

## Effect of different doses of Ajinomoto fertilizer on the dynamics of Cyanobacteria during a rice (*Oryza spp.*) growing cycle in the Korhogo Region, Côte d'Ivoire

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### Abstract

This study investigated the effects of different fertilization treatments on the dynamics of Cyanobacteria in irrigated rice fields in northern Côte d'Ivoire. Sampling was conducted in an experimental Fisher block design comprising four treatments: control (FT1), mineral fertilization (FT2), combined mineral and Ajinomoto fertilization (FT3), and Ajinomoto alone (FT4). Environmental variables and Cyanobacteria communities were monitored throughout the rice-growing cycle.

A total of 21 Cyanobacteria species belonging to 11 genera were identified, all within the class Cyanophyceae. The order Oscillatoriales was dominant. Cyanobacteria abundance was significantly higher in FT3, which also showed the highest nutrient concentrations. *Oscillatoria limosa* was the dominant taxon across all treatments. Statistical analyses revealed significant relationships between Cyanobacteria genera and environmental variables, particularly nutrients and conductivity.

These results highlight the influence of fertilization regimes on Cyanobacteria communities and suggest that combined fertilization enhances cyanobacterial development, with potential implications for sustainable rice production.

**Keywords:** Cyanobacteria; *Oryza Spp*; Ajinomoto Fertilizer; FPCA Analysis; Côte d'Ivoire

### 1. Introduction

Rice (*Oryza spp.*) is one of the world's most important staple crops, feeding nearly half of the global population and providing a critical source of calories and income for millions of smallholder farmers, particularly in developing countries (1; 2; 3). In Côte d'Ivoire, rice is the primary staple for urban and rural populations alike. However, national production meets only about 50% of domestic demand, resulting in substantial import dependency (4; 5). Lowland and rain-fed rice cultivation systems dominate the country, but these systems are highly vulnerable to climatic variability and extreme weather events, which increasingly threaten yield stability (6). Nitrogen (N) and phosphorus (P) are key limiting nutrients in rice production (7). To improve yields, chemical fertilizers such as NPK and urea are commonly applied. While effective in enhancing productivity, over-reliance on mineral fertilizers can lead to several negative consequences, including environmental pollution through nutrient leaching, accumulation of heavy metals in soil, and increased production costs for farmers (8). Furthermore, intensive chemical fertilization can disrupt microbial communities, negatively affecting soil fertility and ecosystem health over the long term. Sustainable alternatives to conventional fertilizers are therefore essential for promoting environmentally friendly and cost-effective rice

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production. Cyanobacteria, also known as blue-green algae, are photosynthetic microorganisms capable of fixing atmospheric nitrogen, thereby improving soil fertility naturally. They can also enhance soil structure, increase water retention, and produce growth-promoting substances for plants (9; 10, 11). In addition, Cyanobacteria contribute to nutrient cycling and primary productivity in flooded rice ecosystems, making them valuable components of integrated soil fertility management strategies (11).

Previous studies in Côte d'Ivoire have documented the diversity of Cyanobacteria in aquatic and soil environments (12; 13). However, their ecological roles and dynamics within rice paddy systems remain largely unexplored. In particular, how different fertilization strategies, including the use of conventional mineral fertilizers and novel formulations such as Ajinomoto fertilizer affect Cyanobacteria community composition and abundance is not well understood. Understanding these dynamics is crucial for designing sustainable rice production practices that enhance soil fertility, minimize environmental impacts, and maintain ecosystem services.

This study aims to investigate the dynamics of cyanobacterial communities in irrigated rice fields in the Korhogo region of northern Côte d'Ivoire. Specifically, the objectives are to:

(i) Identify the Cyanobacteria taxa present in rice paddies, (ii) Assess the effects of different fertilization treatments on Cyanobacteria abundance, diversity, and community structure, and (iii) Examine the relationships between Cyanobacteria taxa and environmental variables.

The results are expected to provide insights into how fertilization regimes influence Cyanobacteria communities and to inform strategies for integrating these microorganisms into sustainable rice production systems in West Africa.

