

# Effect of Combined Application of Subsurface Drainage and Mineral Fertilization on Iron-Reducing Bacterial Populations' Developments and Fe<sup>2+</sup> Uptake by Two Rice Varieties in an Iron Toxic Paddy Soil of Burkina Faso (West Africa)

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## Abstract

Rice is one of the staple crops in Burkina Faso. However, the local production covers only 47% of the population demands. One of the main reasons of the poor productivity in Burkina Faso is iron toxicity which is related mainly to the activity of Iron Reducing Bacteria in the rice field's ecosystems. In order to control the harmful effects of Iron Reducing Bacterial populations and to improve rice productivity, a pots experiment was conducted at the experimental site of the University Ouaga I Pr. Joseph KI-ZERBO. An iron toxic soil from Kou Valley (West of Burkina Faso) and two rice varieties, BOUAKE-189 and ROK-5, sensitive and tolerant to iron toxicity, respectively, were used for the experiment. The pots were drained for 14 days (D2) and amended with chemical fertilizers (NPK + Urea and NPK + Urea + Ca + Mg + Zn complexes). Control pots without drainage and fertilization (D0/NF) were prepared similarly. The kinetics of Iron Reducing Bacterial populations and ferrous iron content in soil near rice roots were monitored throughout the cultural cycle using MPN

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and colorimetric methods, respectively. The total iron content was evaluated in rice plant using a spectrometric method. Data obtained were analyzed in relation to drainage and fertilization mode, rice growth stage and rice yield using the Student's t-test and XLSTAT 2014 statistical software. The experiment showed that the combined application of subsurface drainage and NPK + Urea + Ca + Mg + Zn fertilization, reduced significantly the number of IRB in the soil near rice roots for both rice varieties ( $p = 0.050$  and  $p = 0.020$ ) increased the leaf tissue tolerance to excess amounts of Fe, and rice yield.

## Keywords

Iron Reducing Bacteria, Rice Variety, Iron Uptake, Subsurface Drainage, Fertilization

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## 1. Introduction

Rice is the fourth staple crop after sorghum, millet and maize in Burkina Faso [1]. However, the local production of rice covers only 47% of the population demands. To meet the increasing requirements of rice, the Government of Burkina Faso imports about 260,000 tonnes of rice per year, with more than 30 milliards of CFA francs of currency losses [2]. Sikirou *et al.* [3] reported that one reason for the poor productivity in wetlands in West Africa is the prevalence of biotic and abiotic stresses. The abiotic stresses include drought, submergence and iron toxicity [4] [5] [6]. The iron toxicity is recognized as one of the most widespread nutritional disorders and one of the major edaphic constraints of lowlands in West Africa that affects crop growth, especially rice growth [7] [8] [9] [10]. Haefele *et al.* [11] estimated that 19% of the total rice area in Africa has a potential risk of Fe toxicity [3]. Chérif *et al.* [5] reported also that about 55% of the rice area is affected by Fe toxicity in three West African countries (Guinea, Ivory Coast and Ghana), with about 10% of rice cultivation area abandoned due to severe iron toxicity.

In Burkina Faso, many lowland crop fields were even abandoned due to iron toxicity [12]. Iron toxicity affects many agricultural plains in Burkina Faso like Moussodougou, Tiéfora and Kou Valley [13] [14]. For the latter, since 1986, 300 ha of fields were abandoned because of ferrous intoxication [12], and most among these intoxicated fields remained uncultivated up to date [14].

Iron toxicity is a major nutritional disorder affecting rice production in irrigated and rainfed lowland soils and occurs as a consequence of the reduction of insoluble  $Fe^{3+}$  into soluble  $Fe^{2+}$  under both anaerobic and low pH conditions [10] [15] [16]. Indeed, most mineral soils are rich in Fe, but under aerobic conditions,  $Fe^{3+}$  biodisponibility remains low. In contrast, in flooded soils,  $Fe^{2+}$  is quickly absorbed by plant roots and can lead to cellular Fe overload, inducing numerous metabolic disorders [17].

Iron reduction into ferrous form is largely controlled by microbial processes [18]. In fact, in natural systems, Fe (III) minerals can be reduced by strictly anaerobic or facultative Fe-reducing bacteria using a wide range of organic compounds or  $H_2$  as electron

donors [19] [20]. As soon as a soil becomes flooded, the dissolved oxygen is consumed by aerobic bacteria and chemical oxidation reactions. Oxygen is depleted fast in most regions of the soil and alternative electron acceptors are used [7]. In anoxic conditions, Fe (III)-compounds are reduced and it results in ferrous ions production. Thus, the IRB population produces  $\text{Fe}^{2+}$  by coupling the Fe(III) reduction to the oxidation of substrates to support growth [21] [22] [23] [24] [25].

Therefore, iron toxicity is a condition caused by the microbial reduction under flooded conditions of insoluble iron-III into soluble iron-II, which can be taken up by rice plants in excess amounts [18].

Iron toxicity occurs when the rice plant accumulates a toxic concentration of Fe in the leaves [26] [27] [28]. High concentrations of Fe in soil solution also decrease the absorption by the rice plant of other plant nutrients, especially Phosphorus and Potassium [10] [29]. Excessive Fe uptake results in increased polyphenol oxidase activity, leading to the production of oxidized polyphenols, the cause of leaf bronzing. Large amounts of Fe in plants can give rise to the formation of oxygen radicals, which are phytotoxic and responsible for protein degradation and peroxidation of membrane lipids [30] [31].

The symptoms of iron toxicity vary with rice cultivar [30]. The excess Fe is accumulated as brown dots in plant cells, causing bronzing symptoms of leaves, blackening of roots and damage to cellular membranes [32] [33] [34] [35].

Benckiser *et al.* [36] and Becker and Asch [10] reported that iron-induced yield is frequently associated with a poor nutrient status of the soil. Kosaki and Juo [37] underlined also that the poor level of development of lowlands in West Africa, epitomized with bad water management which generates favourable conditions for the occurrence of iron toxicity [38].

The present experiment was developed in the sensitive site of Kou Valley, to determine the effects of chemical fertilization and subsurface drainage on microbiological and chemical parameters sustaining iron toxicity in paddy fields and on rice yield. Therefore, plastics pots were filled with a sensitive soil, amended by chemicals fertilizers and drained periodically during rice cultivation. The Iron Reducing Bacterial (IRB) populations' density,  $\text{Fe}^{2+}$  content in the paddy soil and iron accumulation in rice plant, were recorded during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties (sensitive and tolerant to iron toxicity, respectively).

## 2. Materials and Methods

### 2.1. Soil Sampling and Experiments Sites Location

The soil used for the experiments was collected at Kou Valley, a site located at the West of Burkina Faso (11°23'12" N and 4°23'25" W) (**Figure 1**). The physical and chemical properties of the soil used in the experiments were described in our previous report [39].

