



# Article Effect of P-Dipping on Growth of NERICA 4 Rice in Different Soil Types at Initial Growth Stages

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**Abstract:** Phosphorus (P) deficiency resulting from P fixation is a major constraint limiting sustainable rice cultivation in sub-Saharan Africa. Soil texture also affects P availability and use efficiency. In a factorial experiment, we evaluated the combined effect of soil texture (sand, clay loam, and clay) and P treatments P-dipping (Pdip) and two other broadcasted P fertilizer levels (Brod1 and Brod2) on the growth of NERICA 4 rice in the initial growth stages. Across all soil textures and P treatments, total plant biomass ranged from 1.06 to 4.63 g pot<sup>-1</sup>. The Pdip treatment significantly increased shoot and root biomass relative to control from 1.27 to 1.98 and 0.23 to 0.38 g pot<sup>-1</sup>, respectively. Mean photosynthetic rate values under Pdip (20.1 µmol m<sup>-2</sup> s<sup>-1</sup>), Brod2 (19.5 µmol m<sup>-2</sup> s<sup>-1</sup>), and Brod1 (19.3 µmol m<sup>-2</sup> s<sup>-1</sup>) treatments showed significant 42%, 37%, and 36% increases over control, regardless of soil texture. In a striking contrast, P-dipping significantly promoted growth of root length under clay soil, but without a commensurate increase in shoot P uptake. Contrary to our hypothesis, the interactive effect of soil texture and P-dipping influenced NERICA 4 shoot and root physiological and morphological characteristics under clay loam soil texture as opposed to clay.

Keywords: phosphorus; Oryza sativa L.; nutrient uptake; photosynthetic rate; root morphology

# 1. Introduction

Phosphorus (P) deficiency is one of the major constraints limiting sustainable rice production globally [1–3]. In sub-Saharan Africa (SSA), this is exacerbated by both limited mineral fertilizer inputs by smallholder farmers and dominant soil types—such as ferralsols and acrisols—within its humid and sub-humid agroecological zones [4,5]. These soil types are inherently low in nutrient contents, low in cation exchange capacities, and low in waterholding capacities [4,6], strongly leached and deeply weathered, with low pH and high Fe and Al oxide contents that increase the soil P fixing capacity [4,7–9]. Large proportions of soil-derived or applied P thus remain unavailable for plant growth, presenting serious agronomic and economic challenges. Improved P acquisition and use by plants are thus of immediate and direct benefit to agriculture in SSA [5,10].

Several approaches to coping with the threats of P depletion have been studied, including the use of phosphate rocks [11,12], breeding of crops that are tolerant to low P conditions [13–15], recycling P from wastewater [16,17], and releasing fixed P in soil [18,19]. Among those approaches, given the limited purchasing capacity of smallholder farmers and highly P-fixing soils in SSA, small-dose and localized P application near the root system has shown promise as a management practice [20–23]. Similarly, the potential of P-dipping for lowland rice production—that is, dipping rice seedling roots into P-enriched slurry just



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). before transplanting—has been found to improve rice seedling resilience to drought and P stresses [24], double applied P use efficiency [25], shorten days to heading, and increase yield grain [26].

On the other hand, soil texture has been widely demonstrated to exert a significant effect on P availability and use efficiency in crop production [27–32]. Improving the opportunity for wider adoption of P-dipping techniques by farmers cultivating rice in diverse soil textures thus implies the importance of understanding the interactive effect of P-dipping and soil texture on rice growth performance. Furthermore, in contrast to excessive chemical fertilizer application rates required for the broadcasting method, which often lead to nutrient losses and cause eutrophication of fresh water, rising nitrous oxide emissions, and degradation of downstream water quality [33,34], P-dipping allows for relatively minimal P fertilizer amounts and employs a localized P application method directly to the roots, thereby contributing less to greenhouse gas emissions while contributing to sustainable rice production. The objective of this study was to evaluate the combined effect of P-dipping and soil texture on the initial growth of rice, focusing on shoot P uptake and root morphological development. The hypothesis was that clay soil, owing to its high water and nutrient retention capacities, is most suited to P-dipping.

# 2. Materials and Methods

# 2.1. Physiochemical Characteristics of the Experimental Soils

The experimental soils with a range of textures were collected from Kagoshima (N31.8549 E130.2086), Tanegashima Island (N30.5331 E130.9586), and Tokunoshima Island (N27.8117 E128.8975), Japan. The soils were analyzed for pH (1:2.5 H<sub>2</sub>O), available P was determined by Truog's method, and total carbon and nitrogen by the dry combustion method using an NC analyzer (JM1000CN/HCN TOC.TN, J-Science Lab Co., Ltd., Kyoto, Japan), the 1 mol L<sup>-1</sup> ammonium acetate extraction method was used to determine exchangeable potassium, and soil texture was determined by the pipette method. We determined the acid oxalate extractable aluminum and iron content by ICP-MS (Eran DRC, PerkinElmer, Shelton, CT, USA) after extraction with an acid ammonium oxalate solution (pH 3.0) for 4 h in darkness [35]. We calculated soil organic matter content by multiplying the percentage of organic carbon with the conventional Van-Bemmelen's factor of 1.724 [36]. The chemical and physical properties of the three experimental soils are presented in Table 1. Briefly, Kagoshima soil was sandy with a pH of 8.8 and low available P content. Tanegashima soil was clay loam with a pH of 5.8 and the lowest content of available P.

