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Long Term Effect of Residue Management, Nitrification and Urease Inhibitor on Non-target Soil Bacterial Community in Rice–Wheat and Maize–Wheat Cropping Systems

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ABSTRACT

An investigation was carried out from November, 2020 to April, 2021 at the Indian Agricultural Research Institute, New Delhi, employing a split-split plot layout with two cropping systems (rice-wheat and maize-wheat), four long-term crop residue management strategies including burning (CRB), removal (CRR), incorporation (CRI), and biochar (BC), and two nitrogen management: neem-coated urea (NCU) and Urea+dual (urease+nitrification) inhibitor (UUINI). Soil DNA was extracted and quantified for 16S bacteria, 16S archaea, nifH, ureC and anammox abundances using quantitative PCR. Additionally, Soil samples were analysed for available nitrogen (urea, NH_4^+, NO_3^-) and water-soluble carbon. Rice-wheat rotations favoured higher 16S bacterial abundance while maize-wheat elevated 16S archaea. Notably, CRI and BC exhibited higher bacterial abundance compared to CRR and CRB, while minimal impact was noticed for archaea. The nifH gene abundance was influenced by all treatments along with their interactions. UreC gene copies exhibited a direct relationship with 16S archaea and an inverse relationship with 16S bacteria; UUINI showed a higher abundance of ureC under CRI and BC in both cropping systems. Moreover, anammox abundance correlated positively with NH_4^+ and NO_3^- but negatively with unhydrolyzed urea, indicating the inhibitory effect of UUINI. These findings underscore the complex relationships among inhibitors, residue management, cropping systems and soil microbial communities, emphasizing the need for tailored approaches to optimise nutrient cycling and soil health in agricultural systems.

KEYWORDS: Anammox, biochar, nitrification inhibitors, nifH, ureC, urease inhibitors

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1. INTRODUCTION

Urease inhibitors (UI) and nitrification inhibitors (NI) are frequently employed to plumate the release of ammonia (NH_3) and nitrous oxide (N_2O) gases from agricultural soils vis-à-vis enhancing N use efficiency (Chakraborty et al., 2023; Kaur et al., 2017; Sutton et al., 2011). However, there has been limited investigation, mostly confined to short-term laboratory experiments into the unintended adverse impacts of NI and UI on non-target soil bacterial communities. Few studies observed an insignificant influence of UIs on soil biomass, and abundance of N cycling communities (Duff et al., 2022; Xi et al., 2017; Fu et al., 2020). Others documented improved ureC genes and alteration in prokaryotic community structure (Li et al., 2019; Fu et al., 2020). Generalizing the effect of NIs on non-target bacteria is more difficult as they vary greatly in composition and mechanism of action (Florio et al., 2016; Zisi et al., 2020). For instance, incubation in the presence of 3,4-Dimethylpyrazole phosphate (DMPP) showed a reduction in bacterial 16S rRNA abundance (Florio et al., 2016), while no significant effects were observed using Dicyandiamide (DCD) (Dong et al., 2021; Guo et al. 2014). Maienza et al. (2014) observed a decline in bacterial growth due to DMPP in the first four weeks. Conversely, in an incubation study, Florio et al. (2014) reported that DMPP applied with organic fertilizer inhibited bacterial transcriptional activity but not 16S rRNA abundance. Recently, Liu et al. (2024) found that after two weeks of incubation, the abundance of the nifH gene decreased when DCD was applied, while it remained largely unchanged with DMPP. Availability of N shows contrasting effects on nifH abundance including stimulation (Chinnadurai et al., 2014a; Poly et al., 2001) or inhibition (Silveria et al., 2021; Tan et al., 2003). A reduction in ureC gene copies was recorded with NI N-(n-butyl) thiophosphoric triamide (NBPT) in two soils with varying pH levels (Fan et al. 2018). Earlier studies indicate ammonium, nitrate, and nitrite in soil have a direct relation with the activity of anammox bacteria (Li et al., 2016; Shen et al., 2017), however, many times this effect is masked owing to the large effect of management practices (Sun et al., 2022; Zhu et al., 2015). Therefore, more comprehensive assessments are warranted before embracing inhibitor technologies on a broader scale.

Moreover, the burning of crop stubbles in fields is a widespread challenge in the predominant rice-wheat and maize-wheat cropping systems of northern and northwestern India, leading to severe air pollution (Khedwal et al., 2023; Ravindra et al., 2023). Some viable alternatives to burning could be the removal of crop residue from the field, incorporation of crop residue in the field during land preparation and converting the residue into biochar. Contrasting reports are available on the impact of organic amendments on 16S rRNA abundance in comparison to fertilizer, including significant improvement (Chinnadurai et al., 2014b) to little effect (Chinnadurai et al., 2014a; Liu et al., 2021). Some studies reported that organic amendments substantially augment the nifH gene abundance (Chinnadurai et al., 2014a; Groß et al., 2022), and ureC gene copies in cotton (Adeli et al., 2019; Brooks et al., 2018). Several reports suggest that the diversity, and abundance of the anammox population were altered by organic amendments (Sun et al., 2022; Nie et al., 2019; Yang et al., 2015; Zhang et al., 2022). Therefore, the present study was undertaken to evaluate the influence of urease and nitrification inhibitors, along with four different long-term (10-year) residue management approaches, on the abundance of non-target microbial communities (bacterial 16S, archaeal 16S, nifH, ureC, and anammox) within rice-wheat and maize-wheat cropping systems.

2. MATERIALS AND METHODS

2.1. Experimental site and soil

The trial was conducted at the research farm of the Indian Agricultural Research Institute, situated in New Delhi, India. Located within the Indo-Gangetic alluvial plain at coordinates 28˚40' N and 77˚12' E, has an elevation of 228 meters, with an average annual rainfall of 750 mm. The soil composition is characterized as alluvial sandy loam, with a slight alkaline reaction (pH 8.1) and a cation exchange capacity of 7.1 c mol (p^+) kg^{-1}. The soil is classified as Typic Haplustept according to Purakayastha et al. (2008).

2.2. Experimental design and crop management

The experiment has been continuing since 2010 with two cropping systems (main plot) and four residue management treatments (subplot) in a 4×2 split-plot design with a 5×3.5 m^2 plot size. The study encompassed two distinct cropping systems: rice (*Oryza sativa*) followed by wheat (*Triticum aestivum*), and maize (*Zea mays*) followed by wheat. Within each cropping system, four different crop residue management practices were implemented: residue removal (CRR), burning (CRB), incorporation (CRI), and biochar application (BC). The winter crop, wheat, was cultivated from November to March. The rice and maize were cultivated as wet-season (monsoon) crops from July to October. Field preparation for aerobic crops involved multiple ploughings using a tractor-mounted rotavator and cultivator in early November, and July for wheat and maize, respectively. For the anaerobic rice, puddling was carried out in July by repeated ploughing, ensuring optimum moisture content. The rice nursery was prepared in a separate place and the seedlings were transplanted to the experimental plots after 28 days. In CRR, the crop

residues were completely removed from the field. In CRB treatment, residues were burnt in situ in the experimental plots, and incorporated into the soil up to 15 cm depth before sowing. For CRI treatment, residues were chopped into small pieces, and for BC, residue were converted into biochar in a low-cost pyrolysis kiln at approximately 400°C, and incorporated into the soil before sowing.

In the *rabi* (November, 2020–April, 2021) season of the 2020-21, cropping year, two nitrogen managements i.e., neem-coated urea (NCU, neem oil 350 ppm) and urea coated with a dual inhibitor (UUINI: UI, NBPT+NI, DMPSA) were superimposed on the existing residue experiment in a 2×4×2 split-split-plot design with three replicates. Each plot was divided into two halves; the cropping systems were considered as the main plots, residue management practices as subplots and nitrogen management treatments were applied in the sub-subplots. The fertilizer dose for rice and wheat was 120-26.8-50 kg ha^{-1} N, P and K and for maize was 180-35.7-66.4 kg ha^{-1} N, P and K. For all crops, the basal dose of N (50%), and the entire dose of P, K was applied after germination; the second N dose (remaining half) was given as a top-dressing, 40 days after the basal N application. Both crops were irrigated as needed, with 4-5 irrigations in wheat, 1-2 in maize, and on every alternate day in rice.