The soil collected was carried out to the experimental site of the University Ouaga I Pr. Joseph KI-ZERBO (12°22'45.7" N and 1°29'52.5" W, **Figure 1**). The experiments

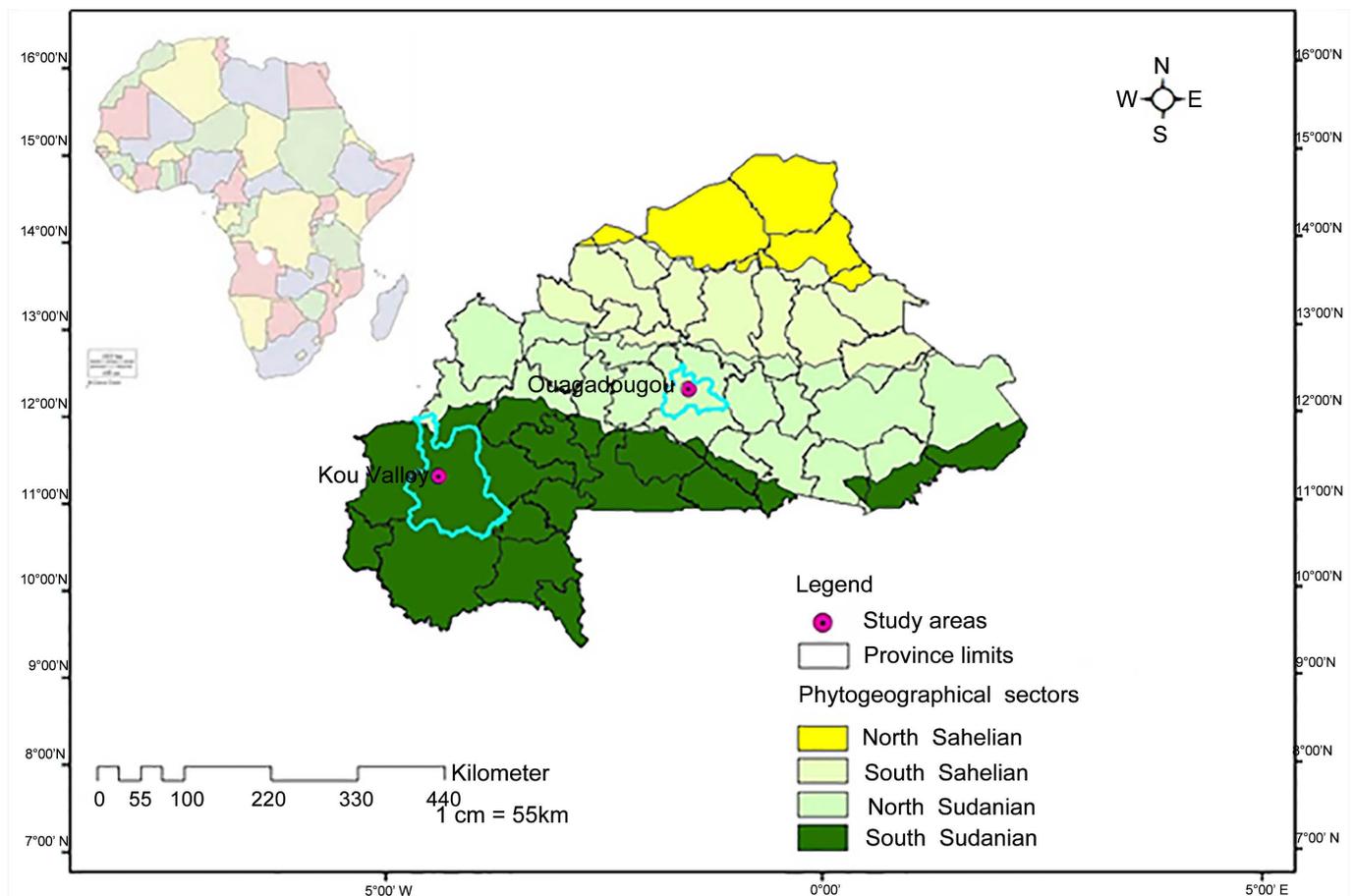
were performed from June to October 2014.

## 2.2. Climatic Characteristics of the Experimental Site Area

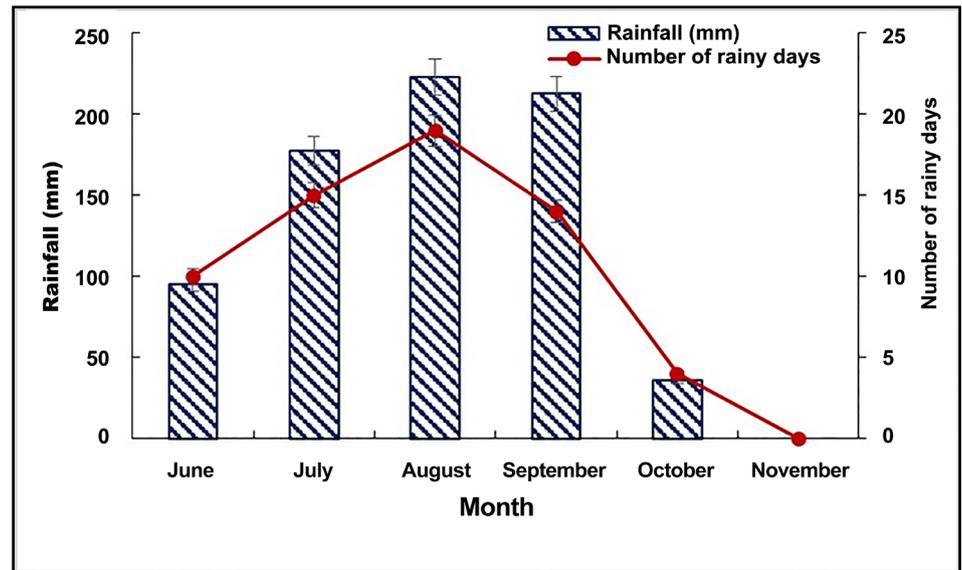
The experiments site was located in the central plateau of Burkina Faso consisting of a rainy season (June-October) with peaks from July to September, and a dry season (November-May). During the experiments period (June-October 2014), sixty-two rainy days were recorded, with an average rainfall of 743.5 mm (Figure 2). The average daily temperature was 29.03°C with minimum at 27.6°C and maximum at 30.7°C (Figure 3). The daily relative hygrometry (RH), varied from 45% to 77% (Figure 3), with a sunniness average of 7.26 hours/day.

## 2.3. Plant Material

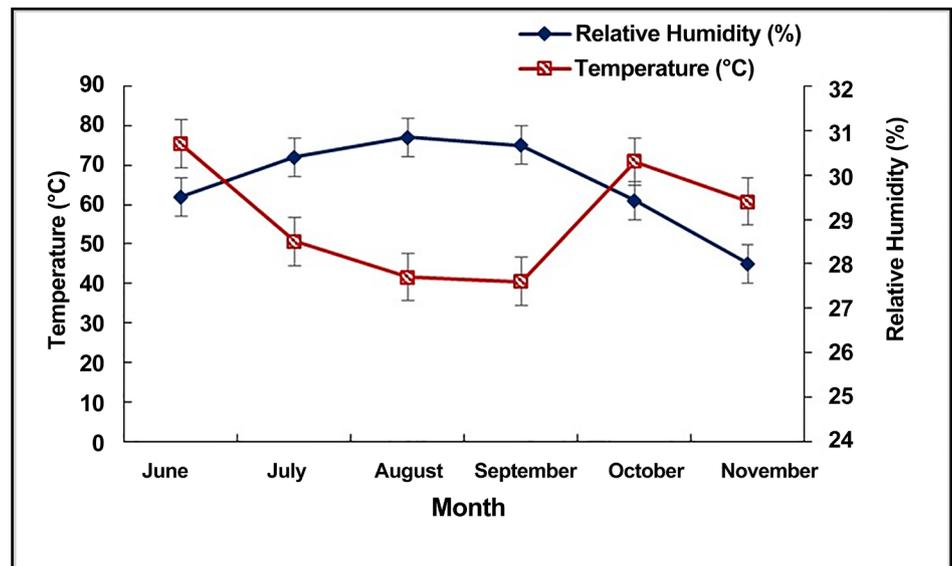
Plant materials used for experiments were BOUAKE-189 and ROK-5 rice varieties, originating from the Asian species *Oryza sativa* L (Indica varietal group), respectively [40]. Native from Indonesia, BOUAKE-189 rice variety, is adapted to the irrigated rice, and is issued from the breeding selection of the National Agricultural Research Center (CNRA, Ivory Coast). This rice has an average yield estimated to 4.5 tons/ha, with a cy-



**Figure 1.** Location of sampling and experimental sites.



**Figure 2.** Monthly rainfall and monthly number of rainy days from June to November 2014.



**Figure 3.** Monthly temperatures and monthly relative humidity from June to November 2014.

cle of seedling-maturity of 125 to 130 days. With an average yield estimated to 5 tons/ha, the ROK-5 rice variety is an inbred rice issued from the West Africa Rice Development Association (WARDA, Rokupr, Sierra Leone) and adapted to mangrove rice.

