad \text{Eq. 6}$$

Where t represents the number of days between the previous sampling ($t1$) and current sampling ($t2$); while (NH_4^+-N_{t1}) and (NH_4^+-N_{t2}) as well as (NO_3^--N_{t1}) and (NO_3^--N_{t2}) denote the concentrations of NH_4^+-N and NO_3^--N at $t1$ and $t2$.

An analysis of variance based on a factorial experiment in a completely randomized design was used to evaluate the effects of neem leaf extract on nitrogen transformation in soil, along with other soil properties and their consequent effects on the growth, yield, and quality of rice planted in varying soil moisture contents. Tukey's honest significant difference test was employed to compare mean values. The statistical significance was at $p \leq 0.05$.

3. Results and discussion

3.1. Soil nitrogen transformation

The peaks of NH_4^+-N (Fig. 1A-C) and NO_3^--N concentrations (Fig. 1D-F), as well as net urea hydrolysis (Fig. 2A-C) and net nitrification rates (Fig. 2D-F) over the experimental period revealed that these soil N statuses increased as lowering soil moisture contents in the order of field capacity>saturated>flooded. The results demonstrated that lowered soil moisture stimulated urea hydrolysis and nitrification because higher aeration enhanced the activity of aerobic microorganisms involved in these processes. The primary contributors to urea hydrolysis were aerobic ureolytic bacteria, including *Sporosarcina pasteurii*, *Lysinibacillus sphaericus*, and *Bacillus sphaericus* (Jiang et al., 2021). Our results aligned with Antil et al. (2006) and Mohanty et al. (2008),