Table 1. Experimental soil physical and chemical properties.

| Property  | Kagoshima <sup>1</sup> | Tanegashima <sup>1</sup> | Tokunoshima <sup>1</sup> |
|---|------------------------|--------------------------|--------------------------|
| WRB classification                              | Arenosols              | Andosols                 | Acrisols                 |
| pH (1:2.5 H <sub>2</sub> O)                     | 8.8                    | 4.9                      | 5.8                      |
| $EC (mS m^{-1})$                                | 44.0                   | 16.3                     | 28.9                     |
| Total N (%)                                     | 0.02                   | 0.19                     | 0.10                     |
| Total organic C (%)                             | 0.05                   | 1.62                     | 0.52                     |
| C:N ratio                                       | 0.9                    | 8.5                      | 5.3                      |
| Organic matter content (%)                      | 0.09                   | 2.79                     | 0.90                     |
| Available P (mg kg <sup><math>-1</math></sup> ) | 24.5                   | 186.5                    | 18.3                     |
| Al oxalate (mg $g^{-1}$ )                       | 9.1                    | 25.2                     | 13.2                     |
| Fe oxalate (mg $g^{-1}$ )                       | 1.7                    | 6.9                      | 1.8                      |
| Sand (%)  | 95.6                   | 30.8                     | 12.6                     |
| Clay (%)  | 2.7                    | 43.4                     | 79.7                     |
| Silt (%)  | 1.7                    | 25.8                     | 7.7                      |
| Textural name                                   | Sand                   | Clay loam                | Clay                     |

<sup>1</sup> Locations from which the experimental soil samples were taken.

# 2.2. Experimental Design and the Environmental Condition

The experiment was conducted in a greenhouse using three soil types and three fertilizer treatments factorially combined in 3 replicates. The soil types included sand, clay loam, and clay soil textures, and the fertilizer treatments consisted of control (no P application), two broadcasts, and one P-dipping. We used perforated plastic pots (11 cm high, 9.5 cm bottom diameter, and 12.5 cm top diameter). We filled the pots with 1.5 kg of the three types of soil (bulk density:  $1.2 \text{ g cm}^{-3}$ ) and placed the pots of each soil type in separate plastic containers (48 cm L × 32 cm W × 8 cm H) lined with black plastic sheets. To correct deficiencies in the soil N and K contents, we homogeneously mixed the experimental soil in each pot with 0.43 g of ammonium sulfate (90 mg N pot<sup>-1</sup>) and 0.12 g of potassium chloride (50 mg K pot<sup>-1</sup>). We filled the plastic containers with water to allow the soil in the pots to absorb by capillarity to the field capacities—volumetric soil moisture contents at 32% for sand soil, 42% for clay loam soil, and 48% for clay soil. Thereafter, we maintained water in the plastic containers holding the pots at 3–4 cm throughout the experiment.

NERICA 4 rice variety—an interspecific progeny between *Oryza sativa* and *Oryza glaberrima*—was grown in seedling trays until the 3–4 leaf stage and with an average of 5 cm of root system length for each seedling. Prior to transplanting, we carefully removed rice seedlings from the seedling tray to avoid root damage, and carefully hand-washed the nursery soil using water in plastic buckets fitted with 1 mm sieves to avoid root loss. For the P-dipping treatment, we dipped the washed seedling roots into the P-enriched slurry for 30 min [37]. To produce the P-enriched slurry, we mixed 45 g of air-dried soil, 14 mL of water, and 1.31 g of single superphosphate (SSP) fertilizer, an equivalent of approximately 68.7 mg  $P_2O_5$  pot<sup>-1</sup> for the P-dipping (Pdip) treatment.