#### 2.4. Pots Experiments

Experiments were carried out as previously described by Otoidobiga *et al.* [39]. Seventy two plastics pots with 25 cm<sup>3</sup> of bulk were used in 3 replications throughout the study. At the bottom of each pot, an external tap was installed to sub-drain the soil. After 2 weeks of flooding, 15 day-old rice plants were transplanted. Two rice varie-

ties, BOUAKE-189 [38] and ROK-5 [41], sensitive and resistant to iron toxicity, respectively were used. The soil was continuously flooded until rice maturity and harvest (120 days after flooding). Three replications and three modes of fertilization were performed throughout the study: without fertilization, NPK + Urea and NPK + Urea + Zn + Ca + Mg, respectively. The doses of N-P-K (14 - 23 - 14), CaCO<sub>3</sub>, ZnO and MgCl<sub>2</sub> application in pots were in the ratio of 720:50:22.4:20 mg/kg of dry soil according to the recommended doses of 300 kg/ha for N-P-K, 10 kg/ha for ZnO, 250 kg/ha for CaCO<sub>3</sub> and 8.92 kg/ha of MgCl<sub>2</sub> at the rice transplanting [1] [36] [38]-[43]. The Urea fertilizer (240 mg/kg of dry soil) was applied in two further dressings (at rice transplanting, and 60 days after transplanting, respectively) according to the recommended dose of 100 kg/ha [1]. Two modes of drainage were applied during the study: without drainage (D0) and drainage for 14 days (D2), respectively by regulating the drained water flow from the bottom [18] as recommended by our previous results [18]. Control pots, without drainage and fertilization (D0/NF) were prepared similarly.

### 2.5. Iron Reducing Bacterial Populations Monitoring

The concentration of Iron Reducing Bacteria (IRB) in the soil were determined by the most-probable-number (MPN) method, and a culture medium adapted from Hammann and Ottow [44] consisting of Glucose (20 g), Sodium acetate (5 g), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.05 g), K<sub>2</sub>HPO<sub>4</sub> (0.25 g), KH<sub>2</sub>PO<sub>4</sub> (0.25 g), NaCl (0.05 g), Na<sub>2</sub>-MoO<sub>4</sub>·2H<sub>2</sub>O (0.005 g), CaCO<sub>3</sub> (5 g), Fe<sub>2</sub>O<sub>3</sub> (1 g) per liter of distilled water. The pH was adjusted to 7.2 by addition of NaOH. The medium (9 ml) was dispensed into 16 ml Hungate tubes and autoclaved at 121°C for 15 min. One milliliter of the 10<sup>-1</sup> to 10<sup>-9</sup> fold of diluted soil suspension was inoculated to 9 ml of the basal medium for enumeration in Hungate tubes. Tubes were incubated at 30°C for five days. Formation of reddish coloration after addition of a reagent containing 0.2% ortho-phenantroline and 10% acetic acid, sustaining the reduction of Fe<sup>3+</sup> (Fe<sub>2</sub>O<sub>3</sub>) into Fe<sup>2+</sup> (reddish coloration) was used for detection of positive tubes after incubation period. The most probable numbers of IRB were calculated from a table of MPN for three tubes. The enumeration of bacteria was performed before flooding when the soil was dried, on transplanting day (two weeks after flooding) and during the rice growth stages until harvest near rice roots [39].

### 2.6. Determination of Ferrous Iron Concentration in Soil

From the soil sample (2 g) for bacterial enumeration and at the same periods during the rice cultural cycle, the ferrous iron was extracted using extraction medium (AlCl<sub>3</sub> 0.5%), according to the method of Vizier and Blanch [45]. The extracted ferrous iron solution (80 µl) was removed by micropipette and rapidly introduced into microplate containing 20 µl of a reagent containing 0.2% ortho-phenantroline and 10% acetic acid. Immediately after mixing (for 5 s), the iron content of the soil was determined by measuring the absorbance at 510 nm using a Spectronic 61 photometer.

## 2.7. Determination of Fe Content in Rice Plant

The Total Fe was analysed in the leaves and roots of the two rice varieties described above. Young leaves were taken from each pot during the cultural cycle of rice. At harvest, the aerial biomass and the roots of each plant were also collected. Completely dried plant parts were digested in a diacid mixture of nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) (15:2) [46]. The dried plant parts (500 mg) were immersed overnight in 15 mL concentrated HNO<sub>3</sub> in a conical flask. On the following day, 2 mL HClO<sub>4</sub> was added and the sample was digested on a hot plate at 60°C for 2 h, followed by further digestion at 90°C until white fumes of HClO<sub>4</sub> effervesced out. The leftover liquid was transferred to a 50 mL volumetric flask and diluted with doubly distilled water. The solution was filtered through Whatman paper No 41 filter paper and the total Fe content was determined by Atomic Absorption Spectrometry [47].

## 2.8. Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) with regard to the IRB populations' development and activity, drainage and fertilization modes and rice yield using XLSTAT 2015 software. Mean variables were compared using the Fishers' test at probability level  $p = 5\%$  [39].

## 3. Results and Discussion

### 3.1. Iron Reducing Bacterial Populations' Dynamics in Soil near Rice Roots

The variance of the numbers of IRB in soil near rice roots, in relation to subsurface drainage and combined application of subsurface drainage and fertilization is presented in **Table 1**. The Fishers' test revealed that the number of bacteria in the soil near BOUAKE-189 and ROK-5 rice roots wasn't significantly related to the subsurface drainage ( $p = 0.891$  and  $p = 0.941$ , respectively). These results can be explained by the survival of IRB in drainage condition [18]. Thus, Ouattara [12], Jacq *et al.* [48] and Liesack *et al.* [49] reported that some IRB can survive and grow in aerobic compartments in the presence of low pressure of oxygen where a surplus of oxygen is released by healthy roots. The IRB number was significantly related to the combined both factors ( $p = 0.050$  and  $p = 0.020$ , **Table 1**). From the experiment, the D2 drainage in the NPK + Urea + Ca +

**Table 1.** Variance of IRB number in soil near rice roots in relation to subsurface drainage and fertilization, during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties.

Source of variation	df	log (IRB number/g dry soil)			
		BOUAKE-189		ROK-5	
		F	p	F	p
Drainage	1	0.019	0.891 <sup>ns</sup>	0.005	0.941 <sup>ns</sup>
Drainage*Fertilization	2	2.914	0.050 <sup>*</sup>	3.998	0.020 <sup>*</sup>

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

Zn + Mg amended pots showed the lowest average number of IRB population in soil near rice roots, during the cultural cycle of both rice varieties ( $2 \times 10^7$  and  $2.6 \times 10^7$  cells/g dry soil, for BOUAKE-189 and ROK-5 rice variety, respectively).