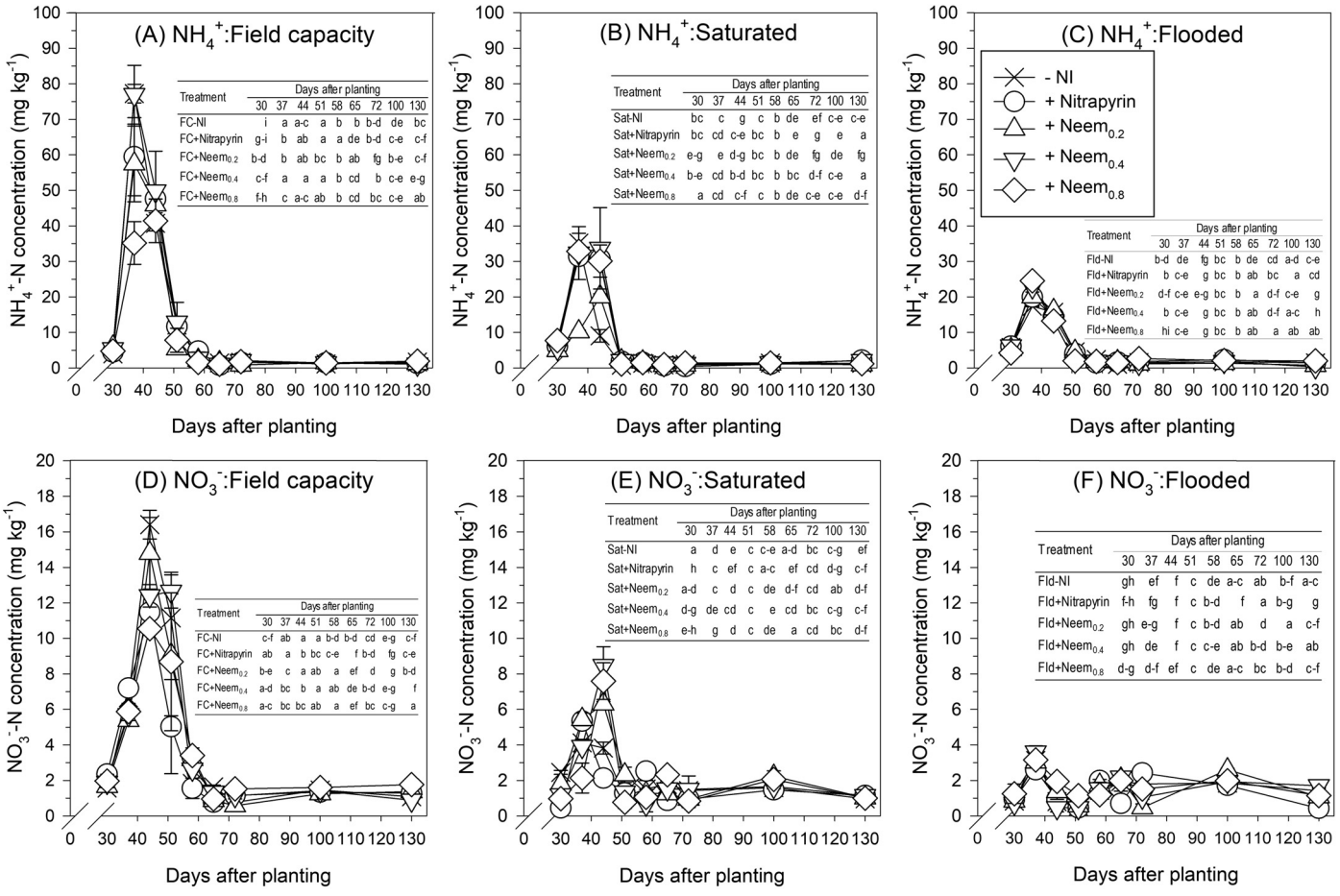

Fig. 1. Soil NH_4^+-N (A, B, C) and NO_3^--N concentrations (D, E, F) as affected by varying soil moisture contents at field capacity(FC) (A, D), saturated (Sat) (B, E), and flooded soils (Fld) (C, F) treated with different nitrification inhibitors, including no inhibitor (-NI), nitrapyrin (+Nitrapyrin), and neem leaf extract at 0.2 (+$Neem_{0.2}$), 0.4 (+$Neem_{0.4}$), and 0.8 (+$Neem_{0.8}$) g of dry neem leaf basis per 100 g soil. The letters within time intervals in the inset tables denote not statistically significant differences across soil moisture contents at $p \leq 0.05$ (Tukey's honest significant different test). The error bars represent the standard deviation

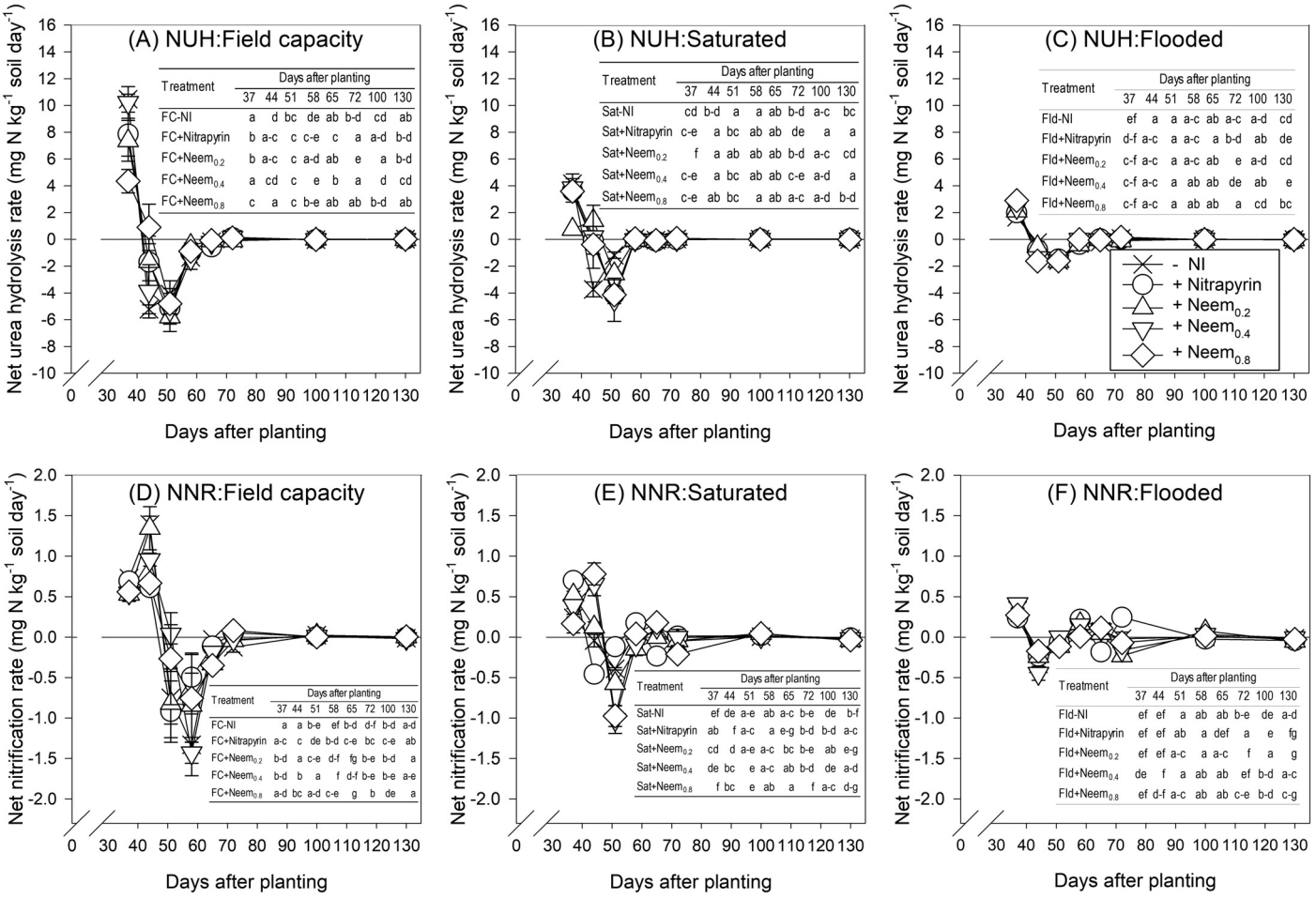

Fig. 2. Net urea hydrolysis (NUH) (A, B, C) and net nitrification rates (NNR) (D, E, F) as affected by varying soil moisture contents at field capacity (FC) (A, D), saturated (Sat) (B, E), and flooded soils (Fld) (C, F) treated with different nitrification inhibitors, including no inhibitor (-NI), nitrapyrin (+Nitrapyrin), and neem leaf extract at 0.2 (+$Neem_{0.2}$), 0.4 (+$Neem_{0.4}$), and 0.8 (+$Neem_{0.8}$) g of dry neem leaf basis per 100 g soil. The letters within time intervals in the inset tables denote not statistically significant differences across soil moisture contents at $p \leq 0.05$ (Tukey's honest significant different test). The error bars represent the standard deviation

who reported peak urease activity at field capacity and a subsequent decline in moisture above the saturated soil.