2.3. Measurement of urea, NH_4^+ and NO_3^-, and water soluble carbon

Approximately 100 g of soil was collected from each plot, corresponding to the peak N_2O emission period (3 days after basal fertilizer application) and stored at -20°C. These samples were analyzed for unhydrolyzed urea, NO_3^-, NH_4^+ concentrations, and water soluble carbon (WSC). For extraction of NO_3^-, NH_4^+ and unhydrolyzed urea, 10 g soil was taken in a 250 ml conical flask, and 100 ml 2 M KCl containing 5 ppm PMA was added (Keeney, and Nelson. 1982). These flasks were shaken at 180 rpm for 1 hour, filtered, and immediately transferred to a -20°C refrigerator. The concentration of NH_4^+ and NO_3^- were measured by an auto-analyzer. The colorimetric method was used for estimating urea concentration based on the formation of imidazolone compound with diacetyl monoxime which gives a red complex with thio-semicarbazide at high temperatures (Mulvenna and Savidge, 1992). For measuring water soluble carbon (WSC), 5 g soil samples were extracted with 25 ml double distilled water, shaken for 1 hour at 200 rpm, centrifuged at 13000 rpm for 30 minutes at 4°C, and then filtered through a 0.45 µm glass filter (Agnelli et al., 2016). 10 ml filtrate was shifted to a 75 ml diffusion tube and heated in a water bath until dryness and the carbon content was quantified using the dichromate digestion method (Snyder and Trofymow, 1984).

2.4. Extraction of soil DNA and quantification of gene copies

For each treatment, around 50 g of soil from 0-15 cm was collected, precisely 3 days after the application of basal fertilizer. These samples were swiftly transported to the laboratory in an ice-filled container and preserved at -20°C until further processing. The extraction of total soil DNA was performed using Nucleopore® G-DNA soil kits (Genetix, New Delhi, India), adhering to the provided guidelines. The extracted DNA concentration was gauged using a Nanodrop 3300 spectrofluorometer. Quantitative PCR (qPCR) assays employing Fast SYBR® Green dye were executed on a Lightcycler 96 Real-Time PCR System. Each reaction comprised 4 µl of the extracted soil DNA as a template, supplemented with gene-specific primers, SYBR Green I Master Mix (10 µl), bovine serum albumin (1 µl of 10 mg ml^{-1} strength), and nuclease-free water (5 µl) in a 20 µl reaction volume. The quantification of gene copies for bacterial and archaeal 16S rRNA, nifH, ureC, and anammox was conducted using qPCR (Table 1). Fluorescence detection took place during the extension step of each cycle, with the specificity of amplification confirmed through melt-curve analysis. Calibration curves were constructed utilizing 10-fold serial dilutions of plasmids containing the targeted genes, and CT values were graphed against gene copies.

2.5. Data management and statistical analysis

To assess the homogeneity of variance and normality, the Levene test and Shapiro-Wilks tests were conducted, respectively, utilizing the 'car' package in R Studio version 4.2.1 (Anonymous, 2022). The 2×4×2 split-split-plot ANOVA was run in the "agricolae" package of R studio for chemical parameters (urea, NH_4^+, NO_3^-, and WSC) and gene copies with cropping system as the main effect, residue managements as sub-effect and nitrogen managements as a sub-sub effect. The separation of mean was done by Tukey's HSD method at probability P=0.05 using the same package. The diagrams were generated using Microsoft Excel 2019 and Pearson's correlation matrix was generated using the "metan" package of RStudio.

3. RESULTS AND DISCUSSION

3.1. Effect of cropping systems, residue and nitrogen management on soil chemical parameters

Significant effects of residue management practices and nitrogen management were discernible in unhydrolyzed urea-N concentration (p<0.01) (Table 2). The concentration of urea-N ranged from 4.70–12.9 mg kg^{-1} in rice-wheat and 4.54–12.9 mg kg^{-1} in maize-wheat (Table 3). In both rice-wheat and maize-wheat, the highest unhydrolyzed urea concentration under UUINI fertilizer practice was recorded in BC, followed by CRR, CRB and the lowest in CRI,

Table 1: Primer details and thermal cycling conditions for PCR reactions

Target gene	Primer	Primer sequence (5' to 3')	Reaction conditions	Amplicon size (bp)	Reference
Bacterial-16S	Eub338 Eub518	ACT CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GG	10 min of 95°C, 35 cycles consisting of 10s at 95°C, 15s at 53°C, and 40s at 72°C	200	Feirer et al., 2005
Archaeal 16S	ARCH-mix1369F Prok-1541R	1:1 mixture of Arch1-1369F: CGGTGAATACGTCCCTGC, and Arch2-1369F: CGGTGAATATGCCCCTGC AAGGAGGTGATCC RGCCGCA	10 min of 95°C, 40 cycles consisting of 10s at 95°C, 15s at 55°C, and 25s at 72°C	172	Suzuki et al., 2000
nifH	nifH-F nifH-R	TGCGAYCCSAARGCBGACTC ATSGCCATCATYTCRCCGGA	10 min of 95°C, 40 cycles consisting of 10s at 95°C, 15s at 55°C, and 40s at 72°C	227	Poly et al., 2001
ureC	ureC-1F ureC-2R	AAGMTSCACGAGGACTGGGG AGRTGGTGGCASACCATSAGCAT	10 min of 95°C, 40 cycles consisting of 10s at 95°C, 15s at 56°C, and 40s at 72°C	300	Koper et al., 2004
Anammox	Amx-818F Amx-1066R	ATGGGCACTMRGTAGAGGGGTTT AACGTCTCACGACACGAGCTG	10 min of 95°C, 40 cycles consisting of 10s at 95°C, 15s at 58°C, and 25s at 72°C	248	Yang et al., 2020

Table 2: Results of analysis of variation to examine urea-N, NH_4^+-N, NO_3^--N, and water soluble carbon (WSC) as affected by residue management, nitrogen treatment, cropping systems (CS) and their interactions

SOV	Df	Urea	NH_4^+-N	NO_3^--N	WSC
CS	1	0.87	0.145	0.012*	0.413
Residue	3	<0.01*	0.669	0.095	<0.01*
CS: Residue	3	0.967	0.945	0.941	0.098
Nitrogen	1	<0.01*	<0.01*	<0.01*	0.92
Nitrogen: CS	1	0.978	0.71	0.792	0.93
Nitrogen: residue	3	0.718	0.89	0.894	0.624
Nitrogen: CS: residue	3	0.994	0.918	0.922	0.659

The statistical significance was determined at the probability level of 0.05; *significant (p<0.05)

however, under NCU nitrogen management, all the residue management practices were at par with each other. Under every residue management practice, urea concentration under UUINI was almost two times higher than NCU, regardless of the cropping system. The hydrolysis of urea by the urease enzyme produces alkalinity (HCO_3^-, and OH^-), and enhances soil pH, leading to high NH_3 volatilization (Mariano et al., 2020; Sommer et al., 2004). However, urease inhibitors like NBPT can chelate with Ni^{+2}, a cofactor of urease enzyme, and thus can hinder hydrolysis of urea to NH_4^+ for 1–2 weeks (Cantarella et al., 2018; Chakraborty et al., 2023).

