

Article

# Architectural Root Responses of Rice to Reduced Water Availability Can Overcome Phosphorus Stress

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**Highlight:** Root plasticity to water availability affects the P uptake efficiency of rice by modifying root architecture. Genotypic variation in root architecture can explain tolerance to low P and water stress.

**Abstract:** Drought and low phosphorus (P) availability are major limitations for rainfed rice production. Crop roots are important for soil resource acquisition and tolerance to P and water limitations. Two pot and two field trials were conducted to evaluate architectural root responses of contrasting rice varieties to combinations of different levels of P (deficient to non-limiting) and water availability (water stressed to submergence) and to identify the interactions with different varieties. Root development was then related to drought and/or low P tolerance. Although shoot and root growth responded more to P than to water availability, architectural root responses to water were much more prominent than responses to P availability. Reduced water availability decreased nodal thickness and increased secondary root branching, both factors partially enhancing P uptake efficiency and even overcoming a decreased root:shoot ratio under reduced water availability. In contrast to root thickness and secondary branching, basal lateral root density was strongly determined by variety and was related to enhanced P uptake. Reduced water availability induces root modifications which—apart from enhancing drought resilience—also affect P uptake efficiency. Future research on rice roots and nutrient uptake may hence take into account the large effects of water on root development.

**Keywords:** root architecture; lateral rooting; root thickness; root plasticity; phosphorus uptake efficiency; water availability

## 1. Introduction

Rice (*Oryza* spp.) is among the most important grain crops in the world [1]. Its annual consumption and demand in sub-Saharan Africa is steadily rising [2,3], and to reduce the dependence on rice imports, there is a large need to increase rice production [2].

Drought and low soil phosphorus (P) availability are two major biophysical limitations to rice production in sub-Saharan Africa [4–9] which often co-occur. For both water and P, root development and root characteristics are crucial for efficient resource (water or P) acquisition under limiting conditions. Especially in low-input agricultural systems, crop roots may provide opportunities for improved resource acquisition, resource use and yield optimization [10]. Hence, it could be stated

that the more successful plants have roots that respond flexibly to soil constraints, to the spatial heterogeneity of soil P [11,12] and to fluctuating water availability [13–15].

Several root architectural traits (i.e., the spatial distribution, arrangement and appearance of different root types) can influence the P uptake efficiency of crops [16–21]. Similarly, several root architectural traits (such as root diameter or root length density) can improve water uptake efficiency and drought tolerance of different crops [13,22–24]. Soil water status and P availability are highly interrelated through diffusion, aeration, and sorption [25] and both resources have specific behavior and dynamics in the soil [26,27]. Due to these complex interactions and the heterogeneous spatial distribution of both P (often stratified in top layers) and water (often more available in deeper layers) in soils, trade-offs and synergisms in P and water uptake efficiency of roots with respect to root architectural traits may exist [28]. It is well established that soil P bioavailability increases as the soil water content increases (due to diffusion and P sorption). However, the net effect of water stress on P bioavailability and crop response is more complex than these chemical and physical processes only, due to the counteracting effects of water stress on increasing the effective root area. In that respect, it might be expected that genotypic variation in response to water stress, related to root traits, are associated in genotypic variation to P stress.

Genotypic variation in root traits provides a potential genetic resource for plant breeders to develop genotypes with enhanced water and/or P uptake efficiency [29,30]. In this view, previous studies focusing on P acquisition have characterized root traits and plasticity of different rice varieties in response to P availability and tried to link these with P uptake capacity and efficiency [9,31–33]. Similarly, a number of studies have focused on screening genotypic variation in root traits of rice under different levels of water availability, irrespective of P availability [34–36]. Under combined P and water limitation, however, roots are expected to simultaneously respond to the availability of both resources. A main concern is that the response to each of both factors (water and P) could vary in strength. As a consequence, the response to one of both factors (water and P) can overrule the responses to the other factor, either in the same or the opposite direction. An example of the latter type of interaction could be that a potentially enhanced deep rooting in response to water limitations would negatively affect topsoil foraging and thus P uptake. In the present study we hypothesize that positive root responses to water availability will have positive effects on P uptake efficiency and vice versa.