The rest of the seedlings were transplanted without P-dipping in pots broadcasted with 0.25 g (43.1 mg  $P_2O_5$  pot<sup>-1</sup> (Brod1)) and 0.49 g (85.9 mg  $P_2O_5$  pot<sup>-1</sup> (Brod2)) of SSP fertilizer. To avoid root damage during transplanting, we made holes approximately 6 cm deep and 3 cm wide in the wet soil within the pots before transplanting the rice seedlings. The daily mean air temperature (29.5 °C) and the daily mean relative humidity (70.5%) in the greenhouse were measured using a sensor equipped with a data logger (RTR-503, T&D Corporation, Tokyo, Japan) throughout the experiment. A summary of the P and soil treatments is presented in Table 2.

| Treatments    | Application Rate (mg $P_2O_5$ pot <sup>-1</sup> ) | Application Method                                     | Timing           |
|---------------|---|--|------------------|
| P application |   |  |                  |
| Pdip          | 68.7  | P-dipping <sup>2</sup>                                 | At transplanting |
| Brod1         | 43.1  | Broadcasting   | At transplanting |
| Brod2         | 85.9  | Broadcasting   | At transplanting |
| Ctrl          | 0   | -  | -                |
| Soil texture  | Quantity (kg soil pot $^{-1}$ )                   | Field condition volumetric moisture content (% $w/w$ ) |                  |
| Sand          | 1.5   | 32   |                  |
| Clay loam     | 1.5   | 42   |                  |
| Clay          | 1.5   | 48   |                  |

Table 2. Overview of the P and soil texture treatments.

<sup>2</sup> The P-enriched slurry for the P-dipping treatment was produced by mixing 45 g of air-dried soil, 14 mL of water, and 1.31 g of SSP fertilizer. To correct deficiencies in the soil N and K contents, 90 mg N pot<sup>-1</sup> and 50 mg K pot<sup>-1</sup>, respectively, were homogeneously mixed with the experimental soil in each pot.

# 2.3. Data Collection and Measurements

At 40 days after transplanting (DAT), we measured the shoot parameters—plant height, leaf age, and Soil Plant Analysis Development (SPAD). We measured plant length from the base of the stem (at the soil surface) to the highest part of the plant. We deter-

mined leaf age by counting the number of fully expanded leaves per plant. We conducted gas exchange measurements on the uppermost fully expanded leaf at 38 DAT between 9:00 AM and 1:30 PM, using a portable gas exchange measurement system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) set at a light intensity of 1200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, a block temperature of 32 °C, and an ambient CO<sub>2</sub> concentration of 410  $\mu$ mol mol<sup>-1</sup>.

At the same time (40 DAT), the plant shoots in each pot were cut, and the leaves were removed to determine the leaf area using a digital image analysis machine (LIA32, Nagoya University, Nagoya, Japan). The leaves and stems were oven-dried at 80 °C for 48 h to determine the shoot dry weight per pot. The oven-dried plant materials were finely ground, and samples (0.5 g each) were wet-digested in 15 mL of di-acid digestion mixture [HNO<sub>3</sub>:HClO<sub>4</sub> (3:2, v/v)]. Thereafter, the total P concentration in plant samples was determined in accordance with the vanadate–molybdate method [38] using a UV-VIS spectrophotometer (V-530, JASCO Co., Tokyo, Japan). We calculated shoot P uptake as the product of shoot dry weight and P concentration.

In preparation for the root analysis, the soil in each pot was carefully removed, placed in a metallic 2 mm gauge sieve, and carefully washed by spraying with low-pressure tap water to rid the roots of all soil particles. The root samples were placed in self-sealing plastic bags containing 50% aqueous ethanol solution and stored in a cold room at 4 °C prior to scanning. Root samples were scanned at 6400 dpi using an Epson scanner (EPSON GT-X830, Epson American Inc., Los Alamitos, CA, USA), and images were analyzed at pixel classification values of 130–150 using the WinRhizo software (WinRHIZO, Regent Instruments Inc., Québec, Canada; Version 2005b) to determine the total root length (RL), root surface area (RSA), and root volume (RV). Following the root morphological analysis, root samples were dried at 80 °C for 48 h in an oven to determine the root dry weight per pot.

#### 2.4. Statistical Analyses

Data analyses were conducted with IBM SPSS Statistics (Version 27.0.1.0) using twoway ANOVA to determine the single and interaction effects of P treatments (Pdip, Brod1, Brod2, and Ctrl) and soil textures (sand, light clay, and clay). The treatment means were compared from replicates at the 5% level of probability using Tukey's HSD test. Where significant interaction effects existed, we ran pairwise comparisons for each simple main effect, modifying statistical significance with a Bonferroni adjustment.

#### 3. Results

#### 3.1. Changes in Shoot Biomass, Root Biomass, and Shoot P Uptake

Soil texture and P application methods significantly affected mean shoot biomass, mean root biomass, and mean shoot P uptake (Figure 1). Across soil textures and P treatments, total biomass ranged from 1.06 to 4.63 g pot<sup>-1</sup>. The Pdip treatment significantly increased shoot biomass relative to Ctrl from 1.27 to 1.98 g pot<sup>-1</sup> (Figure 1a). Similarly, amongst the P treatments, Pdip significantly increased mean root biomass by 53% relative to Ctrl (Figure 1b). Whereas no statistical difference in mean shoot P uptake existed between Ctrl and Pdip, the Pdip treatment resulted in a 49% increase in shoot P uptake relative to Ctrl (Figure 1c). No significant interaction effects between soil texture and P treatments existed for shoot biomass, root biomass, and shoot P uptake.