The NH_4^+-N concentration showed a significant (p<0.01) response to nitrogen managements, while residue management, cropping system, and all the interactions were found to be non-significant (p>0.05, Table 2). The NH_4^+-N concentration ranged from 23.4–31.4 mg kg^{-1} and 26.1–33.9 mg kg^{-1} in rice-wheat and maize-wheat, respectively (Table 3). In both cropping systems, all the residue management yielded statistically similar NH_4^+-N concentrations under the same nitrogen management. However, significantly higher concentrations were recorded under NCU compared to UUINI. In our investigation, NCU consistently exhibited higher NH_4^+ concentration compared to UUINI, which is in similar line with prior reports involving NBPT and DCD (Fu et al., 2020). The lower NH_4^+ levels in UUINI could be attributed to delayed urea hydrolysis due to NBPT (Wang et al., 2020). Additionally, the slightly elevated NH_4^+ concentration observed in maize-wheat systems compared to rice-wheat systems might be attributed to the higher residual nitrogen levels in maize, stemming from the application of higher nitrogen fertilization doses (180 kg ha^{-1} in maize versus 120 kg ha^{-1} in rice).

Table 3: Effect of cropping system, residue management and fertilizer treatments on urea-N, NH_4^+-N, NO_3^--N and Water Soluble Carbon (WSC) (mg kg^{-1})

	Urea-N		NH_4^+-N		NO_3^--N		WSC	
	NCU	UUINI	NCU	UUINI	NCU	UUINI	NCU	UUINI
Rice-wheat								
CRR	5.20^{c}	11.2^{ab}	30.3^{abcd}	24.3^{fg}	22.3^{bcde}	18.6^{e}	84.3^{h}	85.9^{h}
CRB	5.90^{c}	11.6^{ab}	31.0^{abc}	24.7^{fg}	23.8^{abcd}	20.6^{de}	94.8^{efg}	96.3^{def}
CRI	4.70^{c}	10.5^{b}	31.4^{ab}	25.3^{efg}	24.6^{abcd}	21.1^{cde}	110^{ab}	107^{abc}
BC	6.10^{c}	12.9^{a}	29.7^{abcde}	23.4^{g}	23.2^{bcd}	18.2^{e}	102^{bcde}	104^{abcd}
Maize-wheat								
CRR	5.47^{c}	11.3^{ab}	33.7^{a}	28.0^{bcdefg}	25.1^{abc}	21.4^{bcde}	86.9^{gh}	90.0^{fgh}
CRB	5.80^{c}	11.6^{ab}	33.9^{a}	26.7^{cdefg}	25.6^{ab}	22.3^{bcde}	99.5^{cde}	102^{cde}
CRI	4.54^{c}	10.5^{b}	32.2^{ab}	28.3^{bcdef}	28.2^{a}	22.6^{bcde}	111^{a}	111^{a}
BC	6.36^{c}	12.9^{a}	31.6^{ab}	26.1^{defg}	24.8^{abcd}	20.5^{de}	100^{cde}	95.2^{defg}

CRR, CRB, CRI and BC refer to residue removal, burning, incorporation and biochar, respectively. NCU is neem-coated urea, and UUINI refers to urea+dual (*urease+nitrification*) inhibitor. The data followed by no common letters are significant according to Tukey's honestly significant difference (HSD) (p<0.05)

For NO_3^--N concentration, significant effects of cropping systems and nitrogen managements were found (p<0.05), while residue managements showed marginally significant (0.05<p<0.1) effect (Table 2). Compared to NH_4^+-N, slightly lower values of NO_3^--N concentration were recorded, ranging between 18.2 to 24.6 mg kg^{-1} in rice-wheat and 20.5 to 28.2 mg kg^{-1} in maize-wheat (Table 3). The residue management practices within the same cropping system and identical nitrogen management recorded statistically similar NO_3^--N contents. The average NO_3^--N within the NCU treatments were around 20% higher than UUINI in both cropping systems. Cropping systems significantly influenced soil NO_3^--N content, driven by varying preferences for nitrogen uptake, soil conditions, microbial activity, and residue decomposition rates (Yang et al., 2020). Additionally, the marginal effect of residue management underscores the role of organic matter decomposition in modulating nitrate levels by affecting carbon input, microbial activity and rate of organic matter decomposition, which in turn affect nitrogen mineralization and nitrification processes (Vitali et al., 2024). Higher NO_3^--N concentrations in NCU compared to UUINI suggest that the nitrification inhibitor DMPSA might have reduced nitrifying activity by hindering the activity of ammonium monooxygenase enzyme more effectively than neem (Akiyama et al., 2010, Chakraborty et al., 2023).

The WSC content elucidated a significant response to residue management, while cropping systems, nitrogen management, and all associated interactions were determined to be non-significant (Table 2). Within the rice-wheat system, CRI and BC recorded the maximum WSC content followed by CRB and CRR, irrespective of the nitrogen management. Likewise in the maize-wheat system, CRI recorded the highest WSC followed by BC and CRB. WSC, the most labile C pool serves as an immediate C source for soil microbes, making it a sensitive indicator of soil quality (Verma and Mathur, 2009). Its high mobility significantly influences nutrient cycling and distribution in ecosystems (Fujii et al., 2009; Gmach et al., 2019). Our findings suggest that CRI had the greatest positive influence on WSC, followed by BC and CRB. This is likely due to increased microbial activity and organic matter decomposition facilitated by higher levels of residue-derived carbon in CRI (Bhattacharyya et al., 2012; Raheem et al., 2019; Alam et al., 2018). Similarly, fresh biochar, with its readily available carbon substrates, has been associated with higher water-soluble carbon content in previous studies (Angst et al., 2014; Janz et al., 2022).

3.2. Effect of cropping systems, residue and nitrogen management on total bacterial and total archaeal abundance

Cropping systems, residue management and interaction between nitrogen treatments and residue management had a significant impact on the 16S bacterial gene copies (p<0.01; Table 4), while interaction between cropping system and residue was marginally significant (0.1<p<0.05; Table 4). Overall, 16S bacterial abundance was higher in rice-wheat compared to maize-wheat (Figure 1A and 1B). The interaction between residue and nitrogen management exerted a mutual influence. Within the rice-wheat system, CRI and BC exhibited statistically similar values, as did CRR and CRB under NCU nitrogen management (Figure 1A). Conversely, under UUINI nitrogen management,

Table 4: Results of analysis of variation to examine bacterial, archaeal 16S, nifH, ureC, and anammox gene copies as affected by residue managements, nitrogen treatments, cropping systems and their interactions

SOV	Df	Bacterial 16S	Archeal 16S	nifH	ureC	anammox
CS	1	<0.01*	<0.01*	0.018*	<0.01*	0.346
Residue	3	<0.01*	<0.01*	0.015*	0.252	0.025*
CS: Residue	3	0.094	0.097	<0.01*	<0.01*	0.544
Nitrogen	1	0.102	0.152	<0.01*	0.334	<0.01*
Nitrogen: CS	1	0.621	0.39	<0.01*	0.036*	0.608
Nitrogen: residue	3	0.033*	0.462	<0.01*	<0.01*	0.407
Nitrogen: CS: residue	3	0.07	0.082	<0.01*	<0.01*	0.481

The statistical significance was determined at the probability level of 0.05; *significant (p<0.05)

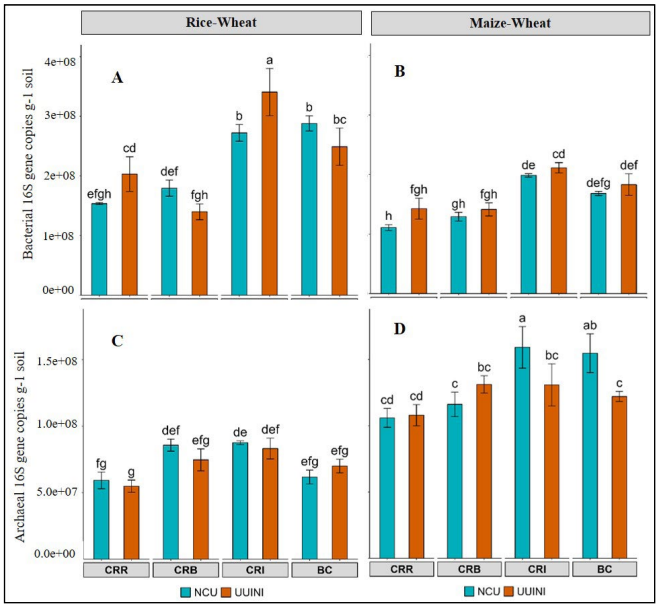

Figure 1: Effect of cropping systems, and different residue and nitrogen managements on 16S bacterial abundance (A-B), and 16S archaeal abundance (C-D). CRR, CRB, CRI and BC refer to residue removal, burning, incorporation and biochar, respectively. NCU is neem-coated urea, and UUINI refers to *urea+dual* (*urease+nitrification*) inhibitor. Different lowercase letters indicate significant differences (p<0.05) based on Tukey's honestly significant difference (HSD)