Currently, the responses of rice roots to different combinations of both water and P availability and their interactions are ill-understood. In this study, we aimed to: (i) assess the responsiveness and robustness of architectural root traits in rice under combinations of different levels of P and water availability; (ii) link the performance of different varieties under water- and P-stressed conditions to their architectural root characteristics and root responses; and (iii) assess how root architectural responses to water availability can affect P uptake efficiency and vice versa.

## 2. Material and Methods

In this study we distinguish between ‘lowland’ and ‘upland’ soils used for rice cultivation. Lowland soils, are logically located in lower areas of the landscape and are considered to be flooded at least once per rice-growing season. They have generally a finer particle size distribution (PSD) compared to upland soils, set in higher and often sloping areas and hence never flooded. We conducted one lowland and one upland field trial, as well as two pot trials with soils originating from a lowland and an upland rice growing field. Soil characteristics are presented in Table 1, and soils were sampled up to a depth of 30 cm. In terms of water availability, the upper limit is considered to be field capacity ( $pF = 2$ , or a water potential  $\psi$  of  $-9.81$  kPa) for upland soils, while for lowland soils soil submergence ( $pF = 0$ , or  $\psi = -0.098$  kPa) can be assumed to be the upper limit. Varieties used in these experiments were carefully selected based on their previously observed performance under low P and drought [9,37–39].

**Table 1.** Soil characteristics from pot and field experiments. Soils were sampled up to a depth of 30 cm.

Pot Trials	Location	Coordinates	pH	Al <sub>oxalate</sub>	Fe <sub>oxalate</sub>	P <sub>oxalate</sub>	—Texture—		
				(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(%sand)	(%silt)	(%clay)
(a) Lowland soil	Dakawa, Tanzania	06°23'56.6'' S 37°33'47.5'' E	6.1 (H <sub>2</sub> O)	670	2600	29	6	71	23
(b) Upland soil	Matombo, Tanzania	07°02'46.8'' S 37°47'11.6'' E	6.1 (CaCl <sub>2</sub> )	1050	1400	31	17	53	30
Field Trials									
(c) Lowland field April–August 2016	Ruvu, Tanzania	06°43'12.0'' S 38°40'48.0'' E	6.4 (H <sub>2</sub> O)	1150	2800	91	6	70	24
(d) Upland field July–November 2016	Morogoro, Tanzania	06°50'55.7'' S 37°39'18.2'' E	6.0 (H <sub>2</sub> O)	925	1410	47	22	61	17

## 2.1. Pot trial with Lowland Soil

### 2.1.1. Trial Establishment and Management

This trial was conducted in a greenhouse at the Sokoine University of Agriculture in Morogoro (6°50'52.8'' S, 37°39'31.2'' E; Tanzania). The average daily minimum and maximum temperatures during the experiment were 22.5 °C and 42.4 °C respectively.

This experimental set-up was a full factorial design with three P rates, three water levels and two rice varieties, having four replicates for each treatment combination.

A P-deficient soil ( $p < 0.01$  mg L<sup>-1</sup> in soil solution) from a lowland rice field (Table 1, a) was shade dried and crushed to an aggregate size of ca. 4 mm. To avoid any nutrient deficiency other than P, the bulk soil was amended with salts of NH<sub>4</sub>NO<sub>3</sub>, CaCl<sub>2</sub>, MgSO<sub>4</sub>, ZnSO<sub>4</sub>, CuSO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub> and Na<sub>2</sub>MoO<sub>4</sub> at rates of 29 mg N kg<sup>-1</sup>, 10 mg Ca kg<sup>-1</sup>, 6 mg Mg kg<sup>-1</sup>, 8 mg S kg<sup>-1</sup>, 0.5 mg Zn kg<sup>-1</sup>, 0.07 mg Cu kg<sup>-1</sup>, 0.03 mg B kg<sup>-1</sup> and 0.02 mg Mo kg<sup>-1</sup> soil.