The present results are in agreement with those obtained in our previous study on Kamboinse paddy soils [18], which showed that subsurface drainage combined to mineral amendment (D2 + NPK + Urea) reduced significantly the number of IRB in microplots ( $p = 0.050$  and  $p = 0.020$ , respectively for the two rice varieties). According to our previous study on the same paddy soil the mean density of IRB population in the soil decreased in the NPK + Urea + Ca + Zn + Mg amended pots for BOUAKE-189 and ROK-5 rice varieties, respectively [39]. Furthermore, Benckiser *et al.* [36] and Jacq *et al.* [48] reported that the number of IRB decreased with increased supply of K, Ca, and Mg for IR22 and IR42 rice varieties (susceptible and tolerant to iron toxicity, respectively). Trolldenier [50] showed also that a sufficient mineral nutrition of potassium was important in maintaining the oxidising power of rice roots and in the reducing of IRB populations in rice fields.

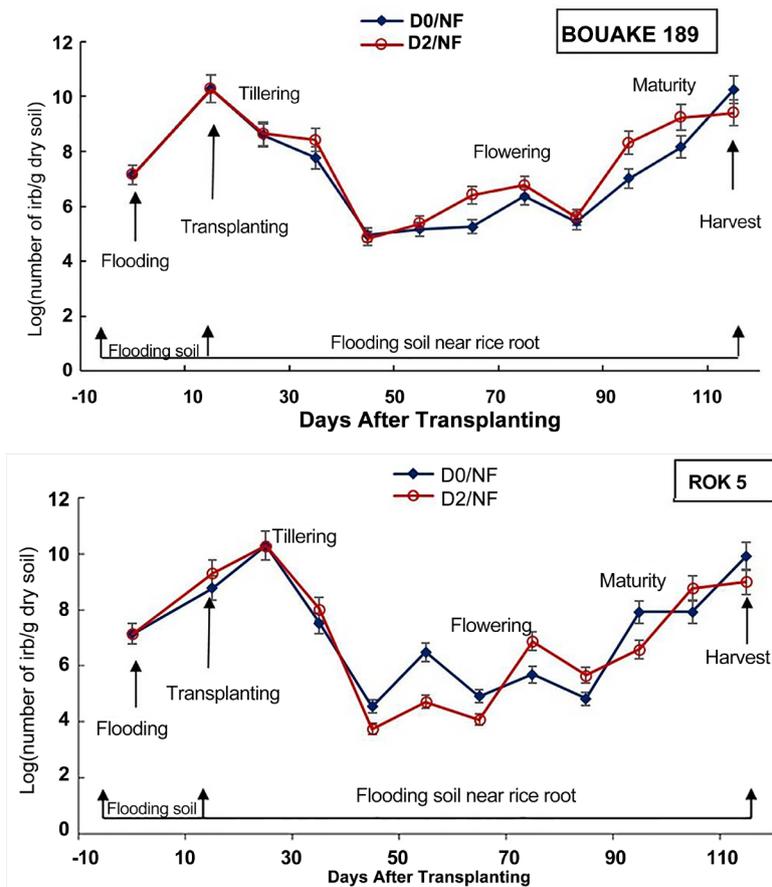
Therefore, combined application of subsurface drainage and fertilization can lead to a significant reduction of IRB number in rice field.

The experiment showed also that the number of IRB in the soil near rice roots increased after two weeks of flooding in all pots for BOUAKE-189 and ROK-5 rice varieties (Figure 4, Figure 5). Soon as a soil is flooded or submerged by stagnant water, the reductive processes start [51]. Ethan and Odunze [6] reported that flooding affects electrochemical and chemical processes which in turn, affect soil fertility in a dynamic manner [12]. Thus, the growth of Iron Reducing Bacteria is stimulated by flooding [18] [39] [48] [52].

It appeared also, that the number of IRB in soil near rice roots increased gradually with fluctuations from transplanting day to rice flowering and maturity stages in all the paddy pots (Figure 4, Figure 5). The highest densities of IRB, in most pots, were recorded from rice tillering and flowering to maturity stages ( $10^8$  to  $10^{10}$  cells/g dry soil). Berthelin *et al.* [48] observed a same evolution of IRB population during rice cultural cycle in a Senegal paddy soil. Our previous results obtained on Kamboinse and Kou Valley paddy soils [18] [39] reported the same pattern. Indeed, Dobermann and Fairhurst [30] reported that the periods of intense metabolic activity of rice plant (e.g., tillering, flowering and maturity), result in an increase of rhizoflora population, which in turn leads to an increased demand for electron acceptors. Thus, the highest level of reduced soil condition corresponds at these stages of growth at which rice plant enhance the exudation of carbohydrates and other metabolites sustaining IRB population growth in soil [18] [21] [48]. These results indicate that in spite of drainage and fertilization mode, rice plant remains the main factor that modulates the dynamic of IRB during rice cultural cycle, as reported by Jacq *et al.* [48].

### 3.2. IRB Activity in Soil near Rice Roots

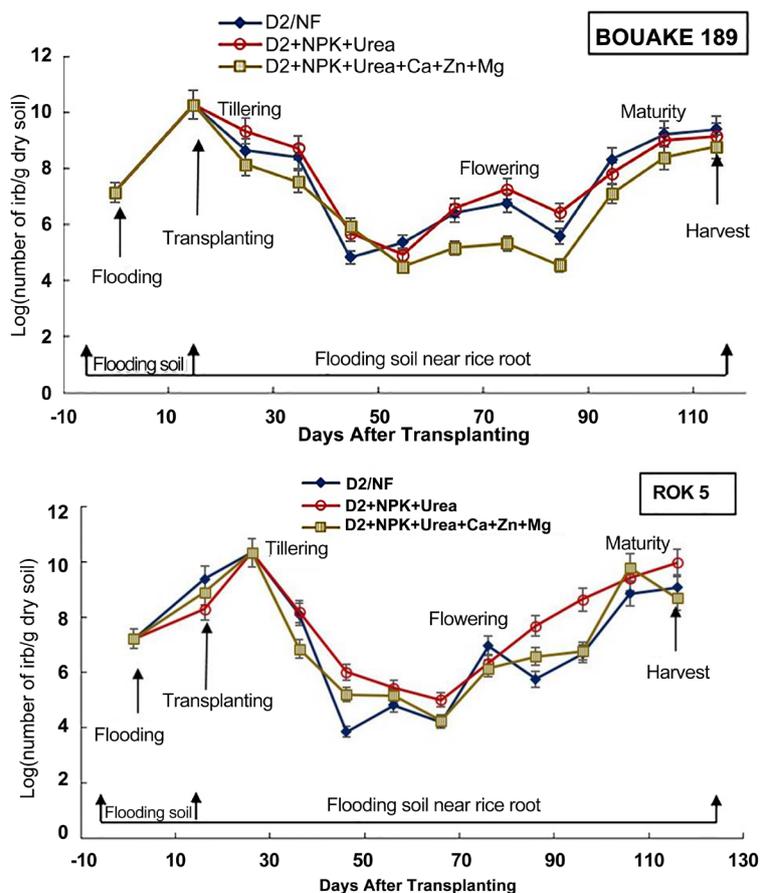
The variance of ferrous iron content in soil near rice roots in relation to subsurface drainage and combined application of subsurface drainage and fertilization for



**Figure 4.** Densities of Iron Reducing Bacteria in soil before flooding, at transplanting day and in soil near rice roots during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in pots without drainage and fertilization (D0/NF) and drained for 14 days (D2/NF), respectively (means of 3 replicates).