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## 2. Material and methods

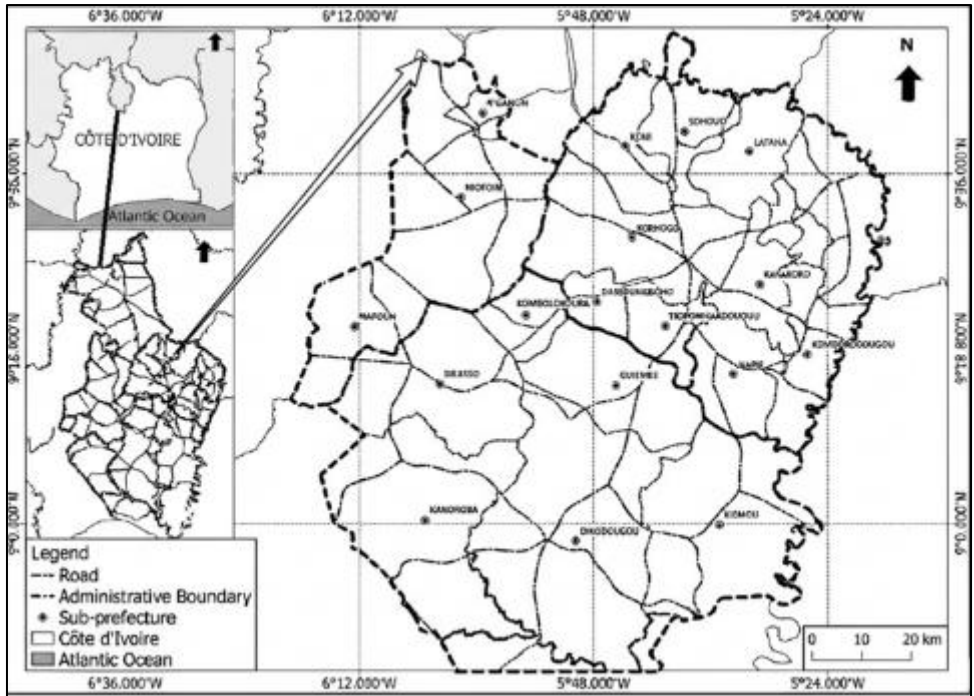
### 2.1. Study area and sampling sites

The experiment was conducted in Nahoualakaha, in the municipality of Tioroniaradougou (Figure 1), in the department of Korhogo region (Northern Côte d'Ivoire) using a Fisher block design (Figure 2) with three replicates and four treatments:

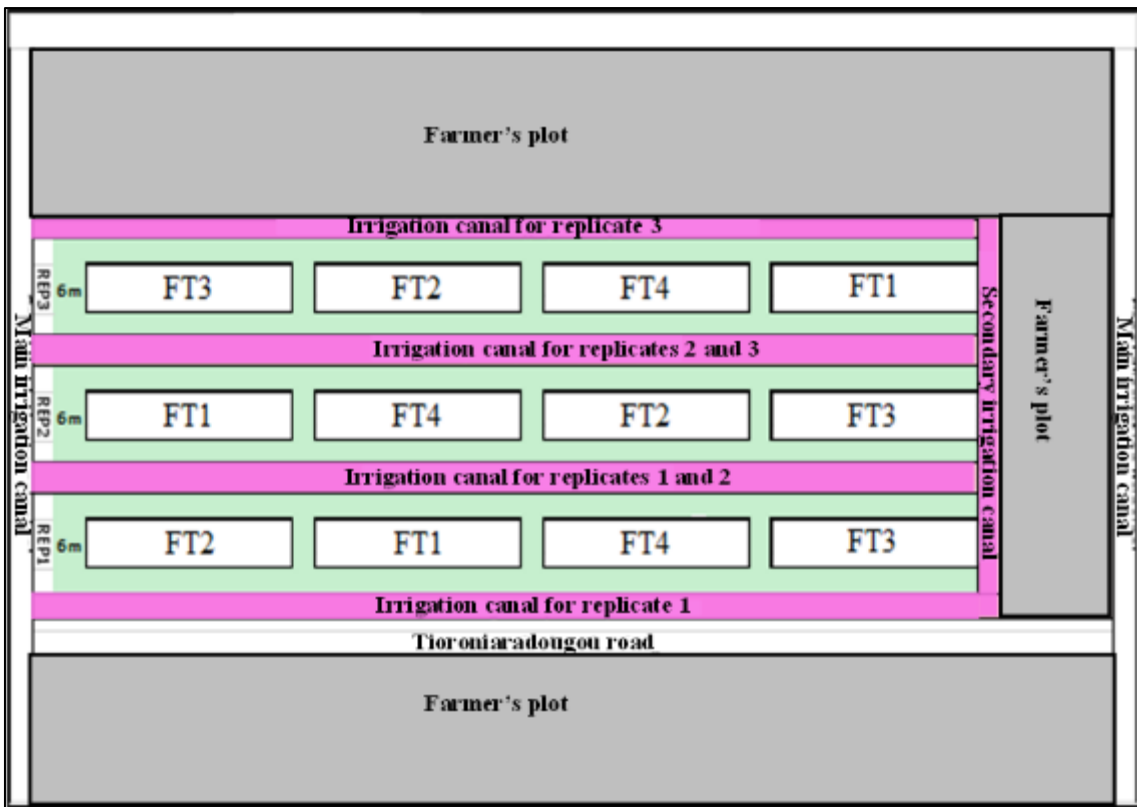
- FT1: Control (no fertilization)
- FT2: NPK + urea
- FT3: NPK + Ajinomoto fertilizer
- FT4: Ajinomoto fertilizer only