CRI demonstrated the highest abundance of bacterial 16S, followed by BC, CRR, and CRB. Meanwhile, in the maize-wheat system, CRI recorded the highest 16S bacterial abundance under NCU nitrogen treatment, but it was statistically similar to BC, while the lowest abundance was found in CRR (Figure 1B). Conversely, under UUINI nitrogen management, CRI, and BC recorded the highest abundance, while CRR and CRB recorded the lowest. In the rice-wheat system, under CRR and CRI residue practices, UUINI recorded higher bacterial 16S gene copies over NCU, while the effects of both nitrogen managements were similar under CRB and BC residue practices (Figure 1A). Conversely, in the maize-wheat system, NCU and UUINI exhibited similar outcomes across all residue practices (Figure 1B). The 16S bacterial abundance was negatively correlated with 16S archaea (r=-0.37, p<0.01) and ureC (r=-0.31, p<0.05) (Figure 3). Soil bacterial abundance and diversity are linked to myriad soil factors including pH, electrical conductivity (EC), soil organic carbon content, and nutrient availability, all of which are influenced by cropping systems, residue management practices, nitrogen management and their interactions (Bragina et al., 2012; Jangid et al., 2008; Reilly et al., 2013). The impact of cropping systems on bacterial abundance is well-documented, attributed to variations in root exudates that affect microbial activity and bacterial composition differently (Navarro-Noya et al., 2013; Xuan et al., 2012). Additionally, contrasting growing conditions in rice-wheat and maize-wheat systems may contribute differently to bacterial abundance as observed in our study. Organic amendments have shown mixed effects on bacterial 16S rRNA abundance in previous studies ranging from improvement (Chinnadurai et al., 2014a) to little effect (Chinnadurai et al., 2014b; Orr et al., 2012). In our investigation, consistently higher bacterial abundance was recorded in CRI and BC, which aligns with findings from previous studies suggesting higher bacterial abundance due to the increased availability of organic material as a carbon source for energy and cell synthesis (Navarro-Noya et al., 2013; Kuramae et al., 2012). Similarly, biochar amendment significantly increased the abundance of bacterial 16S rRNA in rice paddy (Wang et al., 2021). Furthermore, the availability of nutrients, whether in organic or inorganic form, has been shown to have a significant positive influence on bacterial abundance (Esperschutz et al., 2007; Orr et al., 2011).

In the rice-wheat rotation, the ratio of bacterial to archaeal 16S sequences was approximately 3:1, whereas, in the maize-wheat rotation, this ratio was reduced to approximately 5:4 (Figure 1). The abundance of archaeal

16S exhibited significant effects of both cropping system and residue management (p<0.01; Table 4), with a marginally significant effect (0.1<p<0.05; Table 4) observed for the interaction between cropping system and residue. Contrary to bacterial 16S abundance, higher values of archaeal 16S were recorded under maize-wheat compared to rice-wheat (Figure 1C and 1D). In the rice-wheat system, variation among residue management practices was largely negligible, except for higher 16S archaeal abundance observed in CRI compared to CRR under both NCU and UUINI nitrogen managements (Figure 1C). Concurrently, in the maize-wheat system, CRI and BC exhibited higher 16S archaeal abundance compared to CRR and CRB under NCU nitrogen management. Conversely, under UUINI, the residue management practices recorded statistically similar values (Figure 1D). In the rice-wheat system, no significant difference between NCU and UUINI nitrogen management was observed under any residue management practices, however, in maize-wheat, NCU registered higher 16S archaeal abundance under CRI and BC residue management compared to UUINI (Figure 1C and 1D). The archaeal abundance was positively correlated with ureC (r=0.62, p<0.001), NH_4^+ and NO_3^- (r=0.34, p<0.05) and negatively correlated with 16S bacteria (r=-0.37, p<0.01) (Figure 3). The abundance of 16S archaea was lower than 16S bacteria, which corroborates the previous findings (Fisher et al., 2017; Jiang et al., 2023). Conflicting reports are available on the influence of cropping sequence on the abundance and diversity of bacterial and archaeal communities. Few studies hypothesized that the impact of crops on soil microbial communities arises chiefly from the freshly produced organic compounds, so the residual effect of previous crops is negligible (Gregory, 2006; Souza et al., 2023; Vezzani et al., 2018). Conversely, leftover plant residues from previous crops may contribute to a residual effect (Babin et al., 2019). Moreover, root exudates and rhizodeposits released by plant roots harbour a unique microbial community and can have a residual impact on the next crop (Bakker et al., 2018; Lapsansky et al., 2016). Babin et al. (2019) found that the effect of tillage practice on 16S archaeal abundance in winter wheat was negligible when wheat was preceded by rapeseed, while significant differences were observed when wheat followed maize. Our findings support the later findings. Contrasting reports are available on the effect of CRI and BC on archaeal 16S gene copies including no effect (Wang et al., 2021; Paungfoo-Lonhienne et al., 2021), and increase (Paungfoo-Lonhienne et al., 2017). In our study, 16S archaea were mostly unaffected by nitrogen management, except UUINI showed a lower abundance than NCU under CRI and BC in maize-wheat. This is consistent with the mixed findings on the impact of UIs and NIs on 16S archaea including no effects of NBPT or DCD (Fu et al., 2020), inhibitory effects of NBPT and DMPP (Castellano-Hinojosa et al., 2019), and variable response of NBPT and PPD on different incubation interval (Jiang et al., 2023). The reduction in archaeal abundance under UUINI could be due to a reduction in NH_4^+ and NO_3^- availability (Castellano-Hinojosa et al., 2019; Shi et al., 2017), significant correlations among archaeal 16S, NH_4^+ and NO_3^- were also noticed in our study (Figure 3)

3.3. Effect of cropping systems, residue and nitrogen managements on the abundance of nifH, ureC, and anammox genes

Cropping systems, residue and nitrogen management, along with all their interactions, significantly influenced the abundance of the nifH gene abundance (p<0.05; Table 4). Within the rice-wheat cropping system and NCU nitrogen management, nifH gene copies were significantly lower in CRI, compared to CRR, CRB, and BC, with the latter three showing similar values. Conversely, under UUINI nitrogen management, CRI demonstrated the highest value, while CRR had the lowest, and CRB showed levels comparable to both CRI and BC (Figure 2A). Additionally,

