


Can Drainage, Dolomite, and Natural Phosphate Application Control Iron and Sulfide Content in Iron-Toxic Irrigated Rice Fields?

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Abstract

Soil iron (Fe II) and sulfide toxicities are major constraints to irrigated rice productivity in Burkina Faso, West Africa. This study aimed to mitigate these challenges by trialing drainage, fertilization, and iron-tolerant rice varieties, considering seasonal variations. Field experiments were conducted in the dry and rainy seasons using two rice varieties: FKR76 (iron-sensitive) and FKR62N (iron-tolerant). Two drainage modes (D0: no drainage, D2: drainage every 14 days) and eight fertilization treatments were tested: unfertilized (F1), NPK + urea (F2), NPK + Urea + Dolomite + Zn (F3), and NPK + Urea + Natural Phosphate (F4), and each fertilization regime combined with organic manure (FO) and without organic manure (SFO). Soil samples were collected before rice plant transplanting to evaluate the number and activities of Iron-Reducing Bacteria (IRB) and Sulfate-Reducing Bacteria (SRB). At 60 days after transplanting (DAT), the soil Fe II and sulfide concentrations were measured. The results showed that both IRB and SRB activities were higher during the rainy season ($p < 0.001$) than during the dry season. The FKR76 plots showed higher Fe II concentrations than the FKR62N plots. Organic manure increased sulfide content ($p = 0.048$), and NPK + urea raised Fe II concentration in the soil ($p = 0.045$). However, amendments with dolomite and natural phosphate significantly decreased the soil Fe II ($p = 0.005$) and sulfide ($p < 0.001$) contents. The treatments applied had no significant effect on the productivity of the

FKR62N rice variety. However, the D2FOF2, D2FOF3, D2FOF4, and D0FOF3 treatments significantly improved the tillering ($p = 0.000$), panicle development ($p = 0.000$), and grain yield ($p = 0.0001$) of the iron-sensitive rice variety (FRK76). These results indicate that dolomite and natural phosphate can effectively reduce iron and sulfide toxicity in rice fields across seasons, offering a promising strategy for sustainable rice production under challenging soil conditions.

Keywords

Toxicity, Season, Fertilization, Drainage, Rice Variety

1. Introduction

Rice is one of the most produced cereals in the world, with the Asian continent being the dominant producer [1]. In Burkina Faso, West Africa, rice is the fourth most important cereal crop after sorghum, millet, and maize. Rice production in Burkina Faso increased from 113,724 t in 2006 to 438,982.38 t in 2022 [1]; however, local production still covers less than 50% of the country's demand [2]. Despite the availability of lowlands and irrigable land suited to rice production, and alongside government support, rice production in Burkina Faso faces biotic and abiotic constraints, which decrease the production of the best-performing rice varieties developed by researchers [3]. Indeed, rice blast, bacterial blight, rice yellow mottle, parasitic nematodes, and weeds are the main biotic constraints [4]-[6]. In addition, drought, cold, salinity, and iron toxicity are significant abiotic constraints [3].

Iron excess in flooded rice fields could lead to iron toxicity, causing severe yield losses [7] [8]. In West Africa, notably in Burkina Faso, rice yield losses associated with iron toxicity have been estimated at 12% - 100% [9] [10].

Many studies also reported that iron toxicity in rice fields is usually associated with sulfide toxicity [11], leading to the accumulation of iron monosulfides (FeS), which are toxic to rice plants [12] [13]. Indeed, iron toxicity happens when high concentrations of iron (Fe II) and sulfide [14] are accumulated in the lowland soil solution, causing bronzing of leaves, poor growth at the reproductive phase, and poor yields [15].

Previous research has reported that iron and sulfide toxicities result from the *in situ* microbial reduction activities of Iron Reducing Bacteria (IRB) and Sulfate Reducing Bacteria (SRB) present in submerged soils [14] [16]-[18]. In the anaerobic conditions of rice fields, these bacteria produce Fe II, FeS, and sulfide, which can cause damage to rice plant roots, leading to high Fe II absorption and rice plant iron toxicity [11] [18]. Several approaches have been evaluated to control iron toxicity in rice plants growing in West Africa, such as water management [9] [19], the selection of tolerant rice varieties [3] [20], and the use of mineral and organic

fertilizers [19] [21]. Dolomite [22] [23] and phosphate [24] are also applied in acidic soils to increase the soil pH, leading to a reduction in sulfides and Fe II availability in toxic rice fields and thereby improving rice productivity. However, farmers are still facing these soil toxicity issues in West African countries, because studies do not often translate to change on the ground. In addition, some studies note that iron and sulfur toxicity are very often associated and can occur simultaneously, making it difficult to control these stresses.

The current study aims to control the iron and sulfide toxicities in affected rice fields in Burkina Faso by developing integrated methods to reduce Fe II and sulfide content in soil. For this purpose, two rice varieties, organic (compost), mineral (NPK, dolomite, and phosphate) fertilizers, and differing drainage modes were used separately or combined to control the availability of these toxic ions according to the season.

2. Material and Methods

2.1. Study Site

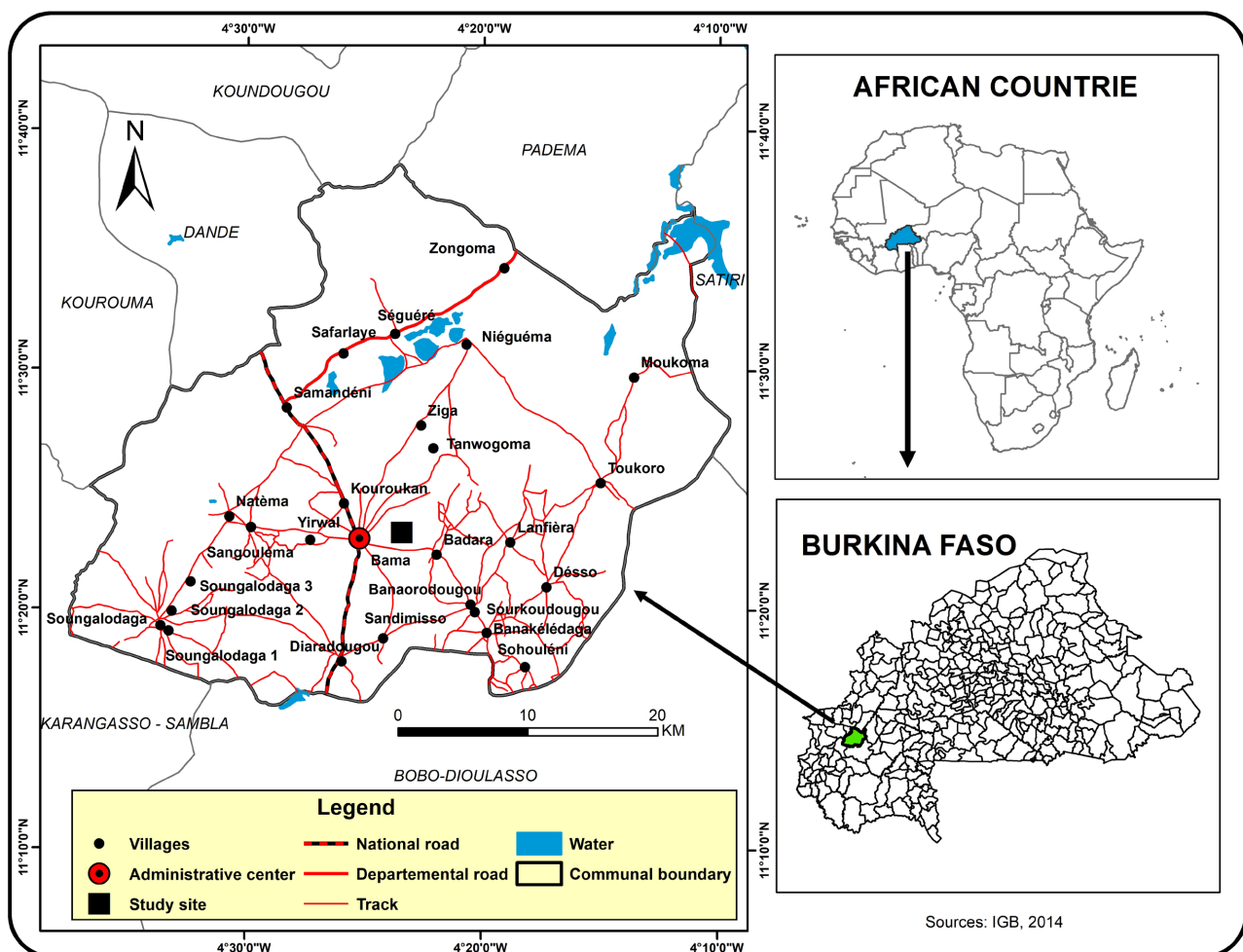


Figure 1. Location of the Kou Valley experimental site in Burkina Faso, West Africa.