Treatments were applied during transplanting, tillering, and panicle initiation stages.

Sampling was conducted on three dates: September, November, and December 2020



**Figure 1** Map indicating the study site in Tioroniaradougou, Korhogo Department, northern Côte d'Ivoire, where the rice fertilization experiment was conducted



**Figure 2** Experimental design showing the Fisher block layout used for the fertilization treatments in the rice paddies in the Tioroniaradougou experimental site

## 2.2. Environmental variables

Environmental variables measured included water temperature, conductivity, pH, dissolved oxygen (DO) and nutrient concentrations (orthophosphate and nitrate).

Water samples were collected *in situ*. Temperature and conductivity were measured using a conductivity meter (WTW COND 340-i model), while pH was measured using a portable pH meter (HANNA Hi 991001 model). Dissolved oxygen (DO) was measured using a portable oximeter (HANNA Hi 9146 model) to prevent atmospheric contamination.

Nutrient concentrations were determined using a HACH DR 2010 spectrophotometer following standard methods (14). Orthophosphate and nitrate were analyzed using standard colorimetric procedures.

## 2.3. Cyanobacteria sampling

They were made in each block using a 10 ml syringe inserted vertically into the submergence water and soil. The sample, consisting of the first centimeter of soil and the submerged water, is transferred to the pill box by pushing the core out of the tube using the piston. The exercise is repeated 20 times per block to obtain the maximum number of taxa in each pill box. These samples are immediately fixed with commercial formalin at a final concentration of 5%.

Cyanobacteria were identified to the lowest possible taxonomic level under a Zeiss microscope at 400× magnification using standard keys and manuals, with names verified in AlgaeBase (15). Cells were counted following Utermöhl (16) with an inverted microscope (200 × 400×) according to NF EN 15204 (17).

## 2.4. Data analysis

The frequency of occurrence (FO) of each specie was calculated using the following formula:  $FO = (Ni/Nts) \times 100$ ; with  $Ni$  = number of samples containing a given specie  $i$  and  $Nts$  = total number of samples collected. The FO was used to classify taxa following Dajoz (18):  $FO > 50$  (common taxa);  $25 < FO < 50$  (occasional taxa);  $FO \leq 25$  (rare taxa).

Differences in environmental variables concentrations, Cyanobacteria abundances among traitement were assessed using the non-parametric Kruskal–Wallis test, followed by pairwise comparisons with the Mann–Whitney test. Data normality was verified using the Shapiro test ( $p > 0.05$  for all sites). All analyses were performed in RStudio (R version 3.1.3; 19) with a significance level set at  $p < 0.05$ .

A Focused Principal Component Analysis (FPCA) (20) was carried to expresses the main relations between Cyanobacteria genres and environmental variables. Six environmental parameters were returned for the analysis. The analyses were computed with the package `psy` (21) for the RStudio.