#### 3.2. Changes in Shoot Physiology and Morphology

Plant height tended to increase with P application rate under sand and clay soil textures, but under the light clay soil texture, plant height decreased with increased P rate from Brod1 to Brod2 (Table 3).



**Figure 1.** Comparison of means from the effect of P treatments (P) on shoot mass (**a**), root mass (**b**), and shoot P uptake (**c**) at 40 days after transplanting. S, soil texture; \*, p < 0.05; ns, not significant according to Tukey's HSD test. Different lowercase letters above P treatments indicate significant differences between P treatments at p < 0.05.

| Soil Texture (S) | Phosphorus<br>Treatment (P) | Plant<br>Height          | Leaf Age Leaf Area      |   | SPAD                    |
|------------------|-----------------------------|--------------------------|-------------------------|---|-------------------------|
|                  |                             | (cm)                     |                         | (cm <sup>2</sup> pot <sup><math>-1</math></sup> ) | Value                   |
| Sand             | Ctrl                        | $55.6\pm1.8~\mathrm{c}$  | $5.0\pm0.01b$           | $197.3\pm10.9~\mathrm{b}$                         | $14.3\pm2.4~\text{b}$   |
|                  | Pdip                        | $63.1\pm2.5\mathrm{b}$   | $5.0\pm0.01~\text{b}$   | $238.4\pm17.4\mathrm{b}$                          | $21.7\pm1.1$ a          |
|                  | Brod1                       | $66.2\pm2.7b$            | $7.3\pm0.58~\mathrm{a}$ | $312.0\pm17.4~\mathrm{a}$                         | $24.0\pm1.3~\mathrm{a}$ |
|                  | Brod2                       | $72.6\pm2.1$ a           | $7.2\pm1.44$ a          | $305.7\pm27.5~\mathrm{a}$                         | $17.6\pm0.1~\mathrm{b}$ |
| Clay loam        | Ctrl                        | $81.7\pm2.8b$            | $9.5\pm0.87~\mathrm{a}$ | $447.2\pm10.7~\mathrm{a}$                         | $43.5\pm0.8b$           |
| -                | Pdip                        | $87.3\pm3.2~\mathrm{ab}$ | $9.3\pm0.58~\mathrm{a}$ | $501.3\pm16.7~\mathrm{a}$                         | $47.1\pm0.3~\mathrm{a}$ |
|                  | Brod1                       | $89.6\pm1.7~\mathrm{a}$  | $9.2\pm0.58~\mathrm{a}$ | $499.5\pm35.6~\mathrm{a}$                         | $45.6\pm0.6~\mathrm{a}$ |
|                  | Brod2                       | $86.9\pm0.9$ ab          | $8.8\pm0.29~\mathrm{a}$ | $500.7\pm12.0~\mathrm{a}$                         | $46.1\pm0.6~\mathrm{a}$ |
| Clay             | Ctrl                        | $65.0\pm5.9\mathrm{b}$   | $4.7\pm0.58~\mathrm{b}$ | $180.1\pm30.9~\mathrm{c}$                         | $39.7\pm1.4~\mathrm{c}$ |
| -                | Pdip                        | $81.0\pm5.5~\mathrm{a}$  | $5.0\pm0.01~\text{b}$   | $265.2\pm28.3\mathrm{b}$                          | $43.5\pm0.6b$           |
|                  | Brod1                       | $84.8\pm5.9~\mathrm{a}$  | $6.8\pm0.29~\mathrm{a}$ | $368.2\pm10.3~\mathrm{a}$                         | $45.8\pm0.4~\mathrm{a}$ |
|                  | Brod2                       | $89.2\pm1.0~\mathrm{a}$  | $7.0\pm0.50~\mathrm{a}$ | $418.4\pm10.1~\mathrm{a}$                         | $47.9\pm0.6~\mathrm{a}$ |
| Two-way<br>ANOVA | S                           | *                        | *                       | *   | *                       |
|                  | Р                           | *                        | *                       | *   | *                       |
|                  | S 	imes P                   | *                        | *                       | *   | *                       |

Table 3. Shoot morphological changes related to soil texture and P treatments.

NERICA 4 shoot morphological changes under sand, clay loam, and clay soil texture and phosphorus treatments including Pdip, Brod1, Brod2, and Ctrl at 40 days after transplanting. \*, p < 0.05 according to Tukey's HSD test. Values are means  $\pm$  standard deviations (n = 3). Different lowercase letters after parameter values indicate significant differences at p < 0.05 within each soil texture.