The study was conducted on the irrigated rice field of the Kou Valley, located 30 km from the city of Bobo-Dioulasso, in the western part of Burkina Faso (**Figure 1**). The site was selected because of its historical presence of rice iron toxicity. The study was conducted at the experimental site of the Institute of the Environment and Agricultural Research (INERA) (11° 23'12" N and 4° 23'25" W). The site previously used for rice production was abandoned because of a drastic decrease in yields. The soil was clay-loamy, acidic, with low organic matter and high iron content. The climate in the Kou Valley is a south-Sudanese climate. The rainfall is very variable, with an annual average of 950 mm [3]. The experiment was performed in 2020, during two consecutive seasons, both in the dry (February to May) and wet seasons (August to November). The monthly climatic data of the experimental site are presented in **Table 1**. During the dry season, the average total rainfall was 162.77 mm. The average monthly temperature was 30.11 °C with a minimum of 28.71 °C and a maximum of 31.26 °C. The monthly relative humidity (RH) varied from 24.11% to 63.09%. In the rainy season, the average total rainfall was 328.4 mm and the average daily temperature was 26.63 °C with a minimum of 25.14 °C and a maximum of 28.12 °C. The monthly relative humidity (RH) in the rainy season varied from 47.67% to 84.91%.

Table 1. Monthly climatic data of the experimental site in 2020.

Month	Temperature (°C)	Relative Humidity (%)	Precipitation Depth (mm)
January	26.23	21.44	0
February	28.71	24.11	44.0
March	30.94	34.85	124.2
April	31.26	44.52	89.3
May	29.52	63.09	393.6
June	27.69	76.14	604.35
July	26.02	77.75	180.65
August	25.14	84.91	374.8
September	25.73	81.85	494.9
October	27.53	73.45	443.9
November	28.12	47.67	0
December	25.97	28.95	52.1

Experimental design: Two rice varieties from the INERA gene bank were tested: FKR76 (iron toxicity-sensitive) and FKR62N (iron toxicity-tolerant), with growth cycles of 90 days and 118 days, respectively [3]. Both varieties have a potential yield of 6 t.ha⁻¹ and are suited to lowland or irrigated rice fields.

Both mineral fertilizers and organic matter were used as soil amendments (**Figure 2**). The mineral fertilizers were formulated as follows: F1 (no fertilization), F2 (NPK + Urea), F3 (NPK + Urea + Dolomite + Zn), and F4 (NPK + Urea + Natural Phosphate). These fertilizers were applied with compost as organic matter (FO) or without organic matter (SFO) as secondary plot treatments. Fertilizer doses followed recommended rates: NPK at 200 kg.ha⁻¹, ZnO at 10 kg.ha⁻¹, dolomite at

200 kg.ha⁻¹, and natural phosphate at 250 kg.ha⁻¹ [25]. Compost was applied at 15 t.ha⁻¹. All fertilizers were applied at transplanting, while Urea (150 kg.ha⁻¹) was split into two applications: 50 kg.ha⁻¹ at transplanting and 100 kg.ha⁻¹ at 60 days after transplanting (Figure 2).

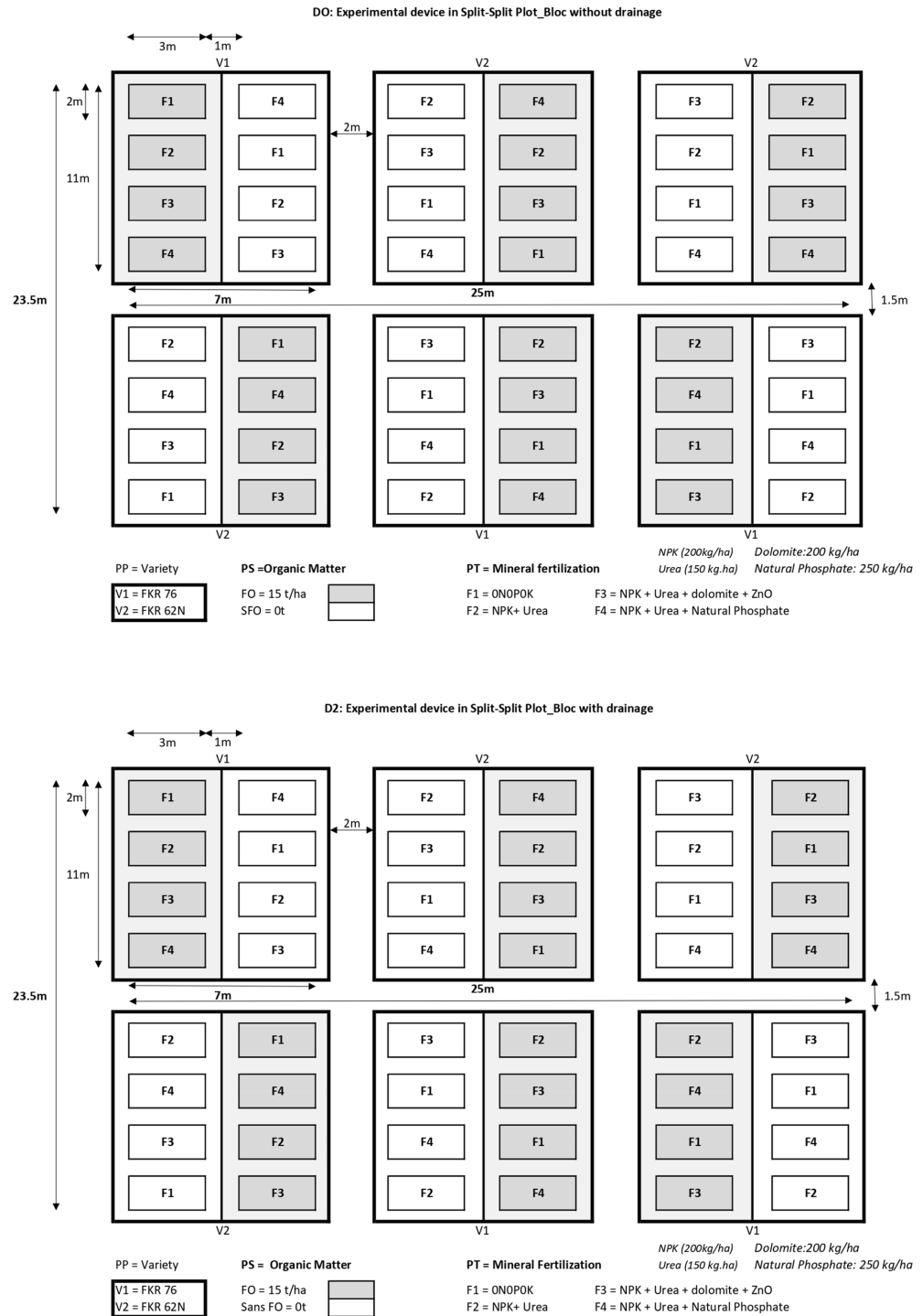


Figure 2. Experimental design of the study in split-split plots. PP = Primary plot, PS = Secondary plot, PT = Tertiary plot. D0 = plots not drained, D2 = drained plot.