## 3. Results

### 3.1. Environmental variables

Table 1 presents the median, minimum, and maximum values of the environmental variables recorded across treatments. The maximum (40.1 °C) and minimum (36.8 °C) water temperatures were recorded in treatment FT1. The highest pH value (7.9) was observed in treatment FT4, while the lowest (6.2) was recorded in FT1. The lowest dissolved oxygen concentration (1.59 mg L<sup>-1</sup>) was recorded in FT2, whereas the highest value (4.9 mg L<sup>-1</sup>) was observed in FT3. Water temperature, pH, and dissolved oxygen did not vary significantly among treatments (Kruskal–Walli's test,  $p > 0.05$ ).

The lowest conductivity value (201 μS cm<sup>-1</sup>) was recorded in treatment FT4, while the highest (512 μS cm<sup>-1</sup>) was observed in FT3. Conductivity was significantly lower in FT4 compared to the other treatments (Mann–Whitney test,  $p < 0.05$ ).

Regarding nutrient availability, the highest concentrations of orthophosphate (PO<sub>4</sub><sup>3-</sup>: 2.5 mg L<sup>-1</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>: 3.14 mg L<sup>-1</sup>) were recorded in treatment FT3. In contrast, the lowest nitrate concentration (0.99 mg L<sup>-1</sup>) and orthophosphate concentration (1.0 mg L<sup>-1</sup>) were observed in treatments FT2 and FT4, respectively. Nutrient concentrations were significantly higher in FT3 than in the other treatments (Mann–Whitney test,  $p < 0.05$ ).

**Table 1** Minimum, maximum and median values of the environmental variables measured at different treatment (FT1, FT2, FT3, FT4) in the Tioroniadougou experimental site

TRETEMENT		T(°C)	pH	OD (mg L <sup>-1</sup> )	CND (μS cm <sup>-1</sup> )	PO <sup>3-</sup> <sub>4</sub> (mg L <sup>-1</sup> )	NO <sup>3-</sup> (mg L <sup>-1</sup> )
FT1	Max	40.1	7.4	4.1	421	2.1	2.2
	Med	37.8a	7 a	3.4 a	412 a	1.96 ab	2.1 b
	Min	36.8	6.2	3.1	391	1.96	1.9
FT2	Max	39.6	7.1	3.54	389	1.4	1.96
	Med	37.9 a	6.8 a	2.54 a	362 a	1.3 ab	1.95 a
	Min	37	6.4	1.59	362	1	1.6
FT3	Max	39.4	7.8	4.9	512	2.5	3.14
	Med	38 a	7.2 a	3.84 a	501 a	2.26 a	3.11 b
	Min	38	7.2	3.12	456	2.26	2.6
FT4	Max	39.1	7.9	4.71	219	0.99	1.1
	Med	38.5 a	7.2 a	4.6 a	215 b	0.98 b	1.2 a
	Min	37.5	6.8	4.1	201	0.95	0.99

T= temperature; CND= conductivity; OD= dissolved oxygen ; NO<sup>3-</sup> = nitrate ; PO<sup>3-</sup><sub>4</sub> = phosphorus; Min = minimum; Max = maximum; Med = median; median values with a letter (a and b) in common do not differ significantly (Kruskal-Wallis test; p > 0.05)

### 3.2. Taxonomic richness and composition

**Table 2** Taxonomic composition and the frequency of occurrence (FO) of Cyanobacteria recorded in the Tioroniadougou experimental site (Côte d'Ivoire): \* = rare taxa; \*\* = occasional taxa ; \*\*\* = common taxa.

TAXON	TREATMENT			
	FT1	FT2	FT3	FT4
<b>Cyanophyceae</b>				
<b>Chroococcales</b>				
<b>Chroococcaceae</b>				
<i>Chroococcus</i> sp.	*			
<b>Microcystaceae</b>				
<i>Microcystis aeruginosa</i> (Kützing) Lemmermann	***	*		***
<i>Microcystis robusta</i> (Clark) Nägeli	*	*		
<i>Microcystis wesenbergii</i> (Komárek) Komárek	**		*	*
<b>Nostocales</b>				
<b>Nostocaceae</b>				
<i>Anabaena constricta</i> (Szafer) Lauterborn			*	*
<i>Anabaena</i> sp.	*		***	*
<i>Anabaena spiroides</i> Klebahn			*	
<i>Cylindrospermum muscicola</i> Kützing ex Bornet and Flahault	***	***	***	*
<b>Aphanizomenonaceae</b>				
<i>Anabaenopsis</i> sp.		*	*	
<b>Oscillatoriales</b>				
<b>Gomontiellaceae</b>				