BOUAKE-189 and ROK-5 rice varieties are reported in **Table 2**. From the study, no significant effect of subsurface drainage and combined application of both factors was observed on the ferrous iron content in soil near rice roots, for BOUAKE-189 rice variety ( $p = 0.676$  and  $p = 0.940$ , respectively), and for ROK-5 rice variety ( $p = 0.746$  and  $p = 0.750$ , respectively), (**Table 2**).

From the present experiment, and in agreement with our previous study [18], the application of subsurface drainage alone, doesn't reduce significantly ferrous iron content in soil near rice roots for both varieties (**Figure 6**). These results can be explained by the production of ferrous iron in the rhizosphere, by surviving facultative anaerobic or aerobic Iron Reducing Bacteria [48]. As reported by Otoidobiga *et al.* [18], ferrous iron production in drained microplots could be also ascribed by chemical reduction of Iron III in the soil [53]. The heterogeneous distribution of oxygen through the drainage can also create anoxic compartments where ferrous iron can be reduced by surviving anaerobic IRB population [26] [49].



**Figure 5.** Densities of Iron Reducing Bacteria in soil before flooding, at transplanting day and in soil near rice roots during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in drained pots for 14 days without fertilization (D2/NF) and combined application of D2 drainage, NPK + Urea and NPK + Urea + Ca + Zn + Mg fertilization (D2 + NPK + Urea and D2 + NPK + Urea + Ca + Zn + Mg), respectively (means of 3 replicates).

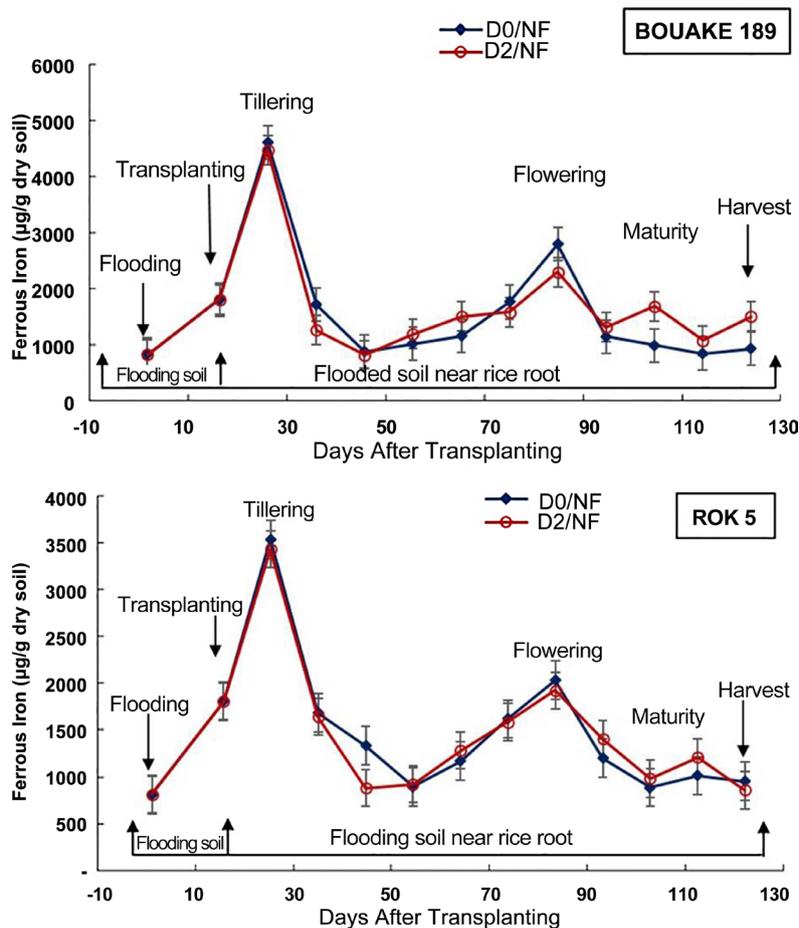
**Table 2.** Variance of ferrous iron content in soil near rice roots in relation to subsurface drainage and fertilization, during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties.

Source of variation	df	Ferrous iron content ( $\mu\text{g/g}$ dry soil)			
		BOUAKE-189		ROK-5	
		F	p	F	p
Drainage	1	0.175	0.676 <sup>ns</sup>	0.006	0.940 <sup>ns</sup>
Drainage* Fertilization	2	0.294	0.746 <sup>ns</sup>	0.288	0.750 <sup>ns</sup>

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

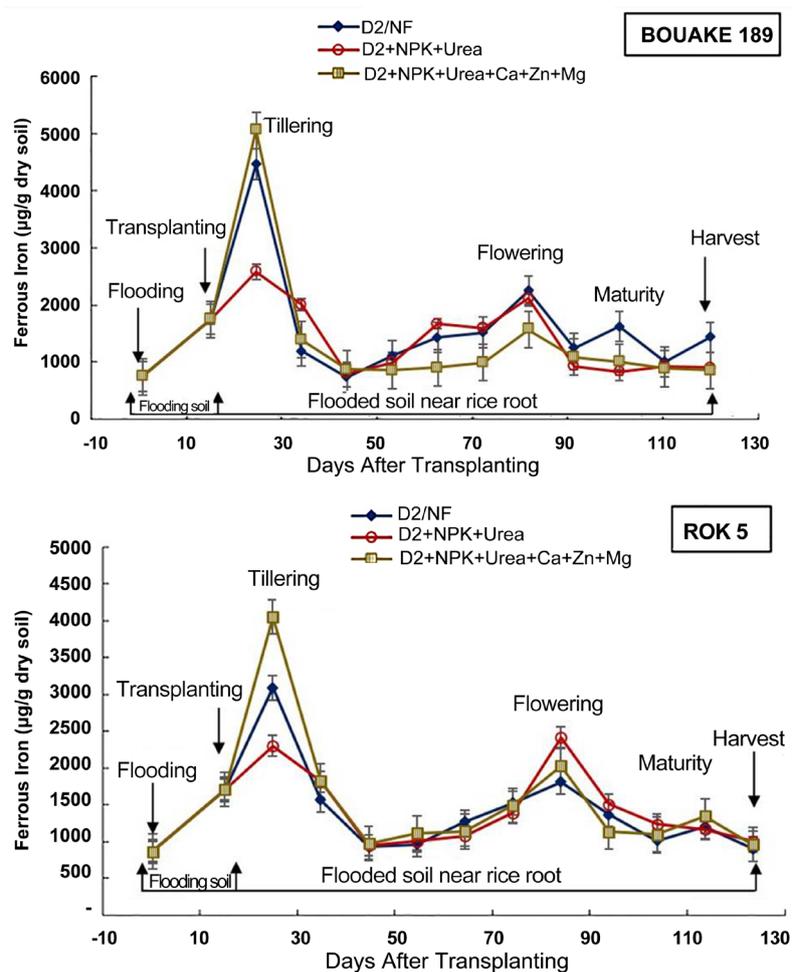
The average of the ferrous iron content in the soil near rice roots was low in D2 + NPK + Urea amended pots, relatively to the control pots, for the two rice varieties (Figure 7). Indeed, Trolldenier [50], revealed that the nutritional status of rice plant influences bacterial activity and the oxidation-reduction conditions around the roots.

Moreover, Jacq *et al.* [48] mentioned that, as long as P and K uptake by roots remains effective and roots are not damaged, the oxygen flow from aerial parts of the rice plant is sufficient to oxidize small amounts of Fe (II), and Fe (III)-oxides may precipitate on the root-soil interface (rhizoplane). Thus, we can deduce that the D2 subsurface drainage combined to NPK + Urea amendment may contribute to the decrement of iron reduction in rice paddy soil.