Aerobic nitrifying bacteria, including *Nitrosomonas* sp., *Nitrosolobus* sp., and *Nitrospira* sp., played a crucial role in converting NH_4^+ to NO_2^-, followed by the immediate transforming of NO_2^- to NO_3^- by *Nitrobacter* sp. (Weil and Brady, 2017). Mohanty et al. (2008) found higher soil NO_3^- concentrations in field capacity soil compared to flooded soil. Soil nitrification rates increased significantly when soil O_2 content was above 20% (Sahrawat, 2008). However, O_2 diffusion dropped drastically in flooded soil, up to 320,000-fold lower (Colmer and Flowers, 2008). Furthermore, aerobic nitrifying bacteria are predominantly Gram-negative (Spieck and Lipski, 2011), consistent with the result of the current study that the abundance of Gram-negative bacteria increases with lowering soil moisture contents (Table 2).

Neem leaf extract stimulated urea hydrolysis, obviously in field capacity soils with better aeration than saturated and flooded soils, as seen in significantly higher net urea hydrolysis rates in specific dose rates of the neem leaf extract than the no inhibitor treatment on 44 and 72 days after planting (Fig. 2A). This stimulation of urea hydrolysis by extract aligned the studies of Mohanty et al. (2008), Kizilkaya et al. (2015), and Butnan et al. (2022); but contrast to Prasad and Power (1995) and Varel (1997). Kizilkaya et al. (2015) explained that neem leaf extract serves as a source of C, N, and energy for soil microorganisms, correspondent to C and N contents of neem leaf extract in this study, which was 216 g C kg^{-1} and 1012 mg N kg^{-1}, respectively.

In contrast to urea hydrolysis, the neem leaf extract exhibited inhibitory effects on the nitrification at specific dosage rates on days 37, 44, 58, and 65 after planting in field capacity soil (Fig. 2D) and days 37 and 72 in saturated soil (Fig. 2F). The nitrification-inhibiting property was attributed to the essential ingredients of neem, *viz* azadirachtin, a well-established nitrification inhibitor (Alves et al., 2009), with 0.393 mg azadirachtin mg^{-1} detected in the neem leaf extract used in this study, and as seen the existence of azadirachtin in soil during rice harvest (Table 2). Neem leaf extract exerted inhibitory effects on nitrifiers, primarily Gram-negative bacteria, i.e., *Nitrosomonas* sp., *Nitrosococcus* sp., *Nitrospira* sp., *Nitrosolobus* sp., and *Nitrobacter* sp. (Mweetwa et al., 2016). However, in this study, the abundance

Table 2
Abundance of Gram-negative bacteria and azadirachtin contents in soils as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	Gram-negative bacteria (x 10^6 CFU kg^{-1} soil)	Azadirachtin (mg kg^{-1})
FC-NI	23.1 ab §	na
FC+Nitrapyrin	29.0 a	na
FC+$Neem_{0.2}$	25.6 a	8.6 f
FC+$Neem_{0.4}$	16.0 bc	10.3 de
FC+$Neem_{0.8}$	29.6 a	12.4 b
Sat-NI	23.5 ab	na
Sat+Nitrapyrin	16.0 bc	na
Sat+$Neem_{0.2}$	26.5 a	11.3 c
Sat+$Neem_{0.4}$	6.4 de	10.6 cd
Sat+$Neem_{0.8}$	22.1 ab	17.4 a
Fld-NI	6.5 de	na
Fld+Nitrapyrin	10.5 cd	na
Fld+$Neem_{0.2}$	5.2 de	9.6 e
Fld+$Neem_{0.4}$	2.4 de	10.5 d
Fld+$Neem_{0.8}$	1.3 e	12.5 b
p-value	< 0.001	< 0.001
F-test	***	***
CV (%)	18.3	3.72

*** $p \leq 0.001$
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +$Neem_{0.2}$, +$Neem_{0.4}$, and +$Neem_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 g of neem leaf dry weight per 100 g soil
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

of Gram-negative bacteria in the neem leaf extract-treated soils was primarily comparable to soil without a nitrification inhibitor, except for $Neem_{0.4}$ in field capacity and saturated soil (Table 2). This could be attributed to the timing of the Gram-negative bacteria determination that occurred on the day of the rice harvest, notably after the application of neem leaf extract. This time gap might have allowed the nitrifiers to revert to their typical levels.

It was also noticed that the urea hydrolysis stimulation and nitrification inhibition, which was caused by specific dose rates of neem leaf extract, were primarily limited to field capacity soil (Fig. 2D). This was likely because microorganisms involved in the soil N transforming processes were mainly aerobic; consequently, in saturated and flooded soils, these microorganisms exhibited lower activity (Weil and Brady, 2017; Jiang et al., 2021). Neem leaf extract, therefore, showed ineffective in stimulating urea hydrolysis and inhibiting nitrification in high-moisture soils, i.e., saturated and flooded treatments.