<i>Komvophoron constrictum</i> (Szafer) Anagnostidis and Komárek	*	*	***	
<b>Oscillatoriaceae</b>				
<i>Oscillatoria chalybea</i> var. <i>insularis</i> Mertens ex Gomont			*	*
<i>Oscillatoria limosa</i> C.Agardh ex Gomont	***	***	***	***
<i>Oscillatoria perornata</i> Kützing Skuja			*	
<i>Oscillatoria princeps</i> Vaucher ex Gomont	*	*	***	*
<i>Oscillatoria</i> sp.1		*		
<i>Oscillatoria</i> sp.2			*	*
<i>Phormidium</i> sp.				*
<b>Microcoleaceae</b>				
<i>Planktothrix compressa</i> (Utermöhl) Anagnostidis and Komárek	***			*
<i>Planktothrix rubescens</i> (De Candolle ex Gomont) Anagnostidis and Komárek	*	*	***	*
<b>Synechococcales</b>				
<b>Leptolyngbyaceae</b>				
<i>Leptolyngbya polysiphoniae</i> (Frémy) Anagnostidis		*	*	
<b>Pseudanabaenaceae</b>				
<i>Pseudanabaena catenata</i> Lauterborn	*		*	*
Total	12	10	15	13

In samples collected from the rice-growing area, 21 species (Table 3) of Cyanobacteria, distributed among 11 genera, 9 families, 4 orders, and 1 class, were identified. All species belonged to the class Cyanophyceae. The order Oscillatoriales was the most represented, with 10 species (47.61%), followed by Nostocales with five species (23.80%), Chroococcales with four species (19.04%), and Synechococcales with two species (9.52%).

In terms of genus distribution, the genus *Oscillatoria*, with 6 species (28.57%), was the most diverse, followed by *Microcystis* and *Anabaena*, each comprising 3 species (14.28%). The remaining genera were poorly represented. Five of the 21 species belonging to the order Nostocales were heterocystous: *Anabaena constricta*, *Anabaena* sp., *Anabaena spiroides*, *Anabaenopsis* sp. and *Cylindrospermum muscicola*.

Regarding the distribution of taxa across treatments, treatment FT3 exhibited the highest number of taxa (15 species), followed by FT4 (13 species), FT1 (12 species), and FT2 (10 species). Four taxa were common to all treatments: *Oscillatoria limosa*, *Oscillatoria princeps*, *Planktothrix rubescens*, and *Cylindrospermum muscicola*.