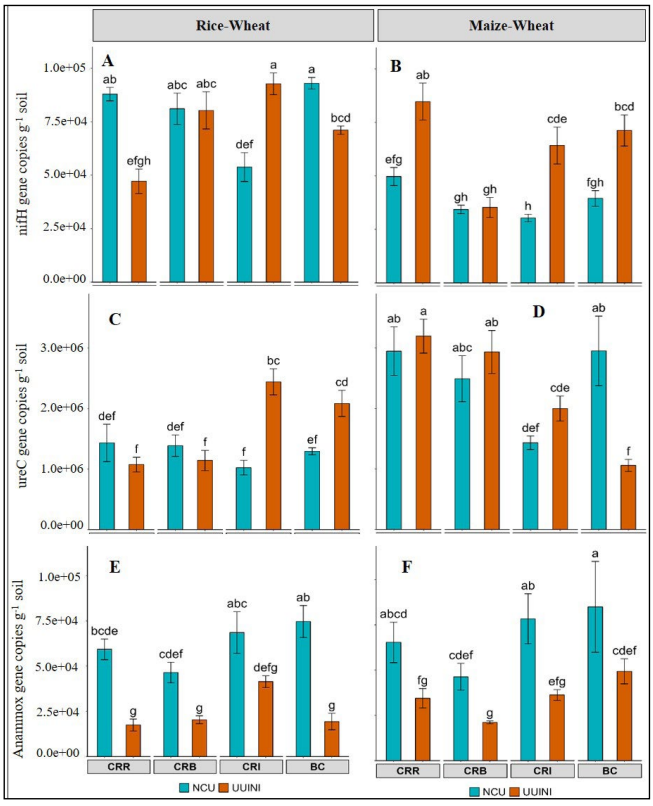

Figure 2: Effect of cropping systems, and different residue and nitrogen managements on abundances of nifH (A-B), ureC (C-D), and anammox (E-F) genes. CRR, CRB, CRI and BC refer to residue removal, burning, incorporation and biochar, respectively. NCU is neem-coated urea, and UUINI refers to urea+dual (*urease+nitrification*) inhibitor. Different lowercase letters indicate significant differences (p<0.05) based on Tukey's honestly significant difference (HSD)

in the maize-wheat system, differences among residue treatments were insignificant except for CRR exhibiting higher values than CRI. Conversely, under UUINI nitrogen management, the trend in residue management followed CRR>BC=CRI>CRB (Figure 2B). In the rice-wheat system, NCU exhibited higher values than UUINI in CRR and BC, whereas in CRI, UUINI surpassed NCU, and in CRB, both were equal (Figure 2A). In maize-wheat, UUINI showed higher values than NCU in CRR, CRI, and BC, while NCU and UUINI were comparable in CRB (Figure 2B). The abundance of nifH gene copies did not exhibit any significant correlation with either chemical parameters or other genes (Figure 3). Nitrogen fixation is a complex and energy-intensive process reliant on easily degradable C sources (Burgmann et al., 2003; Burgmann et al., 2005; Hayden et al., 2010). The abundance of nifH-containing bacteria is controlled by numerous soil chemical, microbiological properties, climate, crop and nutrient management factors (Hayden et al., 2010; Orr et al., 2012). We also observed that the abundance of bacteria containing the nifH gene was significantly affected by crops, residue management, nitrogen management and their interactions which affect soil properties differently. Fertilizer application and organic amendments favour the nifH abundance substantially (Chinnadurai et al., 2014b). No particular trend was observed as the effect of one factor was modified by another. However, contrasting reports are available on the effect of available N on nifH gene abundance including

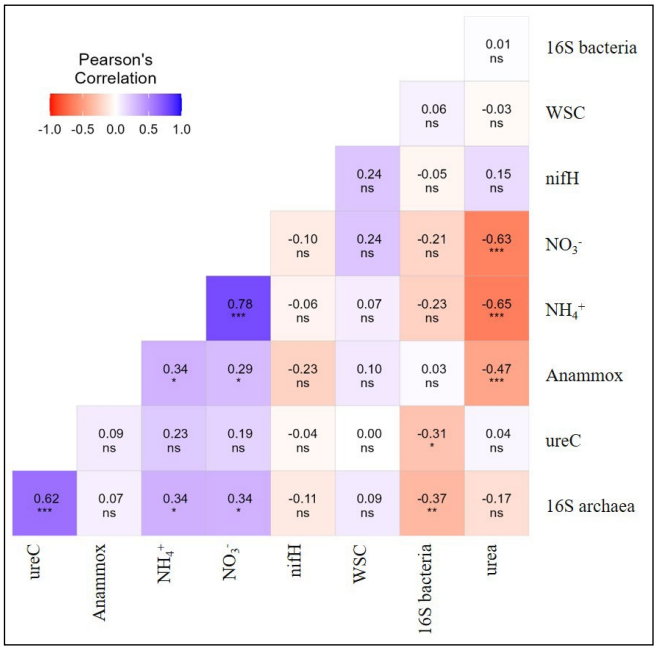

Figure 3: Correlation among bacterial gene abundances, and soil chemical properties. The Pearson correlation coefficients are presented with the following significance levels, NS: non-significant ($p \geq 0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

stimulation (Chinnadurai et al., 2014a; Poly et al., 2001) or inhibition (Tan et al., 2003). The lower nifH population in NCU compared to UUINI under CRI in the rice-wheat system and CRR, CRI, and BC in maize-wheat is likely due to higher N availability in the former that has an inhibitory effect on nifH abundance.

The abundance of the ureC gene was significantly influenced by cropping systems and the interactions among cropping systems and residue; cropping systems and nitrogen management; nitrogen management and residue; as well as three-way interaction among cropping systems, residue, and nitrogen management ($p<0.05$; Table 4). In the rice-wheat cropping system with NCU nitrogen management, all residue management practices yielded statistically similar values of ureC gene abundance, while under UUINI nitrogen management, CRI and BC exhibited the highest values (Figure 2C).

Meanwhile, in the maize-wheat system and under NCU nitrogen treatment, CRR, CRB, and BC showed higher values compared to CRI, whereas conversely, under UUINI treatment, CRR and CRB demonstrated higher ureC abundances than CRI and BC (Figure 2D). In the rice-wheat system, UUINI displayed higher values than NCU in CRI and BC residue practices, whereas, in CRR and CRB, both were equal (Figure 2C). On the other hand, in maize-wheat, NCU and UUINI showed comparable values, except for BC, where NCU exhibited higher ureC abundance than UUINI (Figure 2D). Furthermore, ureC gene copies were positively correlated with total 16S archaea (r=0.62, $p<0.001$) while showed small negative correlation with total 16S bacteria (r=-0.31, $p<0.05$) (Figure 3). Reports suggest that manuring and cover crop use increase ureC gene copies (Adeli et al., 2019; Brooks et al., 2018) due to improved biochemical feedback from healthy crops. However, our findings reveal a more complex effect due to interactions among cropping systems, residue, and nitrogen management. In the rice-wheat system, all residue management was statistically similar under NCU, but CRI and BC showed the highest values under UUINI. Conversely, in maize-wheat, CRI had the lowest ureC abundance under NCU, while CRR and CRB had higher values than CRI and BC under UUINI, illustrating the complex and mutual influence of treatments on urea hydrolysis. Contrasting findings exist regarding the effect of UIs and NIs on ureC abundance. For example, Luchibia et al. (2020) found no significant effect of various inhibitor combinations on ureC abundance initially, but after 28 days, inhibitors recorded lower abundance compared to urea in some soils while showing similar copy numbers in others. However, there is currently no information available on the effect of DMPSA on ureC. We observed higher ureC abundance in UUINI treatment under CRI and BC residue

management in rice-wheat, while under BC in maize-wheat, UUINI had lower values. In all other residue management, NCU and UUINI were comparable. This contrasts with the work of Fan et al. (2018), who found a reduction in ureC abundance with NBPT but supports the work of Jiang et al. (2023) who observed UI NBPT and PPD increase ureC abundance and intracellular urease activity.