3.2. Rice growth, yield, and quality

Lowering soil moisture contents from flooded to saturated and field capacity enhanced rice's vegetative growth, obviously through increased tillering (Fig. 3A-C) and the shoot/root ratio (Table 3). This enhancement primarily stemmed from improved soil N availability, reflected in higher concentrations of soil NH_4^+-N (Fig. 1A-C) and NO_3^--N (Fig. 1D-F). Consequently, rice received more N; indicated by the rises in N uptakes in rice shoot (Table 4) and leaf chlorophyll contents (Fig. 3D-F), in the lowered-moisture soils (saturated and field capacity soils). As for the latter validation, Skudra and Ruza (2017) established a close relationship between leaf chlorophyll and shoot N contents. In addition to enhanced N availability, the increase in rice's vegetative growth might be attributed to alleviated Fe toxicity, in line with the unexpected decrease in soil Fe concentrations as

Table 3
Shoot and root dry weights and shoot/root ratios of rice in soils as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Shoot/root ratio
FC-NI	45.8 ab §	11.3 cd	4.06 a-c
FC+Nitrapyrin	48.5 ab	10.9 d	4.46 ab
FC+$Neem_{0.2}$	52.5 a	11.4 cd	4.63 a
FC+$Neem_{0.4}$	50.5 ab	10.7 d	4.72 a
FC+$Neem_{0.8}$	48.8 ab	11.6 b-d	4.21 a-c
Sat-NI	50.7 ab	12.1 a-d	4.21 a-c
Sat+Nitrapyrin	47.8 ab	13.9 ab	3.47 bc
Sat+$Neem_{0.2}$	52.2 a	12.9 a-d	4.08 a-c
Sat+$Neem_{0.4}$	50.1 ab	12.7 a-d	4.03 a-c
Sat+$Neem_{0.8}$	51.8 a	11.9 a-d	4.35 a-c
Fld-NI	45.3 ab	13.9 ab	3.26 c
Fld+Nitrapyrin	46.4 ab	13.5 a-c	3.42 bc
Fld+$Neem_{0.2}$	46.8 ab	14.2 a	3.31 c
Fld+$Neem_{0.4}$	43.0 b	12.8 a-d	3.37 bc
Fld+$Neem_{0.8}$	46.8 ab	14.0 ab	3.35 bc
p-value	0.007	< 0.001	< 0.001
F-test	**	***	***
CV (%)	5.91	6.5100	9.48

*** $p \leq 0.001$
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +$Neem_{0.2}$, +$Neem_{0.4}$, and +$Neem_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 g of neem leaf dry weight per 100 g soil
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