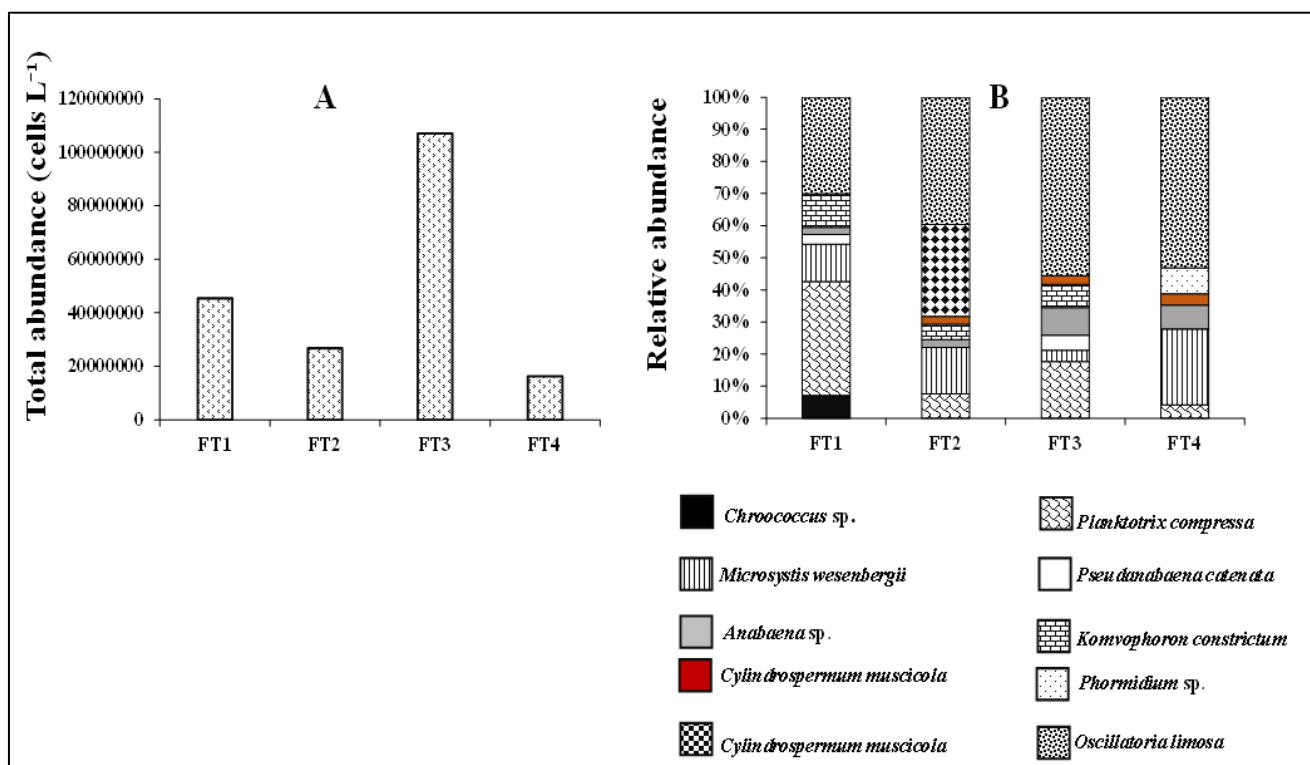
The proportion of frequent taxa (FO > 50%) was the highest across all treatments, with percentages ranging from 58.33% (FT1) to 84.62% (FT4) (Table 4). For common taxa (25% < FO < 50%), the highest proportion (40%) was observed at FT3, whereas the lowest (15.38%) was recorded at FT4. Regarding occasional taxa (FO ≤ 25%), only one taxon was recorded at FT1 (8.33%).

**Table 3** Proportions of common (\*\*\*), occasional (\*\*), and rare (\*) taxa of Cyanobacteria at the four treatments in the Tioroniadougou experimental site

TREATMENT	Common taxa (%)	Occasional taxa (%)	Rare taxa (%)
FT1	33.33	8.33	58.33
FT2	20	0	80
FT3	40	0	60
FT4	15.38	0	84.62