A split-split plot design in a lattice arrangement with three replications was used. Four factors were studied: rice variety (FKR76, FKR62N), organic manuring (FO = with organic matter, SFO = without organic matter), mineral fertilization (four levels), and drainage mode (D0 = no drainage, D2 = 14-day drainage). Varieties were assigned to main plots, organic fertilization to subplots, and mineral fertilization to sub-subplots. Treatments were tested in two blocks under different drainage conditions. Rice was transplanted at 25 × 25 cm spacing (one plant per hill) using 21-day-old seedlings. Each elementary plot measured 3 × 3 m with 1 m spacing, while replications were separated by 2 m (Figure 2).

Drainage method: The 1200-ha Kou Valley irrigation scheme, located in a water-rich watershed, is supplied by gravity through a hierarchical canal system [26] [27]. Previous research results showed that regular 14-day subsurface drainage significantly improves rice yield in iron-toxic conditions [25]. In line with the existing gravity drainage system at the study site, during the current trials, the drainage block was subjected to periodic gravitational drainage every 14 days via quaternary canals directly connected to the plots.

2.2. Soil Sampling and Analysis

2.2.1. Soil Sampling

Before rice plant transplanting, twelve soil samples were collected from all the plots by random sampling. From the soil samples collected, three composite samples were created to evaluate the presence and activities of IRB and SRB. As previous studies showed that the number of IRBs was not correlated to their activities [28] [29], three soil samples were collected in each elementary plot, at sixty (60) days after transplanting (DAT), to determine the effect of the treatments on the bacterial activities (Fe II and sulfide contents). Thus, the soil samples were collected at a depth of 0 to 20 cm using an auger. The sampled soils were immediately introduced into the sampling bags, and the air was removed from the bags before closing to maintain anaerobic conditions. The samples were kept in the refrigerator at +4°C until chemical and microbiological analysis.

2.2.2. Sulfate-Reducing Bacteria Enumeration

The medium used for SRB enumeration was that used by Chaudary and Cornfield [30]. The number of bacteria was determined according to the Most Probable Number (MPN). The medium was inoculated in triplicate (9 ml of the medium for 1 ml of inoculum) with soil dilutions performed (10^{-1} to 10^{-9}) using Hungate tubes under aseptic conditions. The tubes were incubated in an incubator at 30°C for 15 days. Positive cultures were revealed by black staining resulting from iron sulfide (FeS) produced by SRB.

2.2.3. Iron-Reducing Bacteria Enumeration

For IRB enumeration, each sample (10 g) was used to inoculate 90 mL of $MgCl_2 \cdot 6H_2O$ sterile solution at 0.5% (w/v) to obtain a stock solution (S0). The S0 obtained was used for serial dilutions (10^{-1} to 10^{-9}). Hammann and Ottow [31]

modified medium was used to enumerate IRB using the MPN method. The medium was sterilized in an autoclave at 121 °C for 15 minutes and was then inoculated and incubated at 30 °C for five (7) days under nitrogen pressure. After the incubation, a solution of orthophenanthroline (0.2%) was added as the reagent to the medium to indicate IRB growth [31]. A positive result for IRB growth was indicated by the medium turning a reddish color.

2.2.4. Determination of Sulfide Content in the Soil

The Chaudhary and Cornfield [32] method was used to determine the sulfide content of the soil samples. Three (3 g) soil samples were dissolved in 10 ml of an acetate solution (zinc acetate 20 g.l⁻¹ and sodium acetate 12.5 g.l⁻¹) under nitrogen pressure. The mixture was vortex-homogenized for 30 min and centrifuged at 2000 rpm for 10 min. Then, 2 ml of the supernatant was mixed with 4 ml of a reagent containing HCl at 0.5 M and CuSO₄ at 50 mM, to reveal the traces of sulfide. The optical density was measured at 480 nm with the Spectronic 601 spectrophotometer (Milton Roy, USA). The soil was collected at the bottom of the tube after centrifugation, dried, and weighed to determine the sulfide content by weight of the dried soil.

2.2.5. Determination of Iron Content in the Soil

The soil Iron II content was measured using the modified method of Maitte [33]. Therefore, 1 g of soil sample was placed into a Hungate tube containing 9 ml of hydrochloric acid (1N). The mixture was stirred and kept in the dark under nitrogen pressure for 5 min to extract the Fe II. The mixture was centrifuged at 2000 rpm for 30 min and filtered. The soil was collected and dried in an oven at 105 °C for 24 hours, then weighed to determine its dry weight. The content of Fe II was determined using a spectrometer at 510 nm, immediately after the addition of 0.5 ml of orthophenanthroline (0.2%). Solutions of Fe₂SO₄ at 5 to 30 mM concentration, prepared with deaerated water, were used as a standard.

2.2.6. Evaluation of the Effect of the Treatment on the Rice Plant Agromorphological Parameters

To assess the effect of the soil amendments on rice productivity, the number of tillers per m² of area was determined at the 60th DAT by counting the number of stalks per plot. The number of panicles was evaluated before the harvest, and the paddy yield was determined after drying until constant weight and estimated in kg.ha⁻¹.

2.2.7. Statistical Analysis

During the experiment, the data collected were recorded in an Excel spreadsheet and analyzed using both one-way and multi-way analyses of variance (ANOVA) with R 4.3.0 software. The one-way ANOVA was used to evaluate the effects of individual factors such as rice variety, fertilization mode (treatment), drainage mode, and season. For interactions between multiple factors (rice variety, mode, drainage mode, and fertilization), a multi-way ANOVA was used. Pairwise com-

parisons were performed using Tukey's Least Significant Difference (LSD) test at a 5% significance level to assess differences between the parameters.

3. Results

3.1. Evaluation of the Sulphate-Reducing Bacteria's Activities in the Soil during the Experiment

The abundance and activity of the SRB were assessed through the SRB number and the sulfide concentration in the soil sampled. The analysis of the soil collected before the rice plant transplanting showed that it contained 1.68×10^8 SRB numbers.g⁻¹ dry soil and 62.0×10^3 mg.l⁻¹ of sulfide in the soil solution.

The one-factor ANOVA results (**Table 2**) indicated that variety ($p = 0.017$) and season ($p < 0.001$) significantly influenced sulfide concentrations, with higher concentrations observed in the rainy season than in the dry season.

Table 2. Variance of the mean values of soil sulfide and Fe II concentrations by main factors (ANOVA one-factor model).

	Variable	Sulfides (mg.L ⁻¹)	Fe II (mg.L ⁻¹)
Variety	Overall	2346.75 ± 1784.82	6412.15 ± 998.98
	FKR62N	2685.91 ^a ± 2278.70	6234.62 ^b ± 889.69
	FKR76	2011.12 ^b ± 1508.20	6587.84 ^a ± 1129.51
	P-value	0.017 *	0.017 *
Drainage	Drained	2162.73 ^a ± 1950.64	6414.91 ^a ± 959.59
	No drained	2532.71 ^a ± 1951.29	6409.36 ^b ± 1101.66
	P-value	0.192	0.970
Season	Dry	1115.31 ^b ± 441.83	6063.52 ^b ± 775.56
	Rainy	3565.37 ^a ± 2104.99	6757.15 ^a ± 1133.47
	P-value	<0.001 ***	<0.001 ***
Treatment	SFOF1	2888.33 ^a ± 2581.38	6605.56 ^a ± 1185.3
	SFOF2	2559.77 ^a ± 2216.34	6700.26 ^a ± 1083.75
	SFOF3	1505.72 ^a ± 553.74	6451.03 ^{ab} ± 1022.26
	SFOF4	1609.03 ^a ± 479.09	6347.49 ^{ab} ± 856.36
	FOF1	3957.27 ^a ± 3028.98	6448.62 ^{ab} ± 1066.05
	FOF2	2916.19 ^a ± 2149.33	6705.08 ^a ± 1053.33
	FOF3	1736.92 ^a ± 523.85	6131.97 ^{ab} ± 1148.83
	FOF4	1623.34 ^a ± 212.74	5915.25 ^b ± 583.73
	p-value	0.22	0.012 *

df = degree of freedom; F = Fisher F; significant $p < 0.05$; significant $p < 0.01$; significant $p < 0.001$; ns: not significant $p > 0.05$. The values sharing the same letter are not significantly different according to the LSD test, $p < 0.05$.