**Figure 6.** Evolution of soil ferrous iron content during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in pots without drainage (D0) and drained for 14 days (D2), respectively (means of 3 replicates).

Furthermore, the experiment evidenced the highest ferrous iron content in the soil of D2 + NPK + Urea + Ca + Zn + Mg pots (Figure 7), for the two rice varieties. Indeed, the role of Ca, Mg, and Zn fertilizers is the regulation of ferrous iron absorption in the rice plant, both as competing ion and by increasing the plant tolerance to iron toxicity [54] [55]. Therefore, in the present experiment, D2 + NPK + Urea + Ca + Zn + Mg application doesn't reduce ferrous iron production in rice fields, however, NPK + Urea + Ca + Zn + Mg application permits to support rice plant tolerance to the high content of toxic iron in soil as previous underlined by Otoïdobiga *et al.* [39].



**Figure 7.** Evolution of soil ferrous iron content during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in drained pots for 14 days without fertilization (D2/NF), and combined application of D2 drainage NPK+Urea and NPK + Urea + Ca + Zn + Mg fertilization (D2 + NPK + Urea, and D2 + NPK + Urea + Ca + Zn + Mg), respectively (means of 3 replicates).

The concentration of ferrous iron increased after two weeks of soil flooding for all the treatments (Figure 6, Figure 7). This result is in agreement with our previous results obtained in Kamboinse paddy soils [18]. Jacq *et al.* [48] and Betremieux [52] reported also the same increment of ferrous iron content in soil after the flooding of a Senegal paddy soil. Indeed, Becker and Asch [10] and Shahid *et al.* [56], underlined that iron toxicity occurs only in flooded soils and affects primarily the production of lowland rice. Thus, the reducing conditions of waterlogged lowland soils boost iron toxicity through solubilization of almost all iron into its ferrous form ( $\text{Fe}^{2+}$ ) [10] [56]. Moreover, Jacq *et al.* [48] indicated also that a major part of rice crop losses in southern Senegal, were ascribed to primary iron toxicity because of transplanting of seedlings immediately after flooding by most farmers.

In most pots, the highest content of ferrous iron in soil near rice roots was recorded

from rice tillering and flowering to maturity stages ( $10^3$  to  $5 \times 10^3$   $\mu\text{g/g}$  dry soil) (**Figure 6**, **Figure 7**). These results can be explained by enhanced iron microbial reduction in the rizosphere due to intensive exudation during the physiological active phase between heading and flowering [18] [21] [48] [57]. These results are in agreement with those obtained by Dobermann and Fairhurst [30] which showed that in these periods of intense metabolic activity, facultative and obligate anaerobic bacteria reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ . In fact, Ethan and Odunze [6] recorded that in reductive condition, the number of Iron Reducing Bacteria in soil increased almost parallel to the decrease in redox potential and to the increase in iron reducing power [49] [50] [58]. Jacq *et al.* [48] also revealed that despite the aeration mechanism of the roots, iron-reduction processes in the bulk soil are stimulated by the physiological activity and by the growth of rice roots. Moreover, many studies revealed also that redox potential was higher in soils near the plants than in soils away from the plants [50] [55]. These results highlighted that the nutritional status of the rice plant essentially influences the activity of bacteria and the oxidation-reduction conditions around the roots. Furthermore, Trolldenier [50] and Prade *et al.* [57] reported that root debris and/or exudation of carbohydrates at these stages of rice growth can decrease redox potential and stimulate microbial activity and anaerobic respiration (denitrification and/or ferric iron reduction).

### 3.3. Effect of Combined Application Subsurface Drainage and Fertilization on BOUAKE-189 and ROK-5 Rice Plants Total Iron Content

The effects of drainage and fertilization on iron content in BOUAKE-189 and ROK-5 rice plants were recorded during the study (**Table 3**, **Table 4**). The analysis of variance

**Table 3.** Variance of total iron content in rice roots in relation to subsurface drainage and fertilization, during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties.

Source of variation	df	Roots iron content ( $\mu\text{g/g}$ dry soil)			
		BOUAKE-189		ROK-5	
		F	p	F	p
Drainage	1	4.193	0.075 <sup>ns</sup>	2.140	0.182 <sup>ns</sup>
Drainage* Fertilization	2	3.260	0.092 <sup>ns</sup>	2.049	0.191 <sup>ns</sup>

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

**Table 4.** Variance of total iron content in rice plant in relation to subsurface drainage and fertilization, during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties.

Source of variation	df	Total Biomass iron content ( $\mu\text{g/g}$ dry soil)			
		BOUAKE-189		ROK-5	
		F	p	F	p
Drainage	1	6.840	0.011*	6.840	0.011*
Drainage* Fertilization	2	3.034	0.056 <sup>ns</sup>	3.034	0.056 <sup>ns</sup>

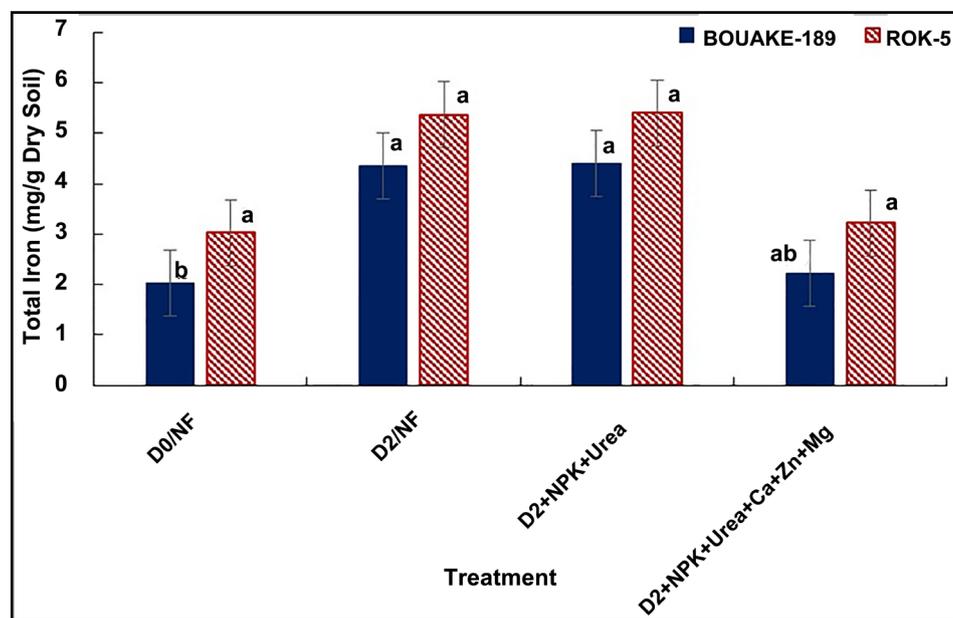
df = degree of freedom; F = Fisher F; \*significant  $p < 0.05$ ; \*\*significant  $p < 0.01$ ; ns: not significant  $p > 0.05$ .

showed that the total iron content in the roots of both rice varieties wasn't significantly related to subsurface drainage ( $p = 0.075$  and  $p = 0.182$ , respectively) and combined application of fertilization and drainage ( $p = 0.092$  and  $p = 0.191$ , respectively) (Table 3). It appeared also that the drained pots (D2, D2 + NPK + Urea and D2 + NPK + Urea + Ca + Zn + Mg) showed the highest total iron contents in roots (Figure 8). These results are in agreement with those of Mullilab [59] and Ethan and Odunze [6] who reported that the drainage of a waterlogged soil oxidizes the soil and precipitates  $Fe^{2+}$  into  $Fe^{3+}$  forms which are insoluble at the root surface.