### 3.3. Densities and relative abundances of Cyanobacterial

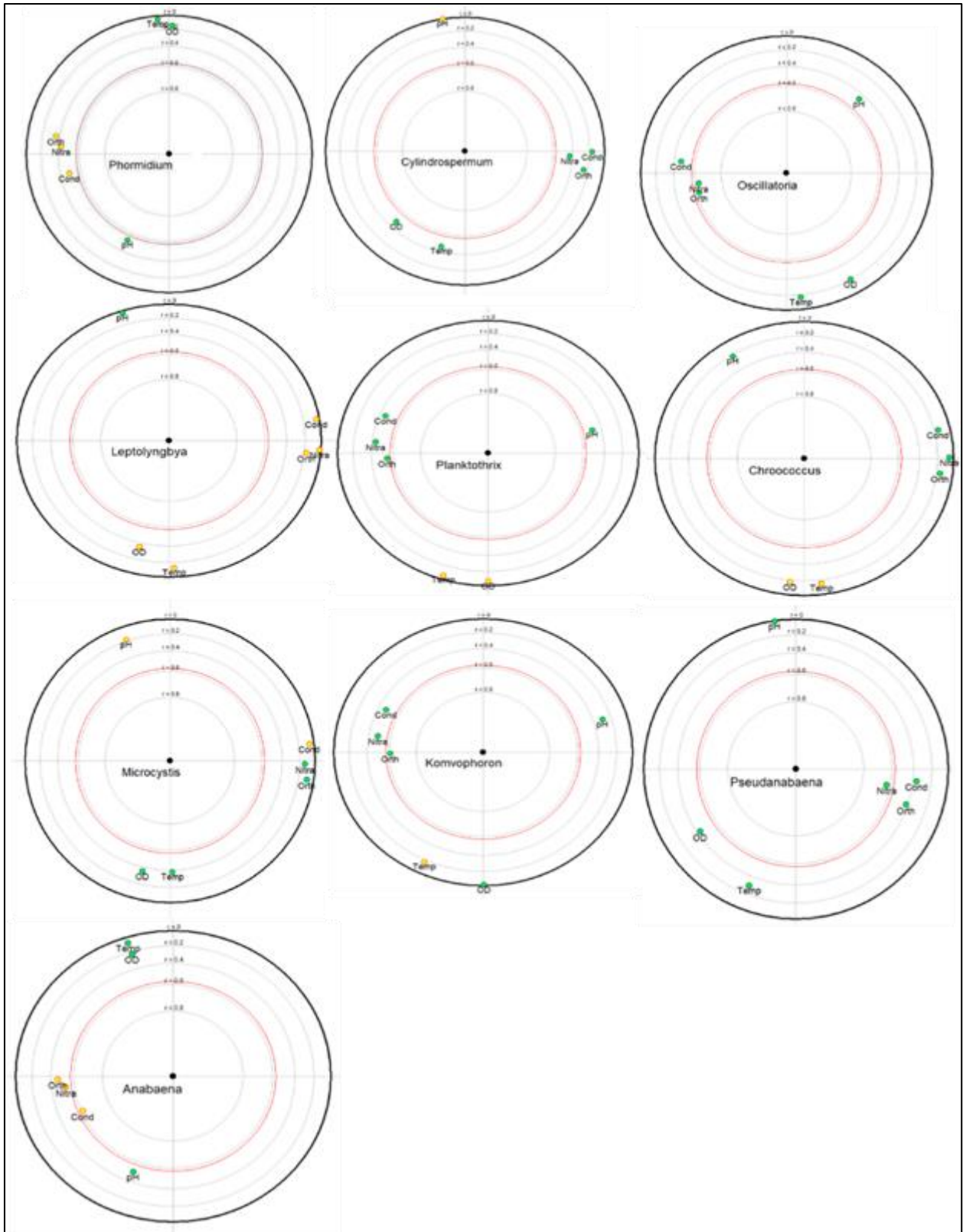
The highest total Cyanobacteria abundance ( $1070.4 \times 10^5 \text{ cells L}^{-1}$ ) was recorded at treatment FT3 (S4), whereas the lowest value ( $163 \times 10^5 \text{ cells L}^{-1}$ ) was observed at treatment FT4 (Figure 3a). Total Cyanobacteria abundance was significantly higher at treatment FT3 than at the other treatments (Mann–Whitney test,  $p < 0.05$ ). Regarding the relative abundance of the identified Cyanobacteria taxa, *Oscillatoria limosa* was the dominant taxon across all treatments, accounting for more than 50% of the total relative abundance (Figure 3b).



**Figure 3** Spatial variations in total abundance (A) and relative abundance of Cyanobacteria in the Tioroniadougou experimental site

### 3.4. Taxa relationships with environmental variables

Relationships between environmental parameters and the main Cyanobacteria genera were assessed using Focused Principal Component Analysis (FPCA) (Fig. 5). Among the ten selected genera, only five showed statistically significant correlations with specific environmental parameters. The analysis revealed that the abundances of Oscillatoriales were significantly and positively correlated with orthophosphate, nitrate, and conductivity. In contrast, the abundances of Anabaena were significantly and negatively correlated with these same parameters. Furthermore, the abundances of the genera *Planktothrix*, *Komvophoron*, and *Pseudanabaena* were significantly and positively correlated with pH and orthophosphate, conductivity, and nitrate, respectively.



**Figure 4** Focused Principal Component Analysis (FPCA) showing the relation between environmental variables and biological variables in the Tioroniadougou experimental site: T= temperature; CND= conductivity; OD= dissolved oxygen; NO<sup>3-</sup> = nitrate; PO<sup>3-4</sup> = phosphorus

#### 4. Discussion

The results showed that the combined NPK + Ajinomoto treatment (FT3) exhibited the highest nutrient concentrations, particularly orthophosphate and nitrate, as well as higher conductivity compared to the other treatments. These observations indicate that fertilization with mineral fertilizers enriched with Ajinomoto improves nutrient availability in paddy water, creating favorable conditions for Cyanobacteria growth (22; 23). Temperature, pH, and dissolved oxygen did not vary significantly among treatments, suggesting that differences in Cyanobacteria communities were mainly driven by nutrient availability and conductivity rather than overall physicochemical conditions.