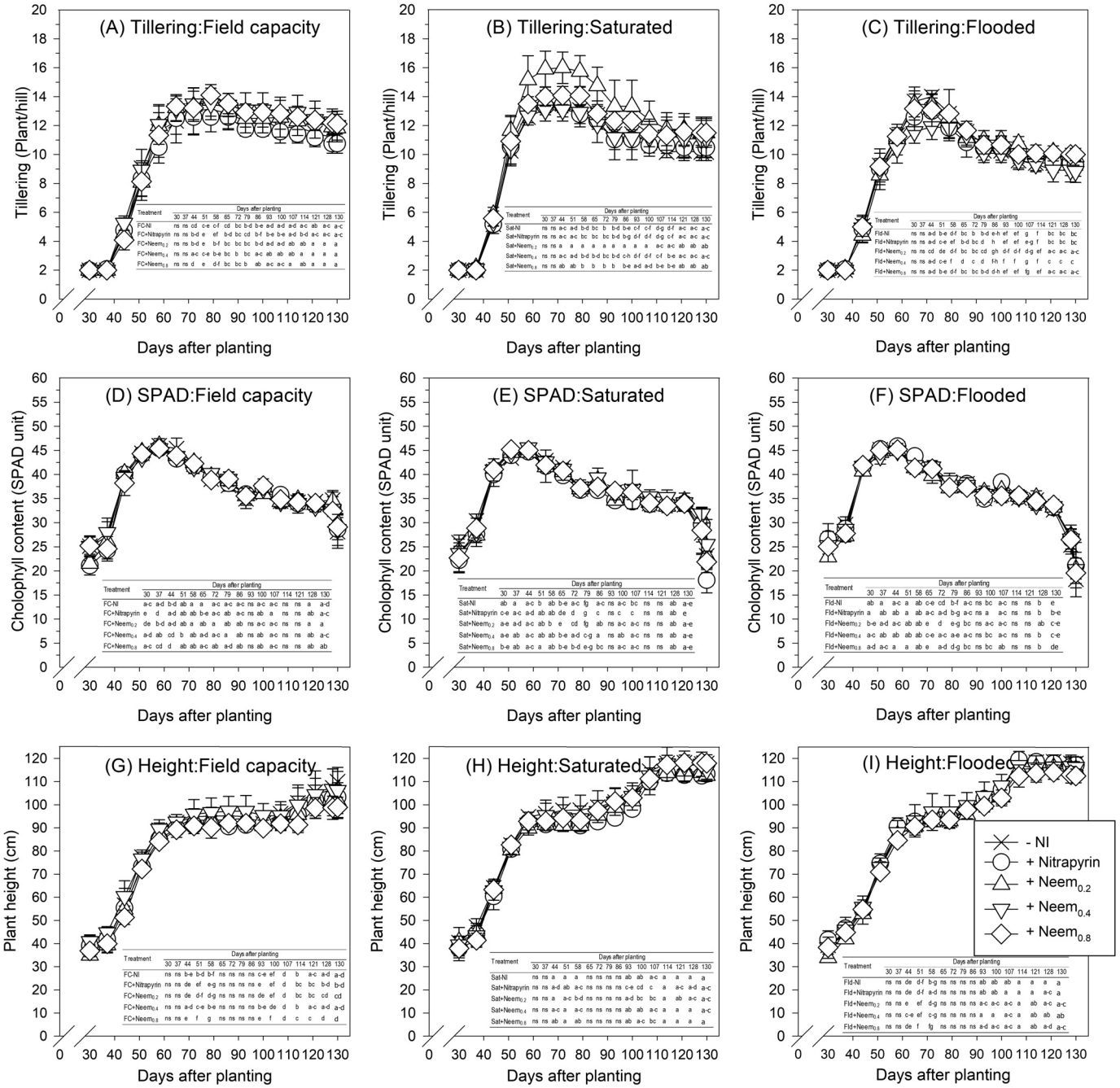

Fig. 3. Rice's tillering (A, B, C), leaf chlorophyll contents (D, E, F), and height (G, H, I) as affected by varying soil moisture contents at field capacity (FC) (A, D, G), saturated (Sat) (B, E, H), and flooded soils (Fld) (C, F, I) treated with different nitrification inhibitors, including no inhibitor (-NI), nitrapyrin (+Nitrapyrin), and neem leaf extract at 0.2 (+$Neem_{0.2}$), 0.4 (+$Neem_{0.4}$), and 0.8 (+$Neem_{0.8}$) g of dry neem leaf basis per 100 g soil. The letters within time intervals in the inset tables denote not statistically significant differences across soil moisture contents at $p \leq 0.05$ (Tukey's honest significant different test). The error bars represent the standard deviation

soil moisture decreased (Table 5). Fageria (2014) reported that the suitable soil Fe concentration for rice growth was 60–100 mg kg^{-1}. Notably, the soil Fe concentrations in the flooded soil in this study consistently exceeded 100 mg kg^{-1} (Table 5).

In soils with lowered moisture, increased tillering (Fig. 3A-C) raised panicle numbers per pot; nonetheless, it deteriorated the rice yield as primarily shown in field-capacity soils (Table 6). Excessive tillering led to later-formed tillers with decreased panicle-forming abilities and deleterious translocation of the assimilates to grains (Yang, Zhang, Wang, Zhu, and Wang, 2001; Fukai and Wade, 2021). This aligned with Matsuo et al. (1995), who noted that excessive N decreased leaf sugar

Table 4
Rice shoot tissue elemental contents and uptakes as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	Tissue elemental content (g kg^{-1})							Plant elemental uptake (mg pot^{-1})						
	N	P	K	Ca	Mg	Fe	Al	N	P	K	Ca	Mg	Fe	Al
FC-NI	4.18 b-e §	1.03 a-c	6.03 a	2.53 cd	2.51 a	0.36 c	0.20 c	186 e-g	45.7 cd	268 a-c	113 d	112 ab	16.0 cd	9.1 c
FC+Nitrapyrin	4.81 a-c	1.10 a-c	6.08 a	2.34 d	2.37 a	0.32 c	0.23 bc	240 a-c	54.8 ab	304 a	117 cd	118 ab	16.0 cd	11.5 bc
FC+Neem$_{0.2}$	4.69 a-d	1.02 a-c	5.92 a	3.24 a-d	2.44 a	0.38 c	0.26 a-c	237 a-d	51.7 a-c	300 ab	163 a-d	123 a	19.4 cd	12.9 a-c
FC+Neem$_{0.4}$	5.13 a	1.05 a-c	5.80 ab	2.83 a-d	2.57 a	0.38 c	0.23 a-c	244 ab	49.8 a-c	275 a-c	134 a-d	122 a	18.1 cd	11.1 bc
FC+Neem$_{0.8}$	4.62 a-d	0.95 a-c	5.71 a-c	2.59 b-d	2.34 a	0.32 c	0.22 bc	225 a-e	46.5 b-d	279 a-c	126 b-d	114 ab	15.8 d	10.9 bc
Sat-NI	3.94 de	0.88 bc	4.87 d	3.80 a-d	2.25 a	0.46 bc	0.30 ab	199 d-g	44.3 cd	246 a-c	193 a-c	114 ab	23.1 b-d	15.0 ab
Sat+Nitrapyrin	4.00 c-e	0.88 bc	5.02 b-d	3.82 a-d	2.09 a	0.42 c	0.28 a-c	186 e-g	40.8 d	234 a-c	178 a-d	97 b	19.4 cd	13.0 a-c
Sat+Neem$_{0.2}$	3.91 de	0.82 c	4.86 d	3.38 a-d	2.20 a	0.43 c	0.25 a-c	212 b-f	44.4 cd	263 a-c	183 a-d	119 ab	23.5 bc	13.4 a-c
Sat+Neem$_{0.4}$	4.85 ab	0.92 a-c	5.42 a-d	3.88 a-d	2.22 a	0.45 c	0.24 a-c	255 a	48.4 a-d	286 a-c	205 a	117 ab	23.6 bc	12.7 a-c
Sat+Neem$_{0.8}$	4.01 b-e	0.89 bc	5.25 a-d	3.51 a-d	2.42 a	0.31 c	0.32 a	202 c-g	45.0 cd	265 a-c	177 a-d	122 a	15.8 d	16.2 a
Fld-NI	4.03 b-e	1.17 ab	4.93 cd	4.36 a	1.31 b	0.69 a	0.25 a-c	180 fg	52.0 a-c	220 c	194 ab	59 c	30.6 ab	11.0bc
Fld+Nitrapyrin	4.24 b-e	1.20 a	4.99 b-d	4.02 a-c	1.29 b	0.63 a	0.23 bc	197 d-g	55.5 a	231 a-c	186 a-d	60 c	29.2 ab	10.5 bc
Fld+Neem$_{0.2}$	4.22 b-e	1.10 a-c	5.55 a-d	4.02 a-c	1.25 b	0.69 a	0.27 a-c	198 d-g	51.5 a-c	259 a-c	188 a-d	58 c	32.1 a	12.8 a-c
Fld+Neem$_{0.4}$	4.02 b-e	1.13 ab	5.34 a-d	4.13 ab	1.33 b	0.75 a	0.30 ab	173 fg	48.7 a-d	230 bc	177 a-d	57 c	32.2 a	12.7 a-c
Fld+Neem$_{0.8}$	3.69 e	1.11 a-c	5.46 a-d	3.67 a-d	1.13 b	0.62 ab	0.24 a-c	165 g	49.6 a-c	244 a-c	164 a-d	51 c	27.7 ab	10.7 bc
p-value	0.044	< 0.001	0.0385	< 0.001	< 0.001	< 0.001	0.0014	<0.001	0.0495	0.0028	<0.001	<0.001	<0.001	<0.001
F-test	*	***	*	***	***	***	**	***	*	**	***	***	***	***
CV (%)	11.78	9.6	9.27	15.2	8.3	11.76	11.51	11.8	10.35	9.44	15.17	8.12	11.05	12.16