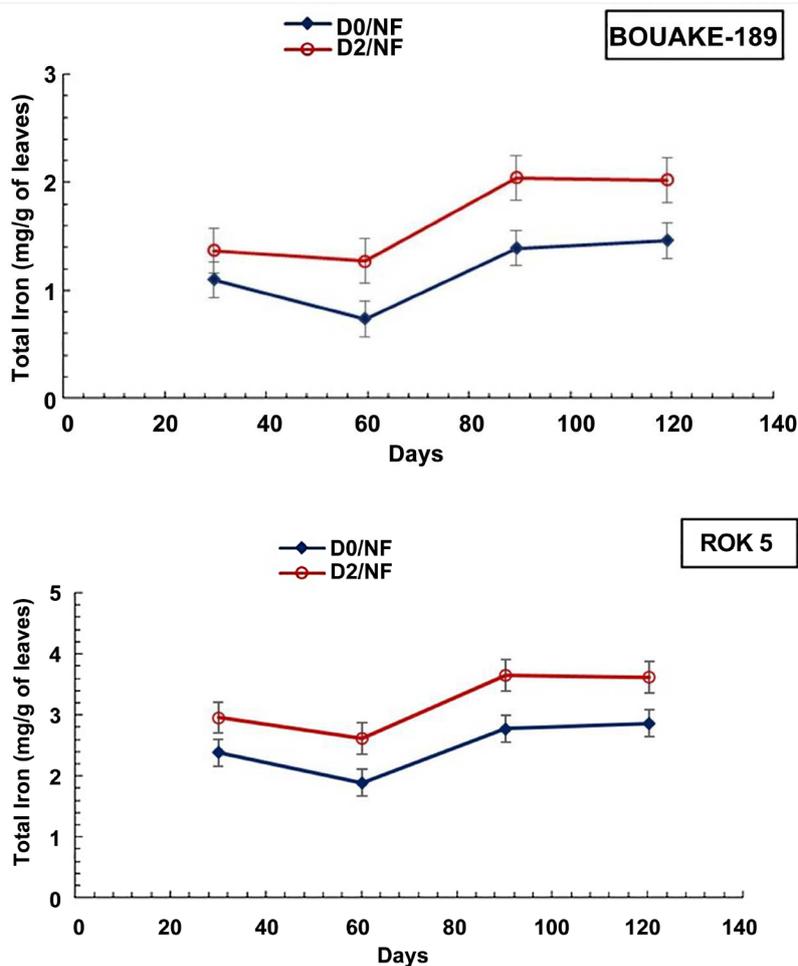
The total iron accumulation in BOUAKE-189 and ROK-5 rice aerial biomass was significantly related to subsurface drainage ( $p = 0.011$ , Table 4). However, no significant effect of combined application of both factors was observed for the two rice varieties ( $p = 0.056$ , Table 4).

Moreover, the total iron contents of aerial biomass in the drained (D2) pots were significantly high comparatively to the controls (D0) pots ( $p = 0.011$ ) (Figure 9). Panda *et al.* [60], explaining the physiological and biochemical mechanisms of Fe uptake by rice plant indicated that rice plants release into the rhizosphere siderophores which bind to  $Fe^{3+}$  in the form of a ligand. The ligand complex enters into the cell, and  $Fe^{3+}$  is reduced into  $Fe^{2+}$  inside the cytoplasm. Therefore, drainage may optimize iron uptake by rice plant by increasing the availability of  $Fe^{3+}$  through the oxidation of ferrous iron.

The experiment revealed also that the total iron content in aerial biomass of pots which received combined application of subsurface drainage and fertilization (D2 +



**Figure 8.** Roots total iron content of BOUAKE-189 and ROK-5 rice varieties in drained for 14 days without fertilization (D2/NF) and combined application of D2 drainage, NPK+Urea and NPK + Urea + Ca + Zn + Mg fertilization (D2 + NPK + Urea and D2 + NPK + Urea + Ca + Zn + Mg), respectively (means of 3 replicates). Yields sharing the same letter are not significantly different according to Fishers' test  $p > 0.05$ .



**Figure 9.** Evolution of the aerial biomass total iron content during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in pots without drainage and fertilization (D0/NF) and in pots drained for 14 days without fertilization (D2/NF) (means of 3 replicates).

NPK + Urea and D2 + NPK + Urea + Ca + Zn + Mg) were higher relatively to D2 drained and non-drained pots (D0) (Figure 9, Figure 10), for both rice varieties. The combined application of subsurface drainage and mineral fertilization seems to have a synergic effect on the rice plant  $\text{Fe}^{3+}$  absorption. Thus, in combined application of subsurface drainage and mineral fertilization condition, the drainage favours oxidation and precipitation of  $\text{Fe}^{2+}$  into  $\text{Fe}^{3+}$  forms, while the mineral fertilization (NPK + Urea + Ca + Zn + Mg) contributes to  $\text{Fe}^{3+}$  acquisition by enabling the plant to synthesize more photosynthetic assimilates (NADPH+, H+) [6] [59].

### 3.4. Effect of Combined Application of Subsurface Drainage and Fertilization on BOUAKE-189 and ROK-5 Rice Varieties Yield

Throughout the study, a significant difference was observed on rice biomass yield for subsurface drainage and combined effects of both factors ( $p = 0.001$  and  $p = 0.000$ , re-

spectively) for ROK-5 rice varieties (Table 5). Furthermore, the experiment revealed that the biomass yield of BOUAKE-189 rice variety was related to subsurface drainage ( $p = 0.007$ , Table 5), while combined effects of both factors showed no significant difference ( $p = 0.108$ , Table 5). However, it appeared that D2 + NPK + Urea + Ca + Zn + Mg treatments showed the highest rice biomass yield, followed by D2 + NPK + Urea ones, for the two rice varieties, respectively (Figure 11). Becker and Asch [10] indicated that various water, crop, and nutrient management options could alleviate the negative effects of Fe toxicity on lowland-rice performance. The findings of our studies are supported by the results of Ethan and Odunze [6], who recorded an increase of rice yield during a study on an iron toxic soil amended with nitrogen fertilizers and subjected to periodical drainage. Our previous study in Kamboinse reported also that microplots, which received D2 subsurface drainage and NPK + Urea amendment showed the highest yield [18].