These findings are consistent with previous studies in tropical rice ecosystems, where nutrient-rich fertilization stimulated algal biomass and primary productivity (7). Higher concentrations of phosphorus and nitrate particularly favor non-heterocystous Cyanobacteria, which rely on dissolved nutrients for growth, whereas heterocystous species like *Anabaena* can thrive under low-nutrient conditions due to their nitrogen-fixing ability (24; 11)

A total of 21 species belonging to 11 genera were identified, all within the class Cyanophyceae. The order Oscillatoriales dominated, followed by Nostocales, Chroococcales, and Synechococcales. The genus *Oscillatoria* was the most diverse, while *Microcystis* and *Anabaena* were less represented. These results align with studies from tropical rice paddies, where filamentous Oscillatoriales often dominate due to their high tolerance to nutrient variations and their ability to form stable floating mats (25, 26).

Treatment FT3 was the highest taxonomic richness (15 species), followed by FT4 (13), FT1 (12), and FT2 (10), suggesting that combined fertilization not only promotes biomass but also enhances Cyanobacteria diversity. The four taxa common to all treatments (*Oscillatoria limosa*, *Oscillatoria princeps*, *Planktothrix rubescens*, and *Cylindrospermum muscicola*) likely represent tolerant species capable of colonizing variable conditions, highlighting their ecological importance for primary productivity in rice paddies (27).

Total Cyanobacteria abundance was significantly higher in FT3, while FT4 had the lowest values. *Oscillatoria limosa* was the dominant taxon across all treatments, accounting for more than 50% of relative abundance. This dominance reflects both high adaptability to fertilization conditions and strong competitive ability for available nutrients, a trait well documented for Oscillatoriales in tropical rice systems (28; 29).

The high abundance observed in FT3 highlights the synergistic effect of combined NPK + Ajinomoto fertilization on Cyanobacteria growth, which may enhance nitrogen fixation and nutrient recycling in paddy soils, contributing to soil fertility.

FPCA analysis revealed that five genera were significantly correlated with environmental parameters. Oscillatoriales were positively correlated with conductivity, nitrate, and orthophosphate, while *Anabaena* showed negative correlations. This indicates that Oscillatoriales benefit from nutrient-rich conditions, whereas heterocystous genera like *Anabaena* are better adapted to low-nutrient environments through nitrogen fixation.

Genera such as *Planktothrix*, *Komvophoron*, and *Pseudanabaena* displayed positive responses to specific parameters (pH, orthophosphate, conductivity, nitrate), confirming that Cyanobacteria community composition is strongly influenced by nutrient availability and water chemistry. These findings are consistent with previous studies in tropical and subtropical rice ecosystems, which showed that fertilization affects both the abundance and structure of Cyanobacteria communities (30; 6; 8).

The results of this study have important implications for sustainable rice cultivation. Stimulation of Cyanobacteria growth by combined fertilization could reduce reliance on chemical fertilizers, improve soil fertility, and enhance microbial biodiversity. Tolerant taxa such as Oscillatoriales may play a key role in maintaining primary productivity and nutrient cycling, particularly in fields with limited or variable fertilization. Optimizing fertilization strategies to support beneficial Cyanobacteria communities could therefore contribute to both higher yields and environmental sustainability in rice production systems in West Africa.

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#### 5. Conclusion

This study demonstrated that fertilization treatments significantly affect Cyanobacteria dynamics in irrigated rice systems. Combined fertilization (FT3) promoted higher nutrient availability, leading to increased Cyanobacteria abundance and diversity.

The predominance of Oscillatoriales and the differential responses of genera such as *Anabaena* highlight the importance of nutrient conditions in structuring cyanobacterial communities.

These findings suggest that optimizing fertilization strategies could enhance beneficial Cyanobacteria populations, contributing to sustainable rice production and reduced dependence on chemical fertilizers.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare no conflicts of interest regarding the publication of this paper.

### *Author's contribution*

The first author collected, processed and drafted this article. The other authors read and corrected the manuscript. All the authors read and approved the final manuscript.

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