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +Neem$_{0.2}$, +Neem$_{0.4}$, and +Neem$_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 g of neem leaf dry weight per 100 g soil
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

Table 5
Soil physicochemical properties as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	BD	Organic C	Total N	P	K	Ca	Mg	Fe	Al
	(g/cm^3)	(g/kg)		(mg/kg)					
FC-NI	1.41	3.85 ab §	0.334	63.3 ab	14.2	224	14.0 d	91.9 d	15.9 d-f
FC+Nitrapyrin	1.38	3.77 ab	0.296	48.3 ab	19.9	202	13.1 d	83.8 d	15.6 d-f
FC+$Neem_{0.2}$	1.36	3.83 ab	0.285	54.0 ab	16.9	226	13.6 d	80.4 d	21.6 bc
FC+$Neem_{0.4}$	1.36	3.85 ab	0.341	50.2 ab	17.0	203	15.7 d	91.5 d	21.6 bc
FC+$Neem_{0.8}$	1.36	3.71 b	0.311	41.9 b	17.9	180	15.2 d	86.0 d	25.8 a
Sat-NI	1.48	3.67 b	0.355	62.9 ab	16.4	202	17.0 cd	116.7 c	7.4 h
Sat+Nitrapyrin	1.43	3.56 b	0.337	67.5 a	15.9	207	16.5 cd	122.9 bc	12.8 fg
Sat+$Neem_{0.2}$	1.50	3.73 b	0.312	60.8 ab	16.7	205	15.2 d	121.7 bc	17.4 de
Sat+$Neem_{0.4}$	1.44	3.73 b	0.326	55.2 ab	17.6	217	17.8 b-d	119.0 c	22.2 ab
Sat+$Neem_{0.8}$	1.44	4.08 a	0.358	59.6 ab	17.8	230	15.9 d	119.0 c	23.4 ab
Fld-NI	1.34	3.81 ab	0.356	56.9 ab	15.3	191	24.5 ab	145.0 ab	9.9 gh
Fld+Nitrapyrin	1.32	3.79 ab	0.340	53.8 ab	18.1	195	23.9 ab	144.6 ab	14.4 d-f
Fld+$Neem_{0.2}$	1.27	3.75 ab	0.292	62.1 ab	19.7	188	23.0 a-c	135.5 a-c	13.5 e-g
Fld+$Neem_{0.4}$	1.30	3.81 ab	0.347	66.0 a	16.0	211	27.0 a	143.5 ab	14.4 d-f
Fld+$Neem_{0.8}$	1.35	3.83 ab	0.303	57.9 ab	16.1	197	25.3 a	148.3 a	18.2 cd
p-value	0.079	0.007	0.604	0.026	0.988	0.054	< 0.001	< 0.001	< 0.001
F-test	ns	**	ns	*	ns	ns	***	***	***
CV (%)	5.87	3.01	14.09	13.9	27.98	8.58	12.26	6.9	7.7

*** $p \leq 0.001$; ns = not significantly different
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +$Neem_{0.2}$, +$Neem_{0.4}$, and +$Neem_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 *g* of neem leaf dry weight per 100 *g* soil; L = Grain length; W = Grain width; ER = Elongation ratio; EI = Elongation index
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

concentration, hindering the translocation of the assimilates to grains. The harvest index, indicating rice partitioning ability, decreased in lower-moisture soils (Table 6). The result corresponded with Du et al. (2022), showing appropriate N promotes assimilate translocation, increasing soluble sugar and starch accumulation in grains. Rice in lower-moisture soils might have therefore received excess N, decreasing partitioning ability.