In contrast, the effects of drainage ($p = 0.192$) and treatment ($p = 0.226$) were not significant in this one-factor model. However, the multifactorial ANOVA results (**Table 3**) provided a more nuanced understanding. The season ($p < 0.001$) had the strongest effect on sulfide content in the soil (**Table 3**). The soil sulfide content was significantly impacted by variety, drainage, and treatment ($p < 0.001$, **Table 3**). Several interactions were highly significant, particularly Variety \times Season, Variety \times Treatment, and Treatment \times Season ($p < 0.001$), indicating that the impact of treatment and variety depends on seasonal conditions (**Table 3**). These findings confirm that sulfide concentrations in the soil were strongly influenced by rainfall, with values significantly higher in the rainy season ($3565.37 \pm 2104.99 \text{ mg.l}^{-1}$) compared to the dry season ($1115.31 \pm 441.83 \text{ mg.l}^{-1}$) (**Table 2, Figure 3**).

Table 3. Variance of the mean values of soil sulfide concentration according to the multifactorial ANOVA (mixed model).

Model term	df1	df2	F.ratio	p.value
Variety	1	120	121.541	<0.001 ***
Drainage	1	120	41.6	<0.001 ***
Treatment	7	120	102.313	<0.001 ***
Season	1	120	1846.824	<0.001 ***
Variety \times Drainage	1	120	0.468	0.495ns
Variety \times Treatment	7	120	16.466	<0.001 ***
Variety \times Season	1	120	117.23	<0.001 ***
Drainage \times Treatment	7	120	6.057	<0.001 ***
Drainage \times Season	1	120	0.056	0.813 ns
Treatment \times Season	7	120	144.69	<0.001 ***
Variety \times Drainage \times Treatment	7	120	11.861	<0.001 ***
Variety \times Drainage \times Season	1	120	18.885	<0.001 ***
Variety \times Treatment \times Season	7	120	9.178	<0.001 ***
Drainage \times Treatment \times Season	7	120	6.901	<0.001 ***
Variety \times Drainage \times Treatment \times Season	7	120	13.853	<0.001 ***

df = degree of freedom; F = Fisher F; significant $p < 0.05$; significant $p < 0.01$; significant $p < 0.001$; ns: not significant $p > 0.05$.

Despite no significant main effect of drainage evident in the one-way ANOVA ($p = 0.192$), the multifactorial model revealed that drainage interacts with both season and treatment to influence sulfide concentrations (e.g., Drainage \times Treatment \times Season, $p < 0.001$), emphasizing the need to consider these interactions. Finally, the Variety \times Treatment interaction demonstrated that the FKR62N variety generally showed higher sulfide concentrations than the FKR76 variety across treatments (**Figure 3(AB)**), particularly under SFOF1 (3859.90 ± 571.92) and

FOF1 (2872.81 ± 632.29). This suggests a combined effect of fertilization mode and the rice plant variety on sulfide production in the rhizosphere.

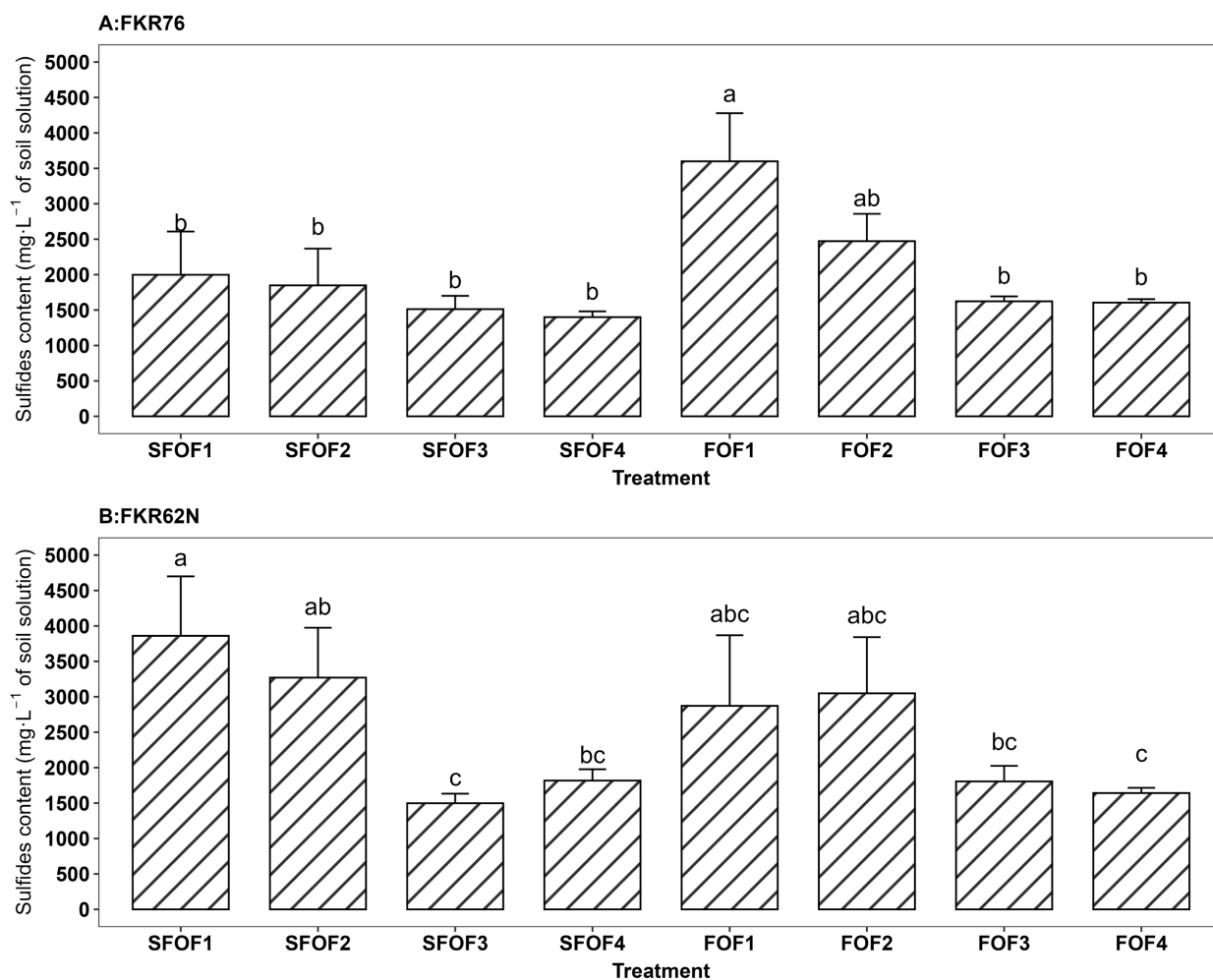


Figure 3. Concentration of soil sulfide according to the treatment and the rice variety at 60 DAT. The values sharing the same letter are not significantly different according to the LSD test, $p < 0.05$. NB: Bars represent standard errors.