Regarding anammox abundance, only residue and nitrogen management showed significant effects (p<0.05, Table 4), while cropping systems and all the interaction effects were deemed non-significant (p>0.05, Table 4). In the rice-wheat cropping system, under NCU nitrogen management, residue management treatments had minor impact, with BC exhibiting higher values compared to CRB, whereas under UUINI, all residue management practices showed statistically similar outcomes (Figure 2E). Similarly, in the maize-wheat system, residue management differences were minimal, except for BC, which demonstrated higher values than CRB under both nitrogen management conditions (Figure 2F). Across both cropping systems, UUINI consistently displayed lower anammox abundance compared to NCU under all residue practices (Figure 2E and 2F). Furthermore, anammox abundance exhibited positive correlations with NH_4^+ (r=0.34) and NO_3^- concentrations (r=0.29), and a negative correlation with unhydrolyzed urea concentration (r=-0.47, p<0.001) (Fig. 3). Anammox, a recently discovered N removal pathway serves as an alternative to heterotrophic denitrification, and can significantly contribute to N loss in agricultural ecosystems (Nie et al., 2019; Long et al., 2013). High N inputs into agricultural soils can stimulate the growth of anammox bacteria, leading to increased activity and diversity of these bacteria and subsequent N loss (Nie et al., 2019; Shen et al., 2015; Shen et al., 2017). Previous studies have reported that 3.2–9.6% of total N emissions in rice-wheat systems occur through anammox mechanism (Gu et al., 2017). Anammox activity relies on the coexistence of both oxidized (NO_3^-) and reduced (NH_4^+) species of nitrogen (Li et al., 2016; Shen et al., 2017; Sun et al., 2022). We observed a significant positive correlation of anammox abundance, NH_4^+ and NO_3^- concentrations. This correlation could explain the lower anammox abundance observed under dual inhibitor treatments, which inhibits the release of NH_4^+ and NO_3^- due to the activity of urease inhibitor (NBPT) and nitrification inhibitor (DMPSA), respectively. Residue management had a minor impact on anammox abundance, with BC showing higher abundance than CRB. This contrasts with previous reports indicating higher abundance and diversity of anammox bacteria under the incorporation of rice straw and green manure (Nie et al., 2018; Sun et al., 2022; Xu et al., 2021; Zhou et al., 2020).

4. CONCLUSION

The study highlights the intricate interplay between inhibitors, residue management, cropping systems, and their effect on non-target microbial abundance by modulating C and N availability. Rice-wheat rotation favoured higher 16S bacterial abundance, while maize-wheat favoured higher 16S archaea. CRI and BC elevated 16S bacteria over CRR and CRB. The nifH gene showed complex interactions. Dual inhibitor increased ureC abundance in CRI and BC. Moreover, anammox abundance correlated positively with NH_4^+ and NO_3^- concentrations and was inhibited by UUINI.

5. ACKNOWLEDGEMENT

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6. REFERENCE

Adeli, A., Brooks, J.P., Read, J.J., Shankle, M.W., Feng, G., Jenkins, J.N., 2019. Poultry litter and cover crop integration into no-till cotton on upland soil. Agronomy Journal 111(4), 2097–2107.

Agnelli, A., Massaccesi, L., De Feudis, M., Cocco, S., Courchesne, F., Corti, G., 2016. Holm oak (*Quercus ilex* L.) rhizosphere affects limestone-derived soil under a multi-centennial forest. Plant and soil 400, 297–314. https://doi.org/10.1007/s11104-015-2732-x.

Akiyama, H., Yan, X., Yagi, K., 2010. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis. Global Change Biology 16(6), 1837–1846. https://doi.org/10.1111/j.1365-2486.2009.02031.x.

Alam, M.K., Bell, R.W., Haque, M.E., Kader, M.A., 2018. Minimal soil disturbance and increased residue retention increase soil carbon in rice-based cropping systems on the eastern gangetic plain. Soil and Tillage Research 183, 28–41. https://doi.org/10.1016/J.STILL.2018.05.009.

Angst, T.E., Six, J., Reay, D.S., Sohi, S.P., 2014. Impact of pine chip biochar on trace greenhouse gas emissions and soil nutrient dynamics in an annual ryegrass system in California. Agriculture Ecosystems

Environment 191, 17–26. https://doi.org/10.1016/j.agee.2014.03.009.

Anonymous, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: https://www.r-project.org. Accessed on: 9th May, 2024.

Babin, D., Deubel, A., Jacquiod, S., Sørensen, S.J., Geistlinger, J., Grosch, R., Smalla, K., 2019. Impact of long-term agricultural management practices on soil prokaryotic communities. Soil Biology and Biochemistry 129, 17–28.

Bakker, P.A., Pieterse, C.M., de Jonge, R., Berendsen, R.L., 2018. The soil-borne legacy. Cell 172(6), 1178–1180.

Bhattacharyya, P., Roy, K.S., Neogi, S., Adhya, T.K., Rao, K.S., Manna, M.C., 2012. Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice. Soil and Tillage Research 124, 119–130. https://doi.org/10.1016/j.still.2012.05.015.

Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., Berg, G., 2012. Sphagnum mosses harbour highly specific bacterial diversity during their whole lifecycle. The ISME Journal 6(4), 802–813.

Brooks, J.P., Tewolde, H., Adeli, A., Shankle, M.W., Way, T.R., Smith, R.K., Pepper, I.L., 2018. Effects of subsurface banding and broadcast of poultry litter and cover crop on soil microbial populations. Journal of Environmental Quality 47(3), 427–435. doi:10.2134/jeq2017.09.0382.

Bürgmann, H., Meier, S., Bunge, M., Widmer, F., Zeyer, J., 2005. Effects of model root exudates on structure and activity of a soil diazotroph community. Environmental Microbiology 7(11), 1711–1724.

Bürgmann, H., Widmer, F., Sigler, W.V., Zeyer, J., 2003. mRNA extraction and reverse transcription-PCR protocol for detection of nifH gene expression by Azotobacter vinelandii in soil. Applied and Environmental Microbiology 69(4), 1928–1935.

Cantarella, H., Otto, R., Soares, J.R., de Brito Silva, A.G., 2018. Agronomic efficiency of NBPT as a urease inhibitor: A review. Journal of Advanced Research 13, 19–27. https://doi.org/10.1016/j.jare.2018.05.008.

Castellano-Hinojosa, A., Gonzalez-Lopez, J., Vallejo, A., Bedmar, E.J., 2020. Effect of urease and nitrification inhibitors on ammonia volatilization and abundance of N-cycling genes in an agricultural soil. Journal of Plant Nutrition and Soil Science 183(1), 99–109.

Chakraborty, R., Purakayastha, T.J., Pendall, E., Dey, S., Jain, N., Kumar, S., 2023. Nitrification and urease inhibitors mitigate global warming potential and ammonia volatilization from urea in rice-wheat system in India: A field to lab experiment. Science of The Total Environment 898, 165479. https://doi.org/10.1016/j.scitotenv.2023.165479.

Chinnadurai, C., Gopalaswamy, G., Balachandar, D., 2014a. Long term effects of nutrient management regimes on abundance of bacterial genes and soil biochemical processes for fertility sustainability in a semi-arid tropical Alfisol. Geoderma 232, 563–572.

Chinnadurai, C., Gopalaswamy, G., Balachandar, D., 2014b. Impact of long-term organic and inorganic nutrient managements on the biological properties and eubacterial community diversity of the Indian semi-arid Alfisol. Archives of Agronomy and Soil Science 60(4), 531–548.

Dong, D., Yang, W., Sun, H., Kong, S., Xu, H., 2021. Nitrous oxide emissions in response to long-term application of the nitrification inhibitor DMPP in an acidic luvisol. Applied Soil Ecology 159, 103861.

Duff, A.M., Forrestal, P., Ikoyi, I., Brennan, F., 2022. Assessing the long-term impact of urease and nitrification inhibitor use on microbial community composition, diversity and function in grassland soil. Soil Biology and Biochemistry 170, 108709.

Esperschutz, J., Gattinger, A., Mader, P., Schloter, M., Fließbach, A., 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiology Ecology 61(1), 26–37.

Fan, X., Yin, C., Yan, G., Cui, P., Shen, Q., Wang, Q., Liang, Y., 2018. The contrasting effects of N-(n-butyl) thiophosphoric triamide (NBPT) on N_2O emissions in arable soils differing in pH are underlain by complex microbial mechanisms. Science of The Total Environment 642, 155–167.

Fierer, N., Jackson, J. A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71(7), 4117–4120.

Fisher, K.A., Yarwood, S.A., James, B.R., 2017. Soil urease activity and bacterial ureC gene copy numbers: Effect of pH. Geoderma 285, 1–8.

Florio, A., Clark, I.M., Hirsch, P.R., Jhurreea, D., Benedetti, A., 2014. Effects of the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) on abundance and activity of ammonia oxidizers in soil. Biology and Fertility of Soils 50, 795–807.