The bulk soil was split into three parts that were amended with three different P rates based on the P adsorption capacity ( $K_d$ ) of the soil. A non-limiting P rate was obtained by amending one part with dry KH<sub>2</sub>PO<sub>4</sub> salt (279 mg P kg<sup>-1</sup>,  $K_d = 139.5$  L kg<sup>-1</sup>) up to an equilibrium P concentration of 2 mg P L<sup>-1</sup> in soil solution. A sub-optimal P rate was obtained by amending a second part up to a P concentration of 0.1 mg P L<sup>-1</sup> (18 mg P kg<sup>-1</sup>,  $K_d = 180.0$  L kg<sup>-1</sup>) and the difference in K application was adjusted with KCl. For the deficient P rate (<0.01 mg P L<sup>-1</sup> in soil solution), only KCl was amended to equalize the K application in each P treatment. Hence total K application was 360 mg K kg<sup>-1</sup> soil.

Subsequently, 72 pots (height: 40 cm; diameter: 16 cm) were filled with 9.5 kg of dry soil (24 pots for each P rate) after covering the bottom with a net and were then placed on trays to prevent leakage of soil and water. Pots meant to be flooded during the experiment were first sealed at the bottom. Pots were re-wet to field capacity (38%  $w/w$ ) and subsequently two rice varieties, NERICA4 and NERICA-L-19, were sown. Both rice varieties are developed by the Africa Rice Center using interspecific crosses between *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). NERICA4 is an upland rice variety known for its drought tolerance and sometimes also used by farmers in drought-prone lowlands. NERICA-L-19 is an irrigated lowland rice variety, not adapted to rain-fed conditions. Seedlings were thinned at 14 days after sowing (DAS) to one plant per pot.

Two top dressings of NH<sub>4</sub>NO<sub>3</sub> were applied at a rate of 317 mg N per pot (33.4 mg kg<sup>-1</sup>) at 20 and 34 DAS. An additional top dressing of ZnSO<sub>4</sub> at a rate of 57 mg Zn per pot (6.0 mg kg<sup>-1</sup>) was applied at 10 DAS in order to preclude possible P induced Zn deficiency.

Pots were daily watered back to field capacity until 18 DAS. Then three different water treatments were imposed based on the predetermined water retention curve. One third of the pots was fully submerged (Sub) (pF = 0; which corresponds with a matric potential  $\psi$  of -0.098 kPa), and another third was kept at field capacity (FC) (pF = 2, which corresponds with a matric potential  $\psi$  of -9.81 kPa, 38%  $w/w$ ) on a daily basis. The third part was subject to water stress (WS) (pF = 3–3.5,

which corresponds with a matric potential  $\psi$  of  $-98.1$  to  $-310.2$  kPa, 21%  $w/w$ ) through gradual drying by withholding water (reached after ca. five days without watering). This sub-optimal water level was reestablished three times a week by weighing the pots and carefully adding distilled water up to the predetermined target weight. Plants in the water-stressed treatment developed symptoms of drought stress (i.e., leaf rolling) prior to watering, indicating that plants were consistently water-limited.

### 2.1.2. Data Collection on Shoot Growth and Root Architecture

At 30 DAS, two young developing leaves of each plant were marked using a thin cotton wire. The marked leaves (matured) were later collected (46 DAS) for  $\delta^{13}\text{C}$  analysis (a measure of the ratio of stable isotopes  $^{13}\text{C} : ^{12}\text{C}$ ) as a proxy for water stress exploiting the differential isotope discrimination during photosynthesis under drought stress [40,41]. The  $\delta^{13}\text{C}$  measurements were done using Isotope Ratio Mass Spectrometry (IRMS) [42] following dry combustion.

The shoots were cut (46 DAS) and oven dried ( $60\text{ }^\circ\text{C}$ ). After weighing, shoots were manually crushed with a mortar to a particle size smaller than 2 mm and P concentrations (Pc) were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis (Thermo Scientific iCAP 7000 series) after digestion in hot  $\text{HNO}_3$  ( $120\text{ }^\circ\text{C}$ ).