In addition, the higher tillering but lower grain yield could be mainly owing to a photosynthesis-respiration imbalance incurred by dense rice canopies that shaded lower-positioned leaves, diminishing their photosynthetic efficiency. Due to the higher biomass, higher respiratory rates resulted in decreased carbohydrate accumulation in the rice plants, leading to a carbohydrate shortage and, consequently, a decrease in assimilate translocation to grains (Fukai and Wade, 2021).

Moreover, the lowered soil moisture, exerting delayed plant growth and an extended time to harvest rice—as shown in the delayed progress in the double sigmoid curve of plant height (Fig. 3G-I) and the delayed harvest of 138 and 151 days for the saturated and field capacity soils compared to 130 days of the flooded soil—was associated with elevated soil N levels. The delayed rice development in drier soils corresponded to higher concentrations of NH_4^+-N (Fig. 1A-C) and NO_3^--N (Fig. 1D-F). Mengel and Kirkby (2001, pp. 286) suggested that N could delay plant maturity, due to its promotion of gibberellin production (Brückner and Blechschmidt, 1991) and enhancement of cytokinin metabolism and translocation (Takei et al., 2001). These hormonal enhancements contributed to delayed plant maturity (Mengel and Kirkby, 2001, , pp. 270–272). Furthermore, elevated N levels in the rice plants led to decreased abscisic acid synthesis (Yang, Zhang, Wang, Zhu, and Liu, 2001), further delaying rice maturity and deteriorating pre-stored biomass remobilization to grains (Yang, Zhang, Wang, Zhu, and Wang, 2001).

The results of tissue elemental contents further revealed that the decrease in rice yield in saturated soil was attributed to a Ca deficiency in the rice plants. The critical Ca deficiency in rice was determined to be 3.2 g kg^{-1} (Reuter et al., 1997), and it is worth noting that the Ca content in rice plants grown in soils at field capacity in the current was generally below 3.2 g

Table 6
Rice yield and yield components as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	Panicle number (Panicle pot^{-1})	Grain number (Grain panicle^{-1})	Grain wt (g pot^{-1}) §	1000-grain wt (g)	HI (%)
FC-NI	11.2 a-c ‡	77.6 g	24.9 d	21.5 b	35.7 f
FC+Nitrapyrin	10.7 a-c	70.9 g	15.0 f	11.9 d	21.5 i
FC+Neem$_{0.2}$	11.8 a	77.5 g	18.9 e	15.7 c	26.3 h
FC+Neem$_{0.4}$	11.9 a	81.0 g	21.1 e	17.8 c	30.1 g
FC+Neem$_{0.8}$	12.1 a	73.8 g	21.4 e	18.1 c	29.9 gh
Sat-NI	10.2 a-c	119.4 de	36.8 b	23.1 ab	41.8 b-e
Sat+Nitrapyrin	10.5 a-c	108.0 ef	36.1 bc	22.7 ab	43.2 b-d
Sat+Neem$_{0.2}$	11.4 ab	100.9 f	37.2 ab	23.0 ab	40.4 c-e
Sat+Neem$_{0.4}$	11.1 a-c	106.4 f	36.3 bc	22.0 ab	39.8 de
Sat+Neem$_{0.8}$	11.4 ab	103.5 f	33.3 c	22.9 ab	39.5 e
Fld-NI	9.4 bc	144.0 ab	35.9 bc	22.7 ab	43.8 bc
Fld+Nitrapyrin	9.3 bc	141.3 ab	34.3 bc	22.6 ab	41.5 b-e
Fld+Neem$_{0.2}$	9.8 a-c	132.5 bc	35.9 bc	22.0 ab	42.7 b-e
Fld+Neem$_{0.4}$	8.8 c	146.2 a	40.3 a	24.1 a	48.0 a
Fld+Neem$_{0.8}$	10.0 a-c	127.3 cd	36.2 bc	23.5 ab	44.4 ab
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
F-test	***	***	***	***	***
CV (%)	7.5	3.59	3.54	4.18	3.29

*** $p \leq 0.001$
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +Neem$_{0.2}$, +Neem$_{0.4}$, and +Neem$_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 g of neem leaf dry weight per 100 g soil
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

kg^{-1} (Table 4). This was because rice's Ca uptake was hindered by high K and Mg availabilities, a phenomenon referred to as the antagonistic effect of K and Mg on Ca uptake (Mengel and Kirkby, 2001). It was validated by the increased levels of K and Mg in rice plants as soil moisture contents decreased, while Ca concentrations decreased with lowered soil moisture. However, soil Mg concentrations (Table 5) contrasted with tissue Mg contents (Table 4), as soil Mg concentration decreased with lowered soil moisture. Given that this study was a pot experiment, the decrease in soil Mg concentration might have resulted from rice uptake.

A further research question emerged whether using neem leaf extract in soils with lowered moisture contents would enhance rice yield. The current study found that neem leaf extract decreased rice yields whenever soil moisture was lowered. Neem leaf extract repressed number of grains in each panicle and 1000-grain weight in well-hydrated soil (Table 6). As neem leaf extract was added to soil approaching field capacity, rice yield decreased even more (Table 6). This could be because neem leaf extract is less effective in moister soils. Moreover, it was the most possibly that the depression of rice yield through the neem extract application was potentially attributed to NH_4^+ toxicity due to the simultaneous effects on stimulating urea hydrolysis and inhibiting nitrification, which was obviously found in lettuce (Sriraj et al., 2022). This hypothesis, however, needs further investigation.