Mean plant height differed significantly (p < 0.05) between clay loam (86.4 cm), clay (79.9 cm), and sand (64.4 cm) soil textures. Mean plant height also differed significantly (p < 0.05) between Brod2 (82.9 cm), Brod1 (80.2 cm), Pdip (77.1 cm), and Ctrl (67.4 cm) treatments. Significant interaction effects (p < 0.05) between soil textures and P treatments emerged for mean plant height, plant leaf age, leaf area, and SPAD values (Table 3). Mean leaf age was significantly affected by clay loam (9.2), sand (6.1), and clay (5.9) soil textures. Plant leaf area was significantly affected by both soil texture and P treatments, with values of 473.4–500.9, 294.2–321.7, and 249.6–277.1 cm<sup>2</sup> pot<sup>-1</sup> under clay loam, clay, and sand soil

textures, respectively. The Pdip treatment showed a significant 47% increase in mean leaf area relative to Ctrl only under clay soil. Under the three soil textures used, the P treatment significantly affected SPAD values, with Pdip showing a 51.7%, 9.6%, and 8.3% increase relative to Ctrl under sand, clay, and clay loam soil textures, respectively.

# 3.3. Gas Exchange Parameters

In Figure 2 we present the changes in the four gas exchange parameters—photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), and intercellular carbon dioxide concentration (Ci)—under soil texture and P treatments, both of which significantly affected all the gas exchange parameters. The effect of soil texture on A, gs, E, and Ci showed a consistent tendency where, under clay loam soil texture and soil texture, we observed the highest and lowest mean values for all the stated parameters, respectively.



**Figure 2.** Boxplots of the responses of NERICA 4 photosynthetic rate (**a**), stomatal conductance (**b**), transpiration rate (**c**), and intercellular CO<sub>2</sub> concentration (**d**) to sand, clay loam, and clay soil textures planted in P treatments including Pdip, Brod1, Brod2, and Ctrl. \*, p < 0.05 according to Tukey's HSD test. Different lowercase letters above P treatments indicate significant differences at p < 0.05 within each soil texture.

While P treatments did not show such consistent changes across the gas exchange parameters, significant differences existed within each parameter. For instance, mean A values under Pdip (20.1  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), Brod2 (19.5  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), and Brod1 (19.3  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) treatments showed significant (p < 0.05) 42%, 37%, and 36% increases over the Ctrl, regardless of soil texture (Figure 2a). Across both soil textures and P treatments, *gs* values ranged from 0.3 to 1.4 mol m<sup>-2</sup> s<sup>-1</sup> while *E* values ranged from 5.4 to 15.5 mmol m<sup>-2</sup> s<sup>-1</sup>. Both *gs* and *E* had similar tendencies where under clay loam soil texture, Pdip treatment

showed the highest values for gs (1.4 mol m<sup>-2</sup> s<sup>-1</sup>; Figure 2b) and E (13.5 mmol m<sup>-2</sup> s<sup>-1</sup>; Figure 2c). We observed significant changes in *Ci* between soil textures (p = 0.017) and P treatments (p < 0.05) (Figure 2d). For all the gas exchange parameters, we observed significant interaction effects (p < 0.05) between soil textures and P treatments.

# 3.4. Changes in Root Morphology and Shoot P Uptake

In Table 4 we present the changes in root morphology related to soil texture and P treatments.