3.2. Evaluation of the Iron-Reducing Bacteria's Activities in the Soil during the Experiment

The IRB activity was assessed through the IRB number and Fe II concentrations in the soil. Initial measurements revealed 8.3×10^4 IRB number.g⁻¹ of dry soil and 6.97×10^4 mg.l⁻¹ of Fe II in the soil solution before rice plant transplanting.

According to the mixed-model ANOVA (**Table 4**), variety ($p = 0.003$), treatment ($p = 0.0034$), and season ($p < 0.001$) significantly influenced Fe II concentration. Although the drainage main effect was not significant ($p = 0.924$), several interactions involving drainage were significant, including Variety \times Drainage ($p < 0.001$), Drainage \times Treatment ($p = 0.002$), and Drainage \times Season ($p < 0.001$). This indicates that drainage effects emerge in combination with other factors rather than in isolation.

Table 4. Results of the multifactorial ANOVA (mixed model) on soil Fe II concentration.

Model term	df1	df2	F.ratio	p.value
Variety	1	175	9.063	0.003**
Drainage	1	175	0.009	0.924 ^{ns}
Treatment	1	175	8.813	0.003**
Season	1	175	34.353	<0.001***
Variety × Drainage	1	175	20.580	<0.001***
Variety × Teatment	1	175	2.040	0.155 ^{ns}
Variety × Season	1	175	18.896	<0.001***
Drainage × Treatment	1	175	9.510	0.002**
Drainage × Season	1	175	12.216	<0.001***
Treatment × Season	1	175	0.107	0.744 ^{ns}
Variety × Drainage × Treatment	1	175	0.251	0.617 ^{ns}
Variety × Drainage × Season	1	175	0.427	0.514 ^{ns}
Variety × Treatment × Season	1	175	0.108	0.743 ^{ns}
Drainage × Treatment × Season	1	175	3.944	0.048*
Variety × Drainage × Treatment × Season	1	175	2.370	0.125 ^{ns}

df = degree of freedom; F = Fisher F; significant $p < 0.05$; significant $p < 0.01$; significant $p < 0.001$; ns: not significant $p > 0.05$.

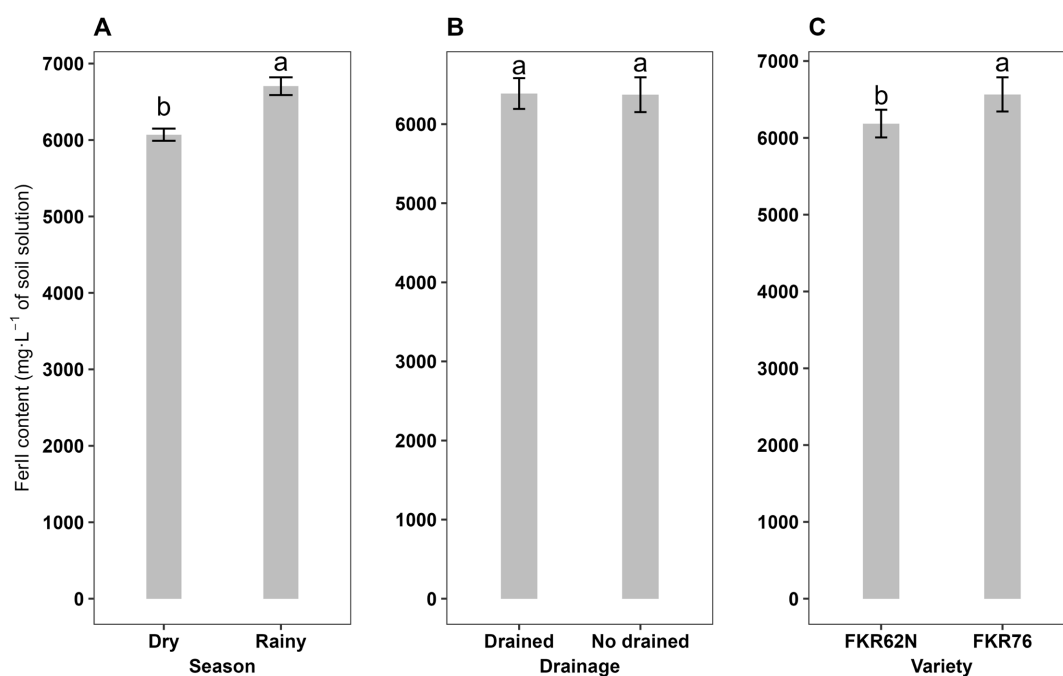


Figure 4. Concentration of soil Fe II according to the season (A), drainage mode (B), and rice variety (C). The values sharing the same letter are not significantly different according to the LSD test, $p < 0.05$. Nb: Bars represent standard errors.

The soil Fe II concentration was significantly higher in the rainy season ($6704.19 \pm 100.09 \text{ mg.l}^{-1}$) than in the dry season ($6069.39 \pm 97.93 \text{ mg.l}^{-1}$, **Figure 4(A)**). Varietal differences were also clear: FKR76 ($6565.85 \pm 101.42 \text{ mg.l}^{-1}$) had a

significantly higher Fe II concentration than FKR62N ($6185.67 \pm 103.65 \text{ mg.l}^{-1}$, **Figure 4(C)**). Drainage conditions alone did not cause substantial differences (**Figure 4(B)**). The Treatment \times Variety interaction ($p = 0.045$) further demonstrated that Fe II concentration varied across combinations. In the FKR76 variety, the highest Fe II concentration was observed under SFOF2 ($7090.35 \pm 307.92 \text{ mg.l}^{-1}$), while FOF4 yielded the lowest Fe II concentration (**Figure 5(A)**). In the FKR62N variety, the FOF2 treatment ($6776.76 \pm 247.79 \text{ mg.l}^{-1}$) led to the highest Fe II concentration, whereas the FOF3 and FOF4 treatments showed the lowest values (**Figure 5(B)**). In addition, the coefficient of variation (CV) for Fe II ranged from 13.28% to 16.24%, indicating moderate variability and good experimental reliability. These results underscore the combined influence of the rice plant variety and fertilization on iron-reducing activity in the soil, modulated by seasonal and hydrological conditions.