Rice quality was examined upon lowering soil moisture and applying neem leaf extract. The study showed that these factors did not affect rice's physical properties, including unmilled, milled, and cooked grains. No influence was seen on chemical quality, notably amylose and amylopectin levels (Table 7). According to Jondhale et al. (2015), genetics play a major effect on rice's physical qualities.

4. Conclusions

The results of this study clearly demonstrated that lowering soil moisture contents amplified soil N availability, thereby enhancing the vegetative growth of rice. Nonetheless, this

Table 7
Rice grain's physical and chemical qualities as influenced by different soil moisture levels and nitrification inhibitors

Treatment †	Grain shape									Elongation of cooked grain		Amylose	Amylopectin
	Unmilled grain			Milled grain			Cooked grain						
	L (mm)	W (mm)	L/W	L (mm)	W (mm)	L/W	L (mm)	W (mm)	L/W	ER	EI	(%)	(%)
FC-NI	11.0	2.56	4.32	8.12	2.17	3.75	9.25	2.35	3.93	1.14	1.05	0.192 a-c §	99.808 b-d
FC+Nitrapyrin	10.6	2.46	4.31	7.95	2.15	3.69	9.04	2.26	3.99	1.14	1.08	0.190 bc	99.810 bc
FC+Neem$_{0.2}$	10.9	2.54	4.29	8.17	2.21	3.70	8.34	2.23	3.73	1.02	1.01	0.207 a-c	99.793 b-d
FC+Neem$_{0.4}$	10.7	2.48	4.31	8.05	2.18	3.69	8.72	2.29	3.81	1.08	1.03	0.195 a-c	99.805 b-d
FC+Neem$_{0.8}$	10.8	2.44	4.42	7.91	2.16	3.67	8.41	2.32	3.63	1.06	0.99	0.147 d	99.853 a
Sat-NI	10.9	2.59	4.24	7.95	2.12	3.74	8.50	2.39	3.57	1.07	0.95	0.202 a-c	99.798 b-d
Sat +Nitrapyrin	10.8	2.56	4.24	8.18	2.21	3.70	8.58	2.33	3.70	1.05	1.00	0.207 a-c	99.793 b-d
Sat +Neem$_{0.2}$	10.9	2.63	4.15	7.91	2.17	3.65	8.67	2.43	3.58	1.10	0.98	0.221 a	99.779 d
Sat +Neem$_{0.4}$	10.7	2.53	4.24	7.96	2.16	3.68	8.86	2.33	3.80	1.11	1.03	0.211 a-c	99.789 b-d
Sat +Neem$_{0.8}$	11.0	2.59	4.25	8.23	2.20	3.74	8.85	2.39	3.70	1.08	0.99	0.199 a-c	99.801 b-d
Fld-NI	10.9	2.54	4.29	7.98	2.16	3.70	9.07	2.27	4.00	1.14	1.08	0.196 a-c	99.804 b-d
Fld+Nitrapyrin	10.5	2.56	4.10	7.98	2.15	3.71	8.67	2.24	3.88	1.09	1.04	0.183 c	99.817 b
Fld+Neem$_{0.2}$	10.9	2.65	4.09	8.16	2.26	3.60	8.48	2.25	3.78	1.04	1.05	0.219 ab	99.781 cd
Fld+Neem$_{0.4}$	10.8	2.52	4.28	8.08	2.16	3.74	9.03	2.30	3.93	1.12	1.05	0.195 a-c	99.805 b-d
Fld+Neem$_{0.8}$	10.9	2.62	4.17	8.01	2.14	3.74	8.98	2.35	3.83	1.12	1.03	0.207 a-c	99.793 b-d
p-value	0.349	0.359	0.855	0.493	0.412	0.850	0.390	0.369	0.309	0.188	0.616	< 0.001	< 0.001
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	***
CV (%)	2.16	3.85	4.65	2.3	2.67	2.46	5.15	4.14	5.78	4.94	6.81	5.23	0.01

*** $p \leq 0.001$; ns = not significantly different
† FC = Soil at the field capacity; Sat =Saturated soil, Fld = Flooded soil; -NI = Untreated nitrification inhibitor; +Nitrapyrin =Nitrapyrin; +Neem$_{0.2}$, +Neem$_{0.4}$, and +Neem$_{0.8}$= Neem leaf extract at the dose rates of 0.2, 0.4, and 0.8 g of neem leaf dry weight per 100 g soil; L = Grain length; W = Grain width; ER = Elongation ratio; EI = Elongation index
‡ The similar letters following the values of each treatment within the same column show not statistically significant differences at $p < 0.05$ (Tukey's Honest Significant Test)

alteration incurred a postponement in the days required for rice harvesting and led to a diminished rice yield.

Instead of enhancing rice grain yield, neem leaf extract led to a decrease in yield. This deleterious effect on the rice yield was more pronounced when the soil was drier. This was attributed to the neem leaf extract's ability to raise soil NH_4^+ concentration by simultaneously stimulating urea hydrolysis and inhibiting nitrification, which could potentially result in NH_4^+ toxicity in rice, which warrants further investigation. As for the rice quality, the effects of soil moisture contents and neem leaf extract were not demonstrated.

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