Immediately after harvesting the shoots, the root system of each plant was carefully washed out by soaking and gently shaking in water and then transferred to a dish with clean water. The basal lateral root density, i.e., the spacing of the lateral S-type branches [33] at the nodal root base (from stubble up to 15 cm depth), was scored using the ‘shovelomics scoreboard’ developed for maize by [43]. These shovelomics scores for lateral root density are also valuable for rice, while the board could not be used to determine rice root angles, as this is modified during soil washing. The number of nodal roots was counted and the average nodal root thickness was measured and scored at the nodal root base using a transparent ruler (to 0.1 mm). The secondary branching degree, i.e., the degree of higher order root branching on L-type roots [33] evaluated over the whole root system, was visually scored. The scores used for the different traits are shown in Table 2. Roots were subsequently oven-dried ( $60\text{ }^\circ\text{C}$ ) and stubble and roots were weighed separately. The root:shoot ( $w/w$ ) ratio, total P uptake in the shoot (Pup), the P uptake in the shoot per unit root weight (Pup<sub>root</sub>), and P uptake per nodal root (Pup<sub>nodal</sub>) was then calculated. The last two variables are considered as indicators of P uptake efficiency as they estimate how efficient a root extracts P from the soil, relative to the metabolic cost (root biomass or number of nodal roots).

**Table 2.** Scoring values used for evaluating architectural root traits in both pot and field trials.

Trait Score	1	2	3	4	5				
Nodal root thickness	Very Thin <0.5 mm	Thin (0.5–0.9 mm)	Average (0.9–1.3 mm)	Thick (1.3–1.7 mm)	Very Thick >1.7 mm				
Secondary branching degree	Very Low (almost no branchings)	Low (only few branchings)	Average (average amount of branchings)	High (many branchings)	Very High (lots of branchings)				
Trait score from shovelomics board	1	2	3	4	5	6	7	8	9
Basal lateral root density	Very Sparse	-	-	-	-	-	-	-	Highly Dense

### 2.1.3. Data Analyses

All statistics were computed in R version 3.2.2, Vienna, Austria [44]. Three-way ANOVAs (Type III, using ‘aov’ from the {stats} package) were performed on the shoot and root variables with water, P, and variety as fixed factors and replicate as random factor. Means ( $\mu$ ) and standard errors ( $\frac{\sigma}{\sqrt{n}}$ , with  $\sigma$  = standard deviation and  $n$  = the number of observations) were calculated for every treatment combination and significance between treatment means was determined by calculating the Least Significant Difference (LSD), after confirming normality of the residuals.

## 2.2. Pot Trial with Upland Soil

This trial was simultaneously conducted at the same location as the pot trial with lowland soil, using a P-deficient soil from a farmer's upland rice field (Table 1, b). The soil preparation was carried out in the same way as described in the previous section. This soil was amended with rates of  $\text{KH}_2\text{PO}_4$  and KCl in order to obtain the same levels of P availability in soil solution ( $<0.01$ ,  $0.1$ , and  $2 \text{ mg P L}^{-1}$ ; corresponding with  $0$ ,  $50$  ( $K_d = 500.0 \text{ L kg}^{-1}$ ) and  $359$  ( $K_d = 179.5 \text{ L kg}^{-1}$ )  $\text{mg P kg}^{-1}$ ).

Pots were filled with  $9 \text{ kg}$  of upland soil, watered to field capacity ( $pF = 2$ ,  $\psi = -9.81 \text{ kPa}$ ,  $28\% w/w$ ) and two rice varieties (NERICA4 and Mudgo) were sown. Mudgo (*Oryza sativa*) is an Indica rice variety known for its superior performance under low P conditions [37]. It is a variety adapted to both lowland and upland conditions.