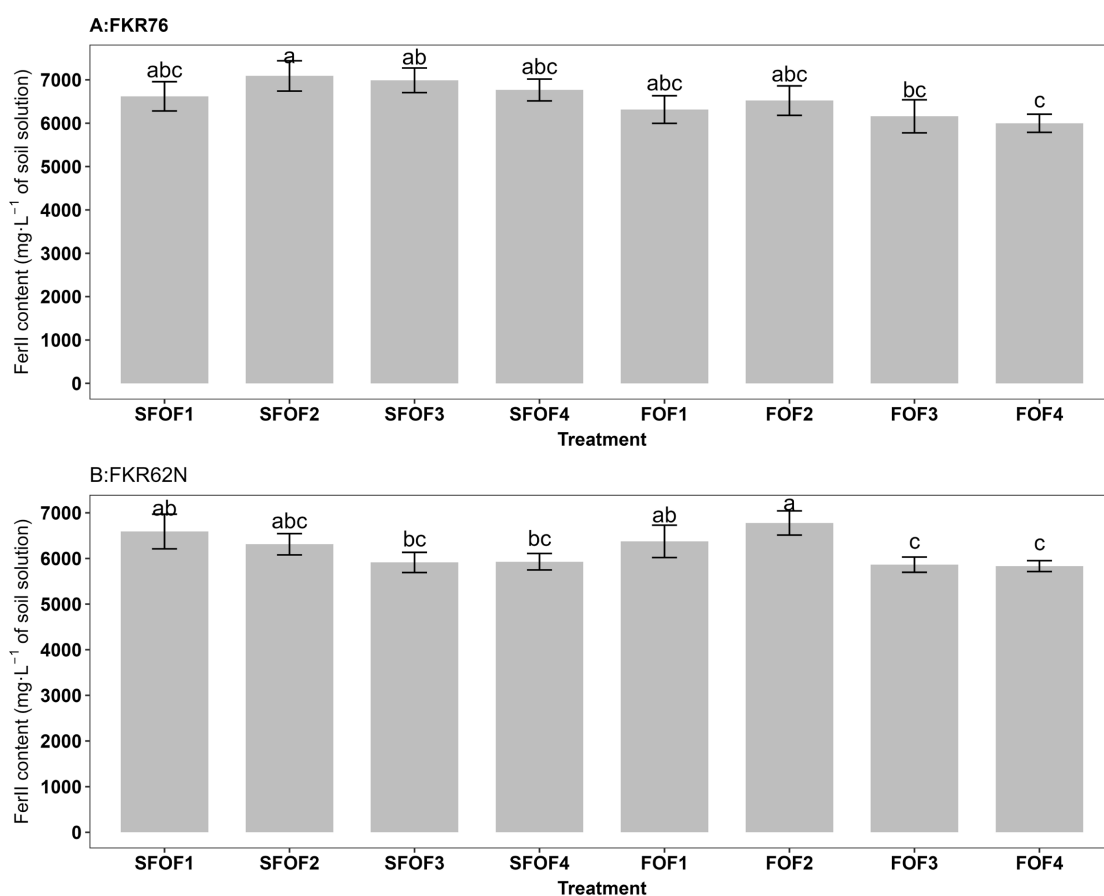


Figure 5. Concentration of soil Fe II according to the treatment and the rice variety. The values sharing the same letter are not significantly different according to the LSD test, $p < 0.05$. SFO = No organic matter, FO = Organic matter, F1 = No mineral fertilizer, F2 = NPK + Urea, F3 = NPK + Urea + Dolomite + Zn, F4 = NPK + Urea + Natural Phosphate. NB: Bars represent standard errors.

3.3. Evaluation of the Effect of Treatments on Rice Productivity

Table 5 presents the effect of treatments on the number of rice plant tillers at 60

days after transplanting (60 DAT), the average number of tillers, and the grain yield for the two rice varieties at harvest, during the two experimental trials. During the experimental trial, 1 to 2 plots, which received D0SFOF1, D0FOF1, and D0FOF3 treatments, were lost due to bird attacks. The results collected evidenced that for the iron-toxicity-resistant variety FRK62N, all treatments induced a high number of tillers at 60 DAT (up to 212 tillers), with no statistically significant differences between them ($p = 0.104$). This suggests that the tillering of this rice variety is less affected by the different fertilization and drainage treatments. In contrast, the sensitive variety FRK76 exhibited a marked variation in the average number of tillers at 60 DAT, ranging from 64 to 159 tillers, with highly significant differences among treatments ($p = 0.000$). These results indicate that the FRK76 variety is more responsive to nutrient inputs, with treatments D0FOF3, D2FOF4, and D2FOF2 particularly promoting enhanced tillering. The overall analysis, considering both varieties, confirms that the effect of treatments on tillering is highly significant ($p = 0.000$), highlighting the important role of integrated organic-mineral fertilization strategies in promoting vegetative growth, particularly tiller development, under iron-toxic conditions.

Furthermore, for the FRK62N variety, the applied treatments did not significantly affect the number of panicles per plant ($p = 0.056$), although an increasing trend was observed with D2FOF4, which recorded a relatively high value (146 panicles). On the other hand, the FRK76 variety showed a strong treatment response, with highly significant effects on panicle production ($p = 0.000$). Indeed, the treatments D0FOF2, D0FOF4, and D0FOF3 stood out as the most effective in enhancing panicle number. The global analysis also revealed a significant treatment effect on panicle number ($p = 0.000$), with a favorable trend observed for treatments combining organic fertilization and absence of water stress, underscoring the importance of these factors in improving rice productivity under iron-rich soil conditions.

Additionally, grain yields for the FRK62N variety ranged from 1376 to 2388 $\text{kg}\cdot\text{ha}^{-1}$, but no significant differences were detected among treatments ($p = 0.875$). This indicates that, despite observed yield differences, the treatments did not lead to statistically significant productivity improvements in this iron-tolerant variety. In contrast, for the iron-sensitive variety FRK76, yields were strongly influenced by treatments, ranging from 982 to 2630 $\text{kg}\cdot\text{ha}^{-1}$, with highly significant differences ($p = 0.0001$). The treatments D2FOF3 and D2FOF2 produced the highest yields, suggesting that this variety responds more favorably to combined drainage and fertilization strategies. Overall, the mean yield was 1985.29 $\text{kg}\cdot\text{ha}^{-1}$, with no statistically significant difference among treatments ($p = 0.485$). This reflects inter-varietal variability, where only certain treatments (notably D2FOF3, D2FOF2, and D0FOF1) showed promising trends, without generating a significant overall effect across both varieties. However, the relatively high coefficients of variation ($\text{CV} > 20\%$ for certain variables) indicate substantial variability, likely influenced by experimental conditions.

Table 5. Variance of the effect of the rice agromorphological parameters with the treatments applied.