| Soil Texture<br>(S) | Phosphorus<br>Treatment (P) | Total Root<br>Length     | Root Surface<br>Area                 | Root Volume                          | Root Length<br>Ratio     | Root Mass<br>Ratio        | Root to<br>Shoot Ratio    |
|---------------------|-----------------------------|--------------------------|--------------------------------------|--------------------------------------|--------------------------|---------------------------|---------------------------|
|                     |                             | (m pot $^{-1}$ )         | (cm <sup>2</sup> pot <sup>-1</sup> ) | (cm <sup>3</sup> pot <sup>-1</sup> ) | (m g <sup>-1</sup> )     | (g g <sup>-1</sup> )      |                           |
| Sand                | Ctrl                        | $33.9\pm1.3~\mathrm{c}$  | $519.7\pm6.8~\mathrm{c}$             | $4.5\pm0.1$ a                        | $29.0\pm1.1~\mathrm{ab}$ | $0.13\pm0.02~\mathrm{a}$  | $0.15\pm0.03~\mathrm{a}$  |
|                     | Pdip                        | $52.0\pm0.8~\mathrm{a}$  | $557.6\pm8.7\mathrm{b}$              | $4.8\pm0.3$ a                        | $38.2\pm2.6$ a           | $0.12\pm0.01~\mathrm{ab}$ | $0.13\pm0.01~\mathrm{a}$  |
|                     | Brod1                       | $52.5\pm0.9$ a           | $588.6\pm9.9~\mathrm{a}$             | $4.9\pm0.4$ a                        | $27.8\pm5.8~\mathrm{ab}$ | $0.11\pm0.01~\mathrm{ab}$ | $0.12\pm0.02~\mathrm{ab}$ |
|                     | Brod2                       | $36.9\pm0.9b$            | $361.6\pm6.4~d$                      | $2.8\pm0.1b$                         | $20.3\pm5.2~\mathrm{c}$  | $0.09\pm0.02~b$           | $0.10\pm0.02~ab$          |
| Clay loam           | Ctrl                        | $27.2\pm6.0\mathrm{b}$   | $404.2 \pm 5.9 \text{ d}$            | $4.1\pm0.1~{ m c}$                   | $13.6\pm1.2$ ab          | $0.25\pm0.04$ a           | $0.13\pm0.06~\mathrm{a}$  |
|                     | Pdip                        | $31.9\pm6.4b$            | $481.6\pm9.4~\mathrm{c}$             | $4.8\pm0.1~{ m c}$                   | $9.8\pm2.9~\mathrm{b}$   | $0.24\pm0.04~\mathrm{a}$  | $0.12\pm0.04~\mathrm{a}$  |
|                     | Brod1                       | $43.0\pm5.9~\mathrm{b}$  | $670.9\pm29.4\mathrm{b}$             | $6.7\pm0.4$ b                        | $12.6\pm2.0~b$           | $0.14\pm0.01~\mathrm{b}$  | $0.08\pm0.01~\mathrm{a}$  |
|                     | Brod2                       | $63.7\pm6.2~\mathrm{a}$  | $852.4\pm29.7~\mathrm{a}$            | $8.1\pm0.9~\mathrm{a}$               | $20.2\pm4.0~\text{a}$    | $0.13\pm0.02~b$           | $0.09\pm0.02~\mathrm{a}$  |
| Clay                | Ctrl                        | $60.2\pm0.2~\mathrm{c}$  | $717.2\pm7.2~\mathrm{c}$             | $6.7\pm0.2$ b                        | $47.1\pm8.4$ a           | $0.11\pm0.05~\mathrm{a}$  | $0.33\pm0.06~\mathrm{a}$  |
|                     | Pdip                        | $115.9\pm3.0~\mathrm{a}$ | $1286.3\pm23.9~\mathrm{a}$           | $11.8\pm0.5$ a                       | $53.2\pm3.9~\mathrm{a}$  | $0.10\pm0.03~\mathrm{a}$  | $0.33\pm0.08~\mathrm{a}$  |
|                     | Brod1                       | $66.1\pm3.7~\mathrm{c}$  | $781.8\pm25.5b$                      | $7.3\pm0.6$ b                        | $25.2\pm1.0~\text{b}$    | $0.08\pm0.01~\mathrm{a}$  | $0.17\pm0.01~\mathrm{b}$  |
|                     | Brod2                       | $75.2\pm3.5b$            | $692.5\pm23.0~\mathrm{c}$            | $6.4\pm0.5b$                         | $27.4\pm6.3b$            | $0.08\pm0.02~\mathrm{a}$  | $0.15\pm0.03~\text{b}$    |
| Two-way<br>ANOVA    | S                           | *                        | *                                    | *                                    | *                        | *                         | *                         |
|                     | Р                           | *                        | *                                    | *                                    | *                        | *                         | *                         |
|                     | S 	imes P                   | *                        | *                                    | *                                    | *                        | *                         | ns                        |

Table 4. Root morphological changes related to soil texture and P treatments.

NERICA 4 root morphological changes under sand, clay loam, and clay soil texture and phosphorus treatments including Pdip, Brod1, Brod2, and Ctrl at 40 days after transplanting. \*, p < 0.05 according to Tukey's HSD test. Values are means  $\pm$  standard deviations (n = 3). Different lowercase letters after parameter values indicate significant differences at p < 0.05 within each soil texture.

Broadly, the values of all root morphological parameters typically increased with an increase in the P rate from Brod1 to Brod2 under clay and clay loam soil textures but decreased under sand soil texture (Table 4). Specifically, we observed a significant difference (p < 0.05) in mean total root length (RL) between clay, sand, and clay loam soil textures. There was also a significant difference (p < 0.05) in the root length between P treatments, with the highest mean RL under Pdip treatment compared to Brod1, Brod2, and Ctrl treatments. RL showed significant interaction effects between soil texture and P treatment (p < 0.05), and analysis of the simple main effects for P treatment showed that Pdip had the highest effect size (partial  $\eta^2 = 0.97$ ). Pairwise comparisons showed the mean RL under Pdip treatment and clay was 83.9 points higher than that under clay loam (p < 0.05), and 63.9 points higher than that under sand (p < 0.05) soil textures.

In striking contrast, whereas the mean RL under clay (79.4 m pot<sup>-1</sup>) was significantly higher than that under clay loam (41.5 m pot<sup>-1</sup>) soil texture, the mean shoot P concentration and shoot P uptake under clay loam soil were significantly higher than those under clay soil texture (Figure 3).