At 18 DAS, one third of the pots was daily watered to field capacity ( $pF = 2$ ,  $\psi = -9.81 \text{ kPa}$ ,  $28\% w/w$ ), another third was subjected to a sub-optimal water level ( $pF = 2.7$ , which corresponds with a matric potential of  $\psi = -49.2 \text{ kPa}$ ,  $24\% w/w$  reached after ca. two days without watering) and another third of the pots was subjected to severe water stress ( $pF = 3-3.5$ ,  $\psi = -98.1$  to  $-310.2 \text{ kPa}$ ,  $16\% w/w$  reached after ca. four days without watering). The two water levels below field capacity were reached through gradual drying by withholding water and they were reestablished three times a week by weighing the pots and carefully adding distilled water up to the predetermined target weight. Plants subjected to the lowest water level showed symptoms of drought stress (i.e., leaf rolling) prior to watering.

In this way, a full factorial design was obtained with three P rates, three water levels and two varieties, having four replicates for each treatment combination. Data were collected and analyzed in the same way as explained for the pot trial with lowland soil.

## 2.3. Lowland Field Trial

### 2.3.1. Trial Establishment and Management

This field trial was conducted in a lowland rice cultivation area (Table 1, c). The upper  $10 \text{ cm}$  of the topsoil was initially removed to reduce P availability. A split plot design with three replicate blocks was established. Within each block,  $12$  plots of  $3 \times 2 \text{ m}$  were constructed, leveled, and well separated by bunds and deep furrows to avoid horizontal water transport between plots.

The set-up resulted in a split plot design with water treatments (field capacity versus soil submergence) in the main plots and P ( $0$  versus  $30 \text{ kg P ha}^{-1}$ )  $\times$  variety (NERICA4, NERICA-L-19, Mudgo) combinations in the sub plots. The reason for blocking the water treatment into main plots was to reduce the risk of water flow from submerged sub plots to sub plots kept at field capacity. Details on the set-up and maintenance of this field trial are given in S1 (Supplementary Information).

### 2.3.2. Data Collection

At 51 DAS, four adjacent rows at one side of each plot were separated wherein four representative plants (in height and tiller number) were selected. The upper part of the root system of each selected plant was excavated by digging out a block of soil ( $20 \times 20 \times 20 \text{ cm}$ ) around the stem. Shoot and root data were collected as described for the pot trials.

Grain yield was determined in a net plot of  $1.6 \times 1.8 \text{ m}$  ( $72$  hills) excluding border rows and rows used for root excavation. In each net plot, a sub sample of  $3 \times 4$  hills ( $12$  hills) was selected for assessment of yield components and plants were cut at soil level. The grains were threshed from the panicles. The straw (including peduncle and rachis) was oven-dried at  $60 \text{ }^\circ\text{C}$  and weighed, while grains were air-dried before determining weight and moisture content. Subsequently, grains were harvested in the remainder of the net plot, and the weight and moisture content was determined. Grain yields are reported at a moisture content of  $14\%$ .

Data were analyzed by ANOVA and LSD, as described for the pot trials. Water was split within the blocks, and plant replicate and replicate blocks were assigned as random factors.

#### 2.4. Upland Field Trial

This field trial was conducted in an upland cultivation area (Table 1, d). Thirty six plots of  $3 \times 2$  m were constructed, separated by bunds and leveled. Land preparation and nutrient application was conducted in the same way (and at equal rates) as for the lowland field trial.

A randomized complete block design (three replicates) was implemented with the factors P, water, and variety. Two rates of P ( $0 \text{ kg ha}^{-1}$  and  $30 \text{ kg ha}^{-1}$ ) were applied and the same three varieties (Mudgo, NERICA4 and NERICA-L-19) were sown. The water treatments were selected based on the relevance for the respective types of rice systems and were hence different from the lowland trial. Plots were daily watered to field capacity until 19 DAS. Then half of the plots were only watered twice a week (average of drying peaks up to  $pF = 3.5$ ) while the other half was maintained daily at field capacity. This treatment mimicked dry periods on the field, and after each drying cycle, plants showed symptoms of drought stress (i.e., leaf rolling). The field was managed in the same way, and the same data were collected and analyzed as for the lowland field trial.