Treatment	N	FRK62N			FRK76			Overall		
		Yield (kg.ha ⁻¹)	Tiller 60DAT	Panicler number	Yield (kg.ha ⁻¹)	Tiller 60DAT	Panicler number	Yield (kg.ha ⁻¹)	Tiller60DAT	Panicler number
D2SFOF1	12	1376.18 ^a ± 307.64	134.5 ^a ± 7.55	120.17 ^a ± 14.43	1699.14 ^{abc} ± 543.94	77.83 ^{de} ± 12.6	62.94 ^e ± 3.83	1618.40 ^a ± 503.71	92.00 ^e ± 27.98	77.25 ^d ± 26.80
D2SFOF2	12	2261.54 ^a ± 546.65	146.67 ^a ± 10.75	133.67 ^a ± 11.54	1869.81 ^{abc} ± 485.40	120.06 ^{abcd} ± 26.04	91 ^{bcd} ± 11.76	1967.74 ^a ± 507.03	126.71 ^{bcd} ± 25.67	101.67 ^{abcd} ± 22.29
D2SFOF3	12	2222.52 ^a ± 766.62	148 ^a ± 44.17	139 ^a ± 11.14	1805.64 ^{abc} ± 497.79	121.06 ^{abcd} ± 29.4	83.83 ^{bcd} ± 15.48	1909.86 ^a ± 567.99	127.79 ^{bcd} ± 33.64	97.62 ^{abcd} ± 28.62
D2SFOF4	12	1963.41 ^a ± 854.09	144.5 ^a ± 28.08	115.67 ^a ± 11.9	2109.83 ^{abc} ± 583.54	106.22 ^{abcde} ± 23.32	79.56 ^{cde} ± 12.08	2073.23 ^a ± 620.22	115.79 ^{cde} ± 28.95	88.58 ^{cd} ± 19.96
D2FOF1	12	1924.85 ^a ± 546.08	146.67 ^a ± 8.1	116.17 ± 3.79	1772.97 ^{abc} ± 726.73	92.89 ^{bcd} ± 18.13	74.06 ^{de} ± 11.47	1810.94 ^a ± 665.62	106.33 ^{de} ± 29.02	84.58 ^{cd} ± 21.47
D2FOF2	12	2320.76 ^a ± 307.25	148.5 ^a ± 19.22	137 ^a ± 6.50	2368.88 ^{ab} ± 774.24	136.11 ^{abc} ± 25.12	90.11 ^{bcd} ± 10.7	2356.86 ^a ± 673.50	139.20 ^{bcd} ± 23.61	101.83 ^{abcd} ± 23.25
D2FOF3	12	1567.23 ^a ± 798.59	162.67 ^a ± 10.77	132.33 ^a ± 3.69	2630.00 ^a ± 866.18	131.5 ^{abcd} ± 28.71	88.11 ^{bcd} ± 17.35	2364.31 ^a ± 944.80	139.29 ^{bcd} ± 28.62	99.16 ^{abcd} ± 24.93
D2FOF4	12	2120.36 ^a ± 251.57	141.83 ^a ± 28.48	146.33 ^a ± 25.11	2073.80 ^{abc} ± 411.90	143.11 ^{ab} ± 33.62	90.22 ^{bcd} ± 9.16	2085.44 ^a ± 367.89	142.79 ^{bcd} ± 31.15	104.25 ^{abcd} ± 28.63
D0SFOF1	11	2057.52 ^a ± 843.76	166.25 ^a ± 38.24	101.25 ^a ± 12.99	982.21 ^c ± 472.47	64.83 ^e ± 11.64	67.67 ^e ± 9.29	1764.25 ^a ± 891.78	138.59 ^{bcd} ± 57.40	92.09 ^{bcd} ± 19.53
D0SFOF2	12	2007.78 ^a ± 689.64	182.06 ^a ± 45.7	116.17 ^a ± 20.33	1339.45 ^{abc} ± 48.60	121 ^{abcd} ± 8.19	118.17 ^a ± 26.6	1840.70 ^a ± 661.58	166.79 ^{abc} ± 47.89	116.67 ^{abc} ± 20.73
D0SFOF3	12	1969.21 ^a ± 605.09	164.83 ^a ± 27.65	109.44 ^a ± 23.69	1391.50 ^{abc} ± 243.40	88.83 ^{bcd} ± 10.05	85.17 ^{bcd} ± 19	1824.78 ^a ± 587.64	145.83 ^{bcd} ± 41.90	103.37 ^{abcd} ± 24.38
D0SFOF4	12	2181.19 ^a ± 633.01	175.11 ^a ± 52.24	114.11 ^a ± 25.72	1416.02 ^{abc} ± 305.16	129.5 ^{abcd} ± 10.64	108.5 ^{abc} ± 14.08	1989.90 ^a ± 654.30	163.70 ^{abc} ± 49.30	112.70 ^{abc} ± 22.88
D0FOF1	12	2388.55 ^a ± 400.57	170.22 ^a ± 45.58	122.61 ^a ± 24.94	1278.81 ^{abc} ± 7.88	79.83 ^{cde} ± 20.84	86.33 ^{bcd} ± 12.39	2111.11 ^a ± 607.13	147.62 ^{bcd} ± 57.10	113.54 ^{abc} ± 27.37
D0FOF2	11	2228.12 ^a ± 776.68	183.75 ^a ± 35.97	116.44 ^a ± 21.55	1033.76 ^{bc} ± 214.91	122 ^{abcd} ± 3.97	99 ^{abcd} ± .36	1902.39 ^a ± 861.82	166.91 ^{abc} ± 41.72	111.68 ^{abc} ± 22.80
D0FOF3	10	2215.89 ^a ± 1108.65	212.69 ^a ± 38.19	129 ^a ± 19.88	1483.53 ^{abc} ± 280.88	159.5 ^a ± 78.49	109 ^{abc} ± 0	2069.42 ^a ± 1029.60	202.05 ^a ± 48.18	125.00 ^a ± 19.45
D0FOF4	12	2322.79 ^a ± 787.2	188.5 ^a ± 45.08	124.94 ^a ± 17.86	1287.39 ^{abc} ± 98.37	134.83 ^{abcd} ± 3.79	110.5 ^{ab} ± 8.89	2063.94 ^a ± 819.58	175.08 ^{ab} ± 45.49	121.33 ^{ab} ± 17.00
Overall mean	188	2119.44	171.48	120.21	1853.95	114.74	86.13	1985.29	142.8112	102.99
CV		33.83	22.59	16.53	31.11	21.75	15.35	35.25	27.93	22.75
DF		15	15	15	15	15	15	15	15	15
p-value		0.875 ^{ns}	0.104 ^{ns}	0.056 ^{ns}	0.0001 ^{***}	0.000 ^{***}	0.000 ^{***}	0.485 ^{ns}	0.000 ^{***}	0.000 ^{***}

***significant $p < 0.001$; ns: not significant $p > 0.05$. The values sharing the same letter are not significantly different according to the LSD test, $p < 0.05$.

4. Discussion

4.1. Effect of the Treatments on Iron-Reducing Bacteria's and Sulfate-Reducing Bacteria's Activities in the Soil

The study evidenced the presence and activities of SRB and IRB populations in rice fields in Burkina Faso. These bacteria have been found in many rice fields in West Africa [34] and especially in Burkina Faso [28] [29]. SRB and IRB are ubiquitous and active in the anaerobic conditions of flooded rice fields [16] [35]. The experiment reported here showed that the soil sulfide concentration was higher than the toxicity threshold (3.1 mg.L^{-1} of soil solution) determined by Fort *et al.* [36]. In addition, the Fe II concentration was higher than the critical limits of 250 mg.L^{-1} reported by De Dorlodot *et al.* [37]. The results also indicated that the soil sulfide and Fe II concentrations were higher in the rainy season than in the dry season. In the dry season, the temperatures are higher and could reach almost 40°C [38]. At this temperature, some forms of sulfide can easily be evaporated from the soil. In addition, during the rainy season, evapotranspiration is low due to low temperatures, and there is a high accumulation of organic matter in the lowlands due to water flow [21], which can result in high sulfide and Fe II production in fields. Indeed, there is a high accumulation of organic matter and chemical components such as iron in lowlands during the rainy season due to the drainage of water from the top slopes [21]. Previous research also exhibited that high organic matter content in submerged rice fields increases IRB activities, leading to Fe II production and rice iron toxicity symptoms [34] [39]. On the other hand, other research has shown that iron toxicity symptoms in rice fields increased during dry seasons [40].

During the study, there was no substantial difference in soil sulfide and Fe II content between drained and non-drained plots. This result confirms that water drainage does not efficiently reduce the activities of SRB and IRB populations, as reported in our previous research [25]. Indeed, the oxygenation of the submerged soils throughout the drainage was not enough to limit the anaerobic IRB and SRB population activities. It could be that there were some microsites in the soil where anaerobic bacteria could survive and grow. Indeed, previous studies have highlighted that some IRB [41] and SRB populations [42] [43] can tolerate oxygen by adapting their metabolic process.

The results of the experiment also showed that the rice variety alone did not have a significant effect on the soil sulfide content. On the other hand, variety alone had a significant effect on the soil Fe II content, with the FKR76 (iron-sensitive) variety showing higher Fe II concentrations than the FKR62N (iron-tolerant) variety. This result can be explained by the mechanisms of tolerance used by some rice varieties in iron toxicity conditions. Indeed, some tolerant rice varieties can resist iron toxicity by excluding Fe from entering root cells through the formation of the Fe plaque in the apoplast [44] [45], Fe retention in roots, root-to-shoot Fe movement restriction [46], Fe compartmentalization in organelles or Fe-storing proteins [47], or by antioxidant accumulation for reactive oxygen species

(ROS) detoxification [44] [48].

During the experiment, plots of the FKR62N rice variety exhibited higher sulfide contents than the FKR76 plots. Moreover, the SFOF1 (control plot with no organic and mineral fertilizers) and FOF1 (organic matter and no mineral fertilizers) treatments showed the highest sulfide content for the FKR62N rice variety. For the FKR76 rice variety, the FOF1 (organic matter and no mineral fertilizers) treatment significantly increased the sulfide concentration. However, for both rice varieties, all the plots that were amended with dolomite and natural phosphate, with or without organic matter amendment (F3 = NPK + Urea + Dolomite + Zn, F4 = NPK + Urea + Natural Phosphate), presented the lowest sulfide content.