Indeed, we had expected the higher RL under clay soil texture to result in higher shoot P concentration and shoot P uptake values under clay soil texture—but that was not the case. The mean shoot P uptake under clay loam was 180% greater than that under clay soil texture.



**Figure 3.** Comparison of means from the effect of soil texture (S) on shoot P concentration (**a**) and shoot P uptake (**b**) across P treatments (P) at 40 days after transplanting. \*, p < 0.05; ns, not significant, both according to Tukey's HSD test. Different lowercase letters above soil textures indicate significant differences between soil textures at p < 0.05.

Root surface area, ranging from 335.6 to 1310.3 cm<sup>2</sup>, showed a similar trend to that observed in the root length, where Pdip treatment gave the highest value under clay soil texture (Table 4). The mean RSA differed significantly (p < 0.05) between that under clay (869.5 cm<sup>2</sup>), clay loam (602.3 cm<sup>2</sup>), and sand (557.9 cm<sup>2</sup>) soil textures. Mean RSA also differed significantly (p < 0.05) between P treatments, with mean RSA under Pdip treatment 17.8% and 41.7% greater relative to the combined broadcasting treatments (Brod1 and Brod2) and Ctrl, respectively, with significant interaction effects between soil texture and P treatment for RSA (p < 0.05). Among P treatments, Pdip treatment showed the highest simple main effect size (partial  $\eta^2 = 0.99$ ). Pairwise comparisons indicated the mean RSA from the Pdip treatment under clay soil texture was 362.6 and 267.2 points higher than that under sand (p < 0.05) and clay loam (p < 0.05) soil textures, respectively.

Root volume showed similar morphological changes to RL and RSA, where significant differences in the mean RV under clay soil (8.1 cm<sup>3</sup>; p < 0.05) were the highest compared to values under the clay loam and sand soil textures. Significant differences (p < 0.05) in RV also existed between P treatments, with Pdip treatments showing the highest value (7.1 cm<sup>3</sup>) among P treatments. Pairwise analysis of the simple main effects among the P treatments showed that Pdip under the clay soil accounted for the highest (partial  $\eta^2 = 0.96$ ) significant interaction effects in RV.

The changes in root length ratio (RLR)—that is, RL per total biomass—express the root's potential for the acquisition of soil resources. On the other hand, root mass ratio (RMR)—that is, root biomass per total biomass—is an indicator of the biomass allocated to the roots. Soil texture and P treatments significantly affected RLR and RMR (Table 4). Significantly, under clay and clay loam soil textures we observed the highest  $(38.3 \text{ m g}^{-1})$ and lowest (14.0 m  $g^{-1}$ ) mean RLR values, respectively. Among the P treatments, Pdip was associated with a significant 51.6% increase in the mean RLR relative to the combined broadcasting treatments (Brod1 and Brod2). Similarly, clay soil showed the significantly highest (0.19 g  $g^{-1}$ ) mean RMR, and relative to the combined broadcasting treatments, the Pdip treatment also showed a significant 46.9% increase in RMR. Both RLR and RMR were significantly affected by interactions between soil texture and P treatments. The mean root-to-shoot ratio under clay soil (0.25) was significantly higher (p < 0.05) than that under sand (0.13) and clay loam (0.10) soil textures (Table 4). Among P treatments, we observed the highest and lowest mean root-to-shoot ratios under Ctrl (0.21) and Brod2 (0.11), respectively. The mean root-to-shoot ratio under Pdip (0.19) was equally high but did not differ significantly from that under Ctrl.

# 4. Discussion

# 4.1. Soil Texture and P-Dipping Effects on Rice Shoot Morphology

Our findings demonstrated that P-dipping and soil texture each separately affected rice shoot biomass and shoot P uptake, and they interactively affected plant height, leaf age, leaf area, and SPAD. The mean P-dipping values for above-ground parameters—including plant height, leaf area, SPAD, and shoot biomass—showed significant increases relative to Ctrl across all soil textures. While we had hypothesized that clay soil, owing to its high water and nutrient retention capacities, is most suited to P-dipping the interactive effects between P-dipping and soil texture on plant height, leaf age, leaf area, and SPAD showed that clay loam soil texture exerted the most significant effect.

The higher quantities of available P, organic matter, and nitrogen initially present in clay loam may have accounted for the better shoot growth performance under the clay loam soil. On the other hand, because fertilizer P added to soil rapidly forms insoluble complexes in acrisols [4,39], we postulate that though P fertilizer was added to the Acrisol clay soils it may have been fixed and its effect may have been neutralized in the shoot. Miller [40] suggested that plant acquisition of P from soil organic matter is enhanced by the secretion of low-affinity enzymes into the soil to provide additional P for plant growth.