Florio, A., Maienza, A., Dell'Abate, M.T., Stazi, S.R., Benedetti, A., 2016. Changes in the activity and abundance of the soil microbial community in response to the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP). Journal of Soils and Sediments 16, 2687–2697.

Fu, Q., Abadie, M., Blaud, A., Carswell, A., Misselbrook,

T.H., Clark, I.M., Hirsch, P.R., 2020. Effects of urease and nitrification inhibitors on soil N, nitrifier abundance and activity in a sandy loam soil. Biology and Fertility of Soils 56, 185–194.

Fujii, K., Uemura, M., Hayakawa, C., Funakawa, S., Kosaki, T., Ohta, S., 2009. Fluxes of dissolved organic carbon in two tropical forest ecosystems of East Kalimantan, Indonesia. Geoderma 152(1–2), 127–136.

Gmach, M.R., Cherubin, M.R., Kaiser, K., Cerri, C.E.P., 2019. Processes that influence dissolved organic matter in the soil: a review. Scientia Agricola, 77, e20180164.

Gregory, P.J., 2006. Roots, rhizosphere and soil: the route to a better understanding of soil science? European Journal of Soil Science 57(1), 2–12. https://doi.org/10.1111/j.1365- 2389.2005.00778.x.

Groß, C., Hossen, S., Hartmann, H., Noll, M., Borken, W., 2022. Biological nitrogen fixation and nifH gene abundance in deadwood of 13 different tree species. Biogeochemistry 161(3), 353–371.

Gu, C., Zhou, H., Zhang, Q., Zhao, Y., Di, H., Liang, Y., 2017. Effects of various fertilization regimes on abundance and activity of anaerobic ammonium oxidation bacteria in rice-wheat cropping systems in China. Science of the Total Environment 599, 1064–1072.

Guo, Y.J., Di, H.J., Cameron, K.C., Li, B., 2014. Effect of application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate, and ammonia-oxidizing bacteria and archaea growth in a grazed pasture soil: an incubation study. Journal of Soils and Sediments 14, 897–903.

Hayden, H.L., Drake, J., Imhof, M., Oxley, A.P., Norng, S., Mele, P.M., 2010. The abundance of nitrogen cycle genes amoA and nifH depends on land-uses and soil types in South-Eastern Australia. Soil Biology and Biochemistry 42(10), 1774–1783.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology and Biochemistry 40(11), 2843–2853.

Janz, B., Havermann, F., Lashermes, G., Zuazo, P., Engelsberger, F., Torabi, S.M., Butterbach-Bahl, K., 2022. Effects of crop residue incorporation and properties on combined soil gaseous N2O, NO, and NH3 emissions-A laboratory-based measurement approach. Science of the Total Environment 807, 151051.https://doi.org/10.1016/j.scitotenv.2021.151051.

Jiang, D., Jiang, N., Jiang, H., Chen, L., 2023. Urease inhibitors increased soil ureC gene abundance and intracellular urease activity when extracellular urease activity was inhibited. Geoderma 430, 116295.

Kaur, J., Kaur, J., 2017. Appropriate nitrogen schedules in direct seeded rice (*Oryza sativa* L.). International Journal of Bio-resource and Stress Management 8(2), 242–246.

Keeney, D.R., Nelson, D.W., 1983. Nitrogen-inorganic forms. Methods of soil analysis: Part 2 Chemical and Microbiological Properties 9, 643–698.

Khedwal, R.S., Chaudhary, A., Sindhu, V.K., Yadav, D.B., Kumar, N., Chhokar, R.S., Dahiya, S., 2023. Challenges and technological interventions in rice–wheat system for resilient food–water–energy-environment nexus in North-western Indo-gangetic plains: a review. Cereal Research Communications 51(4), 785–807.

Koper, T.E., El-Sheikh, A.F., Norton, J.M., Klotz, M.G., 2004. Urease-encoding genes in ammonia-oxidizing bacteria. Applied and Environmental Microbiology 70(4), 2342–2348.

Kuramae, E.E., Yergeau, E., Wong, L.C., Pijl, A.S., Van Veen, J.A., Kowalchuk, G.A., 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiology Ecology 79(1), 12–24.

Lapsansky, E.R., Milroy, A.M., Andales, M.J., Vivanco, J.M., 2016. Soil memory as a potential mechanism for encouraging sustainable plant health and productivity. Current Opinion in Biotechnology 38, 137–142.

Li, H., Yang, X., Weng, B., Su, J., Nie, S.A., Gilbert, J.A., Zhu, Y.G., 2016. The phenological stage of rice growth determines anaerobic ammonium oxidation activity in rhizosphere soil. Soil Biology and Biochemistry 100, 59–65.

Li, W., Xiao, Q., Hu, C., Liu, B., Sun, R., 2019. A comparison of the efficiency of different urease inhibitors and their effects on soil prokaryotic community in a short-term incubation experiment. Geoderma, 354, 113877.

Liu, Y., Xu, Z., Bai, S.H., Fan, H., Zuo, J., Zhang, L., Zhang, M., 2024. Non-targeted effects of nitrification inhibitors on soil free-living nitrogen fixation modified with weed management. Science of the Total Environment 912, 169005.

Liu, Z., Zhou, H., Xie, W., Yang, Z., Lv, Q., 2021. Long-term effects of maize straw return and manure on the microbial community in cinnamon soil in Northern China using 16S rRNA sequencing. PLoS One 16(4), e0249884.

Long, A., Heitman, J., Tobias, C., Philips, R., Song, B., 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. Applied and Environmental Microbiology 79(1), 168–176.

Luchibia, A.O., Suter, H., Hu, H.W., Lam, S.K., He, J.Z., 2020. Responses of ureolytic and nitrifying microbes to urease and nitrification inhibitors in selected agricultural soils in Victoria, Australia. Journal of Soils and Sediments 20, 1309–1322. https://doi.org/10.1007/s11368-020-02562-x.

Maienza, A., Baath, E., Stazi, S.R., Benedetti, A., Grego, S., Dell'Abate, M.T., 2014. Microbial dynamics after adding bovine manure effluent together with a nitrification inhibitor (3, 4 DMPP) in a microcosm experiment. Biology and Fertility of Soils 50, 869–877.

Mariano, E., de Sant Ana Filho, C.R., Bortoletto-Santos, R., Bendassolli, J.A., Trivelin, P.C., 2019. Ammonia losses following surface application of enhanced-efficiency nitrogen fertilizers and urea. Atmospheric Environment 203, 242–251.

Mulvenna, P.F., Savidge, G., 1992. A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent. Estuarine, Coastal and Shelf Science 34(5), 429–438. https://doi.org/10.1016/S0272-7714(05)80115-5.

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biology and Biochemistry 65, 86–95.

Nie, S., Zhu, G.B., Singh, B., Zhu, Y.G., 2019. Anaerobic ammonium oxidation in agricultural soils-synthesis and prospective. Environmental Pollution 244, 127–134.

Nie, S.A., Lei, X.M., Zhao, L.X., Brookes, P.C., Wang, F., Chen, C.R., Yang, W.H., Xing, S.H., 2018. Fungal communities and functions response to long-term fertilization in paddy soils. Applied Soil Ecology 130, 251–258.

Orr, C.H., James, A., Leifert, C., Cooper, J.M., Cummings, S.P., 2011. Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. Applied and Environmental Microbiology 77(3), 911–919.

Orr, C.H., Leifert, C., Cummings, S.P., Cooper, J.M., 2012. Impacts of organic and conventional crop management on diversity and activity of free-living nitrogen fixing bacteria and total bacteria are subsidiary to temporal effects. PLoS One 7(12), e52891.

Paungfoo-Lonhienne, C., Wang, W., Yeoh, Y.K., Halpin, N., 2017. Legume crop rotation suppressed nitrifying microbial community in a sugarcane cropping soil. Scientific Reports 7(1), 16707. DOI: https://doi.org/10.1038/s41598-017-17080-z.