The study showed that the field amendment with organic matter increased sulfide content in the soil in the plots of both varieties tested. Organic matter can be used as a nutrient source by the IRB and SRB populations, resulting in sulfides and Fe II release in the fields. Indeed, according to previous studies, sulfate availability and reduction, soil temperature, redox potential, pH, organic matter content, CO₂ and bicarbonate accumulation, and sulfide ion immobilization by Fe II are the main factors influencing the sulfide accumulation [49]. Gao *et al.* [50] also proved that organic matter amendment (such as straw) in sulfide-toxic rice fields may induce soil sulfide toxicity. Moreover, the experiment showed that mineral fertilizers such as NPK + urea, natural phosphate, and dolomite significantly decrease the sulfide content in fields. Jacq *et al.* [51] revealed that NPK applied in rice fields reduces the number and activities of SRBs in sulfide-toxic paddy soil. Indeed, a previous study indicated that soil amendment with NPK improves soil oxygenation due to increasing oxygen release by rice roots [51]. Therefore, soil aeration could lead to decreased SRB population growth, which is mostly anaerobic. Some research also showed that applying dolomite in acid-sulfate soils can significantly increase the soil pH, resulting in increased rice growth and yield [52] [53]. Shaabanet *et al.* [54] also evidenced that dolomite liming in acidic rice paddy soils impacts the microbial populations and soil biochemistry in these fields. In the present study, the dolomite applied in the field could have influenced the activity of the SRB population. The study has also shown that natural phosphate can control sulfide content in rice field soil. Indeed, Ahmad *et al.* [55] indicated that the application of natural phosphate in sulfide-toxic paddy soil reduces proton availability, leading to a pH increase, which results in decreases in sulfide production and accumulation in the field.

During the study, the SFOF2 treatment for the FKR76 rice variety and the FOF2 treatment for the FKR62N rice variety resulted in the highest Fe II concentrations. On the other hand, for both rice varieties, all the plots that were amended with dolomite and natural phosphate, with or without organic matter amendment (F3 = NPK + Urea + Dolomite + Zn, F4 = NPK + Urea + Natural Phosphate), presented the lowest Fe II content. Indeed, NPK + Urea amendment increased the soil Fe II content. The results showed that natural phosphate and dolomite application reduced the soil Fe II content for both of the rice varieties, whether with

drainage or without drainage. This result could be explained by the fact that the natural phosphate and dolomite amendments could reduce Fe II bioavailability and decrease IRB population activity. Indeed, the phosphate application reduced the Al and Fe toxicity through precipitation and formation of Al-P and Fe-P compounds [56] [57]. Moreover, Suriyagoda *et al.* [23] revealed that the application of dolomite in iron-toxic rice fields led to a decrease in the exchangeable soil Fe II concentration and an increase in the soil pH and phosphate availability. Indeed, the natural phosphate, by increasing the pH of acidic soil [55], can modulate the bacterial community structure and activities [58]. Likewise, other studies have highlighted that soils containing high clay and free Fe₂O₃ contents, and flooded acid sulfate soils, similar to the soils encountered in the current study, have the highest P sorption capacity, thereby improving the Rock Phosphate dissolution [59] [60].

The study's results reveal differential responses of the rice varieties to the treatments applied, with the FRK76 variety showing greater sensitivity to the type of fertilizers used. Indeed, the treatment effects were significant for this iron-sensitive variety, with combinations such as D0FOF3, D2FOF4, and D2FOF2 significantly enhancing tillering, panicle number, and grain yield. This observation could be explained by the high sensitivity of the FRK76 variety to iron toxicity. The application of mineral fertilizers (NPK + urea), in combination with organic manure (FO), dolomite (NPK + urea + dolomite + Zn), and/or natural phosphate (NPK + urea + natural phosphate), appears to improve the variety's tolerance to iron and/or sulfur-induced toxicity. Indeed, many studies have reported that some mineral fertilizers [61], organic matter [44], dolomite [23], and rock phosphate [55] amendments can improve rice productivity in iron or sulfide toxicity conditions. Also, Hu *et al.* [62] and Martinengo *et al.* [63] investigated the impact of different iron and phosphate treatments on the formation and morphology of iron plaque on rice roots. Their results showed that increased phosphate (PO₄³⁻) content reduced the quantity and thickness of iron plaque—a mechanism known to alleviate iron toxicity by limiting iron uptake at the root surface.

4.2. Effect of Treatments on Rice Productivity

In the current study, treatments D2FOF2, D2FOF3, D2FOF4, and D0FOF3 proved promising in enhancing productivity across both tested rice varieties, with a more pronounced effect on the iron-sensitive FRK76 variety. These results highlight the crucial role of organic matter (FO), which, whether applied under drained or undrained conditions, significantly promotes tillering and panicle development—particularly in the iron-sensitive variety. The positive impact of organic fertilization may be attributed to its ability to supply essential nutrients, helping to counteract deficiencies exacerbated under iron-toxic soil conditions [44]. This effect appears to be maximized when organic inputs are combined with periodic soil drainage and/or supplementary inputs such as dolomite and natural phosphate. Furthermore, dolomite (F3) and natural phosphate (F4) application in

iron and sulfate-toxic rice fields could improve rice productivity in those edaphic conditions. Indeed, Farhati *et al.* (2023) showed that incorporating dolomite into iron-toxic soils helps to raise the soil pH and subsequently reduces the availability of Fe II to rice plants, thereby limiting the manifestation of iron toxicity symptoms. Similarly, studies by Ahmad *et al.* [55] and Bagayogo *et al.* [64] indicate that applying phosphate fertilizers to acidic or iron-toxic soils contributes to an increase in pH, which positively influences rice growth parameters such as plant height and grain yield. However, the relatively high coefficients of variation ($CV > 20\%$ for some variables) reflect considerable variability, likely due to fluctuating environmental factors such as seasonal effects or drainage conditions.

5. Conclusions

This study highlighted the presence and activity of SRB and IRB populations in rice fields in Burkina Faso, West Africa. The analyses showed that soil sulfide and Fe II content exceeded critical toxicity thresholds. These concentrations were higher during the rainy season compared to the dry season due to low evapotranspiration and organic matter accumulation. In contrast, high temperatures in the dry season likely favored sulfide evaporation, reducing its accumulation.

The study also confirmed that drainage alone was not effective in mitigating SRB and IRB activity. Additionally, regarding soil iron toxicity, the tested rice varieties responded differently. Plots with the FKR76 variety (iron-sensitive) indicated higher Fe II concentrations than plots with the FKR62N (iron-tolerant) variety. This could be explained by iron tolerance mechanisms specific to certain rice varieties, such as Fe II exclusion, root storage, and antioxidant mobilization.

The experiment revealed that organic matter amendments significantly increased soil sulfide content, while the addition of mineral fertilizers such as NPK, urea, natural phosphate, and dolomite helped reduce the sulfide content. On the other hand, NPK + Urea amendment increased the soil Fe II content. Furthermore, plots amended with dolomite and natural phosphate had significantly lower Fe II and sulfide content.

This study reveals that the iron-sensitive rice variety FRK76 is more responsive to the treatments applied than the FRK62N variety, under iron and sulfide toxicity conditions. The application of natural phosphate and dolomite reduced the sulfide content in soil by up to 39.86% (F3) and 43.80% (F4), respectively. These treatments reduced the soil Fe II by up to 7.16% (F3) and 10.45% (F4), respectively. Treatments combining mineral fertilizers (F2 = NPK + Urea) with organic matter (FO), dolomite (F3), and natural phosphate (F4) and drainage (D2FOF2, D2FOF3, D2FOF4, and D0FOF3) proved most effective in enhancing tillering, panicle formation, and grain yield, particularly for the FRK76 variety. The beneficial effects are likely due to improved nutrient availability and reduced toxic ion availability (iron and sulfides), which is supported by findings from previous studies. These results emphasize the importance of considering environmental factors, rice varieties, and fertilization, and their interactions, to develop strategies to optimize the

agricultural performance of rice in soils affected by iron and sulfide toxicity.

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Conflicts of Interest

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

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