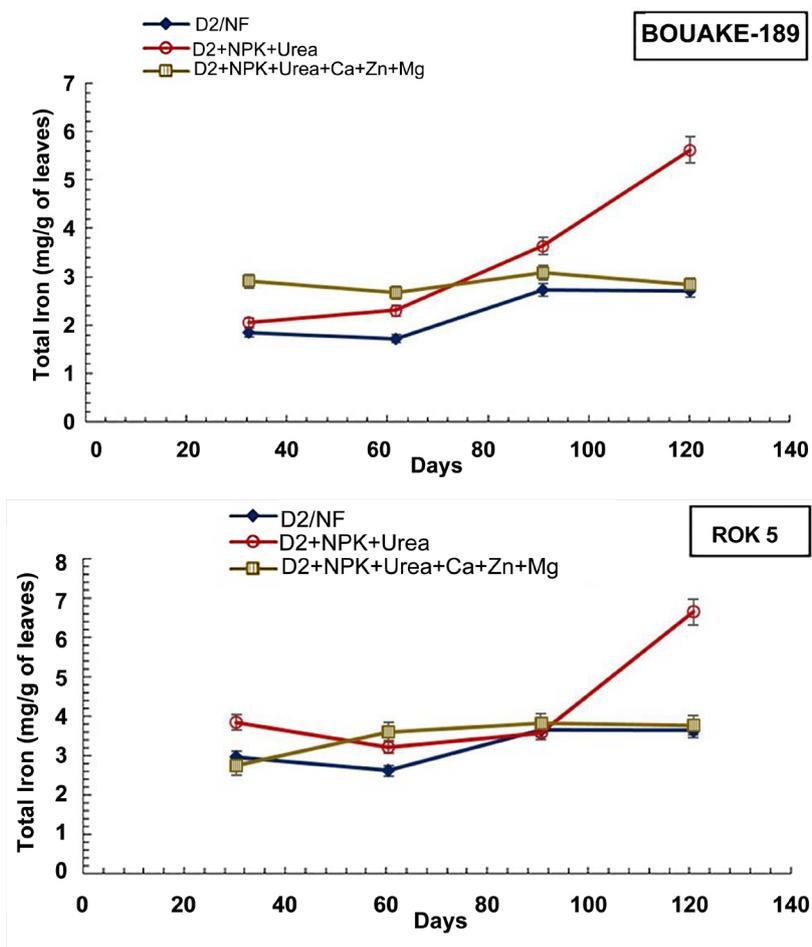
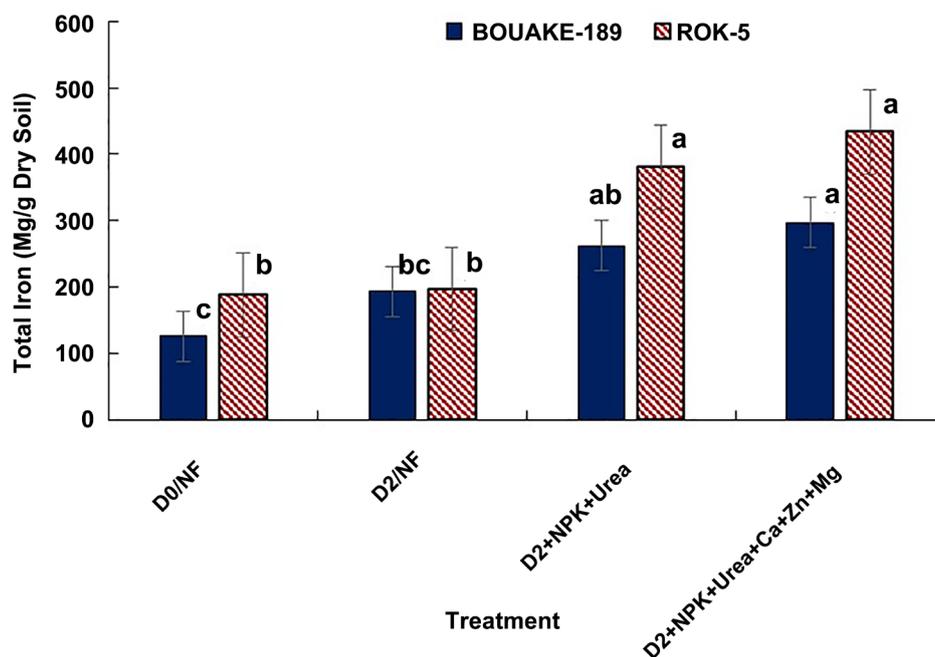


Figure 10. Evolution of the aerial biomass total iron content during the cultural cycle of BOUAKE-189 and ROK-5 rice varieties in drained pots for 14 days without fertilization (D2/NF), and in pots with combined application of D2 drainage NPK + Urea and NPK + Urea + Ca + Zn + Mg fertilization (D2 + NPK + Urea, and D2 + NPK + Urea + Ca + Zn + Mg), respectively (means of 3 replicates).

**Table 5.** Variance of total biomass yield in relation to subsurface drainage and fertilization, for BOUAKE-189 and ROK-5 rice varieties.

Source of variation	df	Yield of plant total biomass (g/pot)			
		BOUAKE-189		ROK-5	
		F	p	F	p
Drainage	1	12.867	0.007**	28.469	0.001**
Drainage* Fertilization	2	2.981	0.108 <sup>ns</sup>	26.482	0.000**

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



**Figure 11.** Total biomass yield of BOUAKE-189 and ROK-5 rice varieties in drained (D2) in drained pots for 14 days without fertilization (D2/NF) and combined application of D2 drainage, NPK + Urea and NPK + Urea + Ca + Zn + Mg fertilization (D2 + NPK + Urea and D2 + NPK + Urea + Ca + Zn + Mg), respectively (means of 3 replicates). Yields sharing the same letter are not significantly different according to Fishers' test  $p > 0.05$ .

Keita [61] and Ethan *et al.* [6] indicated that drainage in iron-toxic soils increased grain yield of lowland rice by the reduction of ferrous iron absorption of rice plant. Indeed, many studies showed that soil oxygenation through water drainage affects the iron profiles by inducing fast chemical and microbial oxidization reactions of the iron II in the zones where oxygen is present [49] [62].

Our previous experiment, on the effect of fertilization on the rice yield in the same paddy soil, reported also that the pots which received NPK + Urea + Ca + Zn + Mg amendment, recorded the highest yield, relatively to NPK + Urea fertilization [39]. In fact, the application of plant essential nutrients counteracts negative effects of excess amounts of iron, by competing with  $Fe^{2+}$  uptake at the sites of ion adsorption on roots

or by enhancing plants' defence or tolerance mechanisms [10] [55] [63].

However, the D2 + NPK + Urea + Ca + Zn + Mg treatment showed the highest yield (296.6 and 433.7 g/pot, respectively) relatively to solely application of NPK + Urea + Ca + Zn + Mg (250.017 and 362.87 g/pot, respectively), as previously reported in the same paddy soil, for BOUAKE-189 and ROK-5 rice varieties, respectively [39]. Thus, in the present study, the oxidation power of the subsurface drainage [6] combined to the mineral (Ca, Mg, Mn P, K and Zn) fertilization may optimize the acquisition of Fe by rice plant for an improved growth and yield [55] [63] [64].

#### 4. Conclusions

From the results obtained in this study, subsurface drainage combined with NPK + Urea + Ca + Zn + Mg fertilization decreases the number of IRB in an iron toxic paddy soil under BOUAKE-189 and ROK-5 rice varieties cultivation. Thus, oxygenation throughout drainage and mineral fertilization of the soil maintains the oxidising power of rice roots and reduces the IRB population's number. Moreover, the low level of ferrous iron content in the soil near rice roots, for both rice varieties in the D2 + NPK + Urea pots, indicates that the combination of drainage and NPK + Urea fertilization may decrease iron reduction and/or optimize the absorption of Fe(III) complexes into the rice plant. However, the subsurface drainage combined to NPK + Urea + Ca + Zn + Mg complex amendment recorded high level of ferrous iron content in the soil near rice roots, high content of total iron in the aerial biomass and the highest mean of rice biomass yield for both rice varieties. Indeed, combined application of drainage and mineral fertilization seems to have a synergic effect on improving rice yield. The oxidation power of the subsurface drainage combined to the mineral (Ca, Mg, Mn P, K and Zn) fertilization may optimize the acquisition of Fe by rice plant for an improved growth and yield. In fact, for the two rice varieties, NPK, Ca, Mg, and Zn amendment seems to increase the leaf tissue tolerance to excess amounts of Fe and to optimize Fe<sup>3+</sup> absorption, produced through the oxidation of ferrous iron during the subsurface drainage, at the root surface in the rice plant, for a better growth and better yield.

The experiment showed also that the rice plant remains the main factor which modulates the dynamic and activity of IRB during rice cultural cycle.

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