Paungfoo-Lonhienne, C., Wang, W., Yeoh, Y.K., Reeves, S., Halpin, N., Daly, J., 2021. Effects of different rotational legume crops and residue management regimes on soil microbial properties and functions in a sugarcane farming system. Journal of Plant Nutrition and Soil Science 184(3), 398–408.

Poly, F., Monrozier, L.J., Bally, R., 2001. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. Research in Microbiology 152(1), 95–103.

Purakayastha, T.J., Rudrappa, L., Singh, D., Swarup, A., Bhadraray, S., 2008. Long-term impact of fertilizers on soil organic carbon pools and sequestration rates in maize–wheat–cowpea cropping system. Geoderma 144(1–2), 370–378. https://doi.org/10.1016/J.GEODERMA.2007.12.006

Raheem, A., Zhang, J., Huang, J., Jiang, Y., Siddik, M.A., Deng, A., Zhang, W., 2019. Greenhouse gas emissions from a rice-rice-green manure cropping system in South China. Geoderma, 353, 331–339. https://doi.org/10.1016/j. geoderma.2019.07.007.

Ravindra, K., Singh, T., Singh, V., Chintalapati, S., Beig, G., Mor, S., 2023. Understanding the influence of summer biomass burning on air quality in North India: Eight cities field campaign study. Science of The Total Environment, 861, 160361.https://doi.org/10.1016/j.scitotenv.2022.160361.

Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., Griffiths, B.S., 2013. Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. Journal of the Science of Food and Agriculture 93(15), 3700–3709.

Shen, L.D., Wu, H.S., Gao, Z.Q., Xu, X.H., Chen, T.X., Liu, S., Cheng, H.X., 2015. Occurrence and importance of anaerobic ammonium-oxidising bacteria in vegetable soils. Applied Microbiology and Biotechnology 99, 5709–5718.

Shen, L.D., Wu, H.S., Liu, X., Li, J., 2017. Vertical distribution and activity of anaerobic ammonium-oxidising bacteria in a vegetable field. Geoderma 288, 56–63.

Shi, X., Hu, H.W., Kelly, K., Chen, D., He, J.Z., Suter, H., 2017. Response of ammonia oxidizers and denitrifiers to repeated applications of a nitrification inhibitor and a urease inhibitor in two pasture soils. Journal of Soils and Sediments 17, 974–984. https://doi.org/10.1007/S11368-016-1588-X.

Silveira, R., de Mello, T.D.R.B., Sartori, M.R.S., Alves, G.S.C., de Assis Fonseca, F.C., Vizzotto, C.S., da Cunha Bustamante, M.M., 2021. Seasonal and long-term effects of nutrient additions and liming on the nifH gene in cerrado soils under native vegetation.

Iscience 24(4).

Snyder, J.D., Trofymow, J.A., 1984. A rapid accurate wet oxidation diffusion procedure for determining organic and inorganic carbon in plant and soil samples. Communications in Soil Science and Plant Analysis 15(5), 587–597. https://doi.org/10.1080/00103628409367499.

Sommer, S.G., Schjoerring, J.K., Denmead, O.T., 2004. Ammonia emission from mineral fertilizers and fertilized crops. Advances in Agronomy 82(557622), 82008–4. https://doi.org/10.1016/S0065-2113(03)82008-4.

Souza, J.P., Vezzani, F.M., Lazeris, T.S., Kaschuk, G., de Souza, E.M., Balsanelli, E., Bayer, C., 2023. Composition of soil organic matter and the structure and diversity of soil bacteria and archaea, in crop systems under no-till in a subtropical ecosystem. Soil and Tillage Research 234, 105813.

Sun, G., Sun, M., Luo, Z., Li, C., Xiao, X., Li, X., Nie, S.A., 2022. Effects of different fertilization practices on anammox activity, abundance, and community compositions in a paddy soil. Soil Ecology Letters, 1–10.

Sutton, M.A., Oenema, O., Erisman, J.W., Leip, A., Van Grinsven, H., Winiwarter, W., 2011. Too much of a good thing. Nature 472(7342), 159–161. https://doi.org/10.1038/472159a.

Suzuki, M.T., Taylor, L.T., DeLong, E.F., 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Applied and Environmental Microbiology 66(11), 4605–4614.

Tan, Z., Hurek, T., Reinhold-Hurek, B., 2003. Effect of N-fertilization, plant genotype and environmental conditions on nifH gene pools in roots of rice. Environmental Microbiology 5(10), 1009–1015.

Verma, G., Mathur, A.K., 2009. Effect of integrated nutrient management on active pools of soil organic matter under maize-wheat system of a typic haplustept. Journal of the Indian Society of Soil Science 57(3), 317–322.

Vezzani, F.M., Anderson, C., Meenken, E., Gillespie, R., Peterson, M., Beare, M.H., 2018. The importance of plants to development and maintenance of soil structure, microbial communities and ecosystem functions. Soil and Tillage Research 175, 139–149. https://doi.org/10.1016/j.still.2017.09.002.

Vitali, A., Russo, F., Moretti, B., Romani, M., Vidotto, F., Fogliatto, S., Said-Pullicino, D., 2024. Interaction between water, crop residue and fertilization management on the source-differentiated nitrogen uptake by rice. Biology and Fertility of Soils, 1–16. https://doi.org/10.1007/s00374-024-01794-0.

Wang, C., Chen, D., Shen, J., Yuan, Q., Fan, F., Wei, W., Wu, J., 2021. Biochar alters soil microbial communities and potential functions 3–4 years after amendment in a double rice cropping system. Agriculture, Ecosystems Environment 311, 107291.

Wang, H., Kobke, S., Dittert, K., 2020. Use of urease and nitrification inhibitors to reduce gaseous nitrogen emissions from fertilizers containing ammonium nitrate and urea. Global Ecology and Conservation 22, e00933. https://doi.org/10.1016/j.gecco.2020.e00933.

Xi, R., Long, X.E., Huang, S., Yao, H., 2017. pH rather than nitrification and urease inhibitors determines the community of ammonia oxidizers in a vegetable soil. Amb Express 7, 1–14.

Xu, L., Zhang, B., Wang, E., Zhu, B., Yao, M., Li, C., Li, X., 2021. Soil total organic carbon/total nitrogen ratio as a key driver deterministically shapes diazotrophic community assemblages during the succession of biological soil crusts. Soil Ecology Letters, 1–14. https://doi.org/10.1007/s42832-020-0075-x.

Xuan, D.T., Guong, V.T., Rosling, A., Alstrom, S., Chai, B., Högberg, N., 2012. Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, *Oryza sativa*. Biology and Fertility of Soils 48, 217–225.

Yang, T., Siddique, K.H., Liu, K., 2020. Cropping systems in agriculture and their impact on soil health-A review. Global Ecology and Conservation 23, e01118.

Yang, X.R., Li, H., Nie, S.A., Su, J.Q., Weng, B.S., Zhu, G.B., Zhu, Y.G., 2015. Potential contribution of anammox to nitrogen loss from paddy soils in Southern China. Applied and Environmental Microbiology 81(3), 938–947.

Zhang, Q., Lin, J.G., Kong, Z., Zhang, Y., 2022. A critical review of exogenous additives for improving the anammox process. Science of the Total Environment 833, 155074.

Zhou, G., Gao, S., Lu, Y., Liao, Y., Nie, J., Cao, W., 2020. Co-incorporation of green manure and rice straw improves rice production, soil chemical, biochemical and microbiological properties in a typical paddy field in southern China. Soil and Tillage Research 197, 104499.

Zhu, G., Wang, S., Zhou, L., Wang, Y., Zhao, S., Xia, C., Qu, J., 2015. Ubiquitous anaerobic ammonium oxidation in inland waters of China: an overlooked nitrous oxide mitigation process. Scientific Reports 5(1), 17306.

Zisi, X., Karydogianni, S., Kakabouki, I., Stavropoulos, P., Folina, A., Bilalis, D., 2020. Effects of nitrogen fertilizers with two different inhibitors (urease and nitrification) on the survival and activity of earthworms (*Octodrilus complanatus*). Journal of Elementology 25(4), 1449–1461.