Studies have also shown that hydrolysis of organic matter contributes to the amounts of soluble P in the soil solution [41–43]. Thus, the low organic matter and nutrient contents in sand soil on the one hand, and the possible diffusion away of the applied soil P from the point of application, on the other, may have contributed to the overall low shoot growth response to P application in sand soil [44,45]. The high pH in sand soil may have also contributed to the decline in root activity [46,47], which could in turn have negatively impacted nutrient and water absorption, leading to low shoot growth under sand soil.

# 4.2. Changes in Photosynthetic Rate under Different Soil Textures

In this study, results of the gas exchange measurements showed that soil texture and P treatments significantly affected the photosynthetic rate of NERICA 4, with the highest mean values obtained under the clay loam soil texture (24.6  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and the P-dipping treatment (20.1  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), respectively. With reference to the conclusion by Yang [48] that photosynthetic capacity is closely related to the leaf N content, our findings regarding the photosynthetic rate may be explained by the differences in the SPAD values as an estimate of leaf N content, where the highest mean SPAD values were equally obtained under clay loam (45.6) soil texture treatment, and Brod1 (38.5) and Pdip (37.4) P treatments (Table 3). The high N content of clay loam soil may have been taken up to the plant leaves, resulting in a high photosynthetic rate under clay loam soil texture. On the other hand, P-dipping may have boosted root growth [45], leading to an enhanced P uptake under the Pdip treatment compared to that under Brod1 and Brod2 P application treatments.

#### 4.3. Changes in Root Morphology and the Effect on Shoot P Uptake

Plant roots are directly exposed to the rhizosphere soil, thereby providing the primary channel for nutrient acquisition and its subsequent utilization for plant growth. Root growth and development depend on several soil factors, including texture and density, water and nutrient contents, and concentration of oxygen [49–51]. Our findings here showed that the combined effects of soil texture and P-dipping significantly influenced NERICA 4 root morphology. Specifically, the mean values for RL, RSA, RV, and root biomass under clay soil texture and P-dipping treatment were significantly higher than those for other treatments. The low available P content in the clay experimental soil may have triggered the observed extensive root growth, as the relieved P constraints possibly led to increased soil microbial mass, and consequently an increased microbial utilization of soil carbon for increased root development [52,53].

Increased root morphological characteristics under P-deficient conditions have been reported for enhanced P absorption [54–56]. This has further been evidenced by high root-to-shoot ratios, which are generally inversely related to soil nutrient and water availability,

as plants allocate more photosynthates to their roots for increased soil exploration [57–59]. While some studies have also shown strong positive linear relationships between root morphological characteristics and P acquisition under P-deficient conditions [60–62], our results showed the opposite—particularly under P-deficient clay soil texture. In our findings, the mean shoot P concentration under the clay texture was -48.8% lower than that under clay loam soil, yet the mean RL under clay texture was 91.4% higher than the mean RL under clay loam soil texture (Table 4; Figure 3). This suggests that enhanced root morphology does not necessarily enhance P uptake in the initial rice growth stages, and thus, further research needs to be carried out to evaluate the potential of NERICA 4 rice to increase its P acquisition and utilization efficiencies at later stages of the cropping cycle for increased grain yield.

The lower shoot P content of plants under clay soil texture—despite having the most robust root biomass—could be due to the remobilization of the shoot P into the roots. Similar studies by Abdallah [63] and Irfan [64] found that in P-deficient soils, shoot P was remobilized or translocated from metabolically inactive to active sites such as the roots; in our study, the clay soil was P-deficient (Table 1). On the other hand, we think that the combination of higher root biomass with low plant tissue P concentration in the P-deficient clay soil can be explained by the Piper–Steenbjerg effect [65], summarized concisely by De Bauw [20] as low tissue P concentrations when the fast growth of plants grown in an initially higher P medium (locally after placement) eventually leads to a more rapid depletion of external P than the slow growth of plants grown in an initially lower P medium, as was the case in our study.

# 5. Conclusions

We evaluated the combined effect of soil texture and P-dipping on NERICA 4 rice shoot and root physiology and morphology, with a major focus on shoot P uptake in the initial growth stages. Contrary to our hypothesis, the interactive effect of soil texture and P-dipping influenced NERICA 4 shoot and root physiological and morphological characteristics mainly under clay loam rather than clay soil. The clay loam soil examined in our study showed higher shoot morphological characteristics despite the relatively lower root biomass. On the other hand, P-dipping significantly promoted rice root morphology under clay soil, but without a commensurate shoot P concentration and uptake. This suggests that enhanced root morphology does not necessarily enhance P uptake in the initial rice growth stages; thus, further research is necessary to evaluate the potential of NERICA 4 rice to increase its P acquisition and utilization efficiencies at later stages of the cropping cycle for increased grain yield. The findings of our study provide new insights into the existing body of knowledge on the widely adapted NERICA 4 rice variety across SSA, which should ultimately contribute to improving sustainable food security among smallholder farmers in the region.

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