### 3. Results

#### 3.1. Pot Trial with Lowland Soil

Shoot growth differed more among the three P levels within each water availability treatment than among the three water levels within the P levels (Table 3). The largest source of variation in the dry shoot production, tiller number and plant height was explained by P level (sum of squares from ANOVA, plant height, and tiller number not shown).

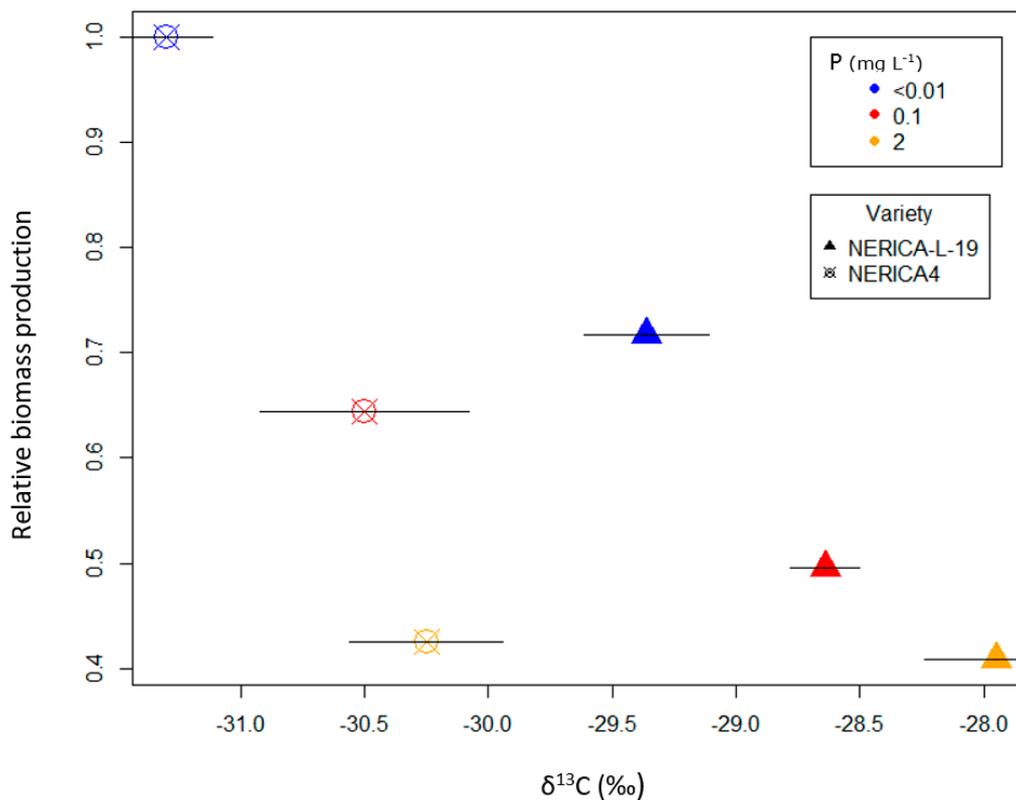
In every treatment combination (P  $\times$  variety or water  $\times$  variety), NERICA-L-19 produced more biomass than NERICA4, but these varietal differences were smaller under non-optimal conditions (low P or water stress) (Table 3). Lowering P availability had more impact percent-wise on NERICA4 than on NERICA-L-19 relative to optimal growth without P limitation. Sub-optimal P availability ( $0.1 \text{ mg P L}^{-1}$ ) decreased shoot biomass more in NERICA4 (by 50%) than in NERICA-L-19 (by 39%). Water stress in turn affected NERICA-L-19 more than NERICA4, with reductions of 47% and 36% respectively (Table 3).

Under water stress and field capacity,  $\delta^{13}\text{C}$  values in NERICA-L-19 were higher (less negative) than those in NERICA4 ( $-30.03\text{‰}$  versus  $-31.02\text{‰}$  for FC and  $-28.65\text{‰}$  versus  $-30.68\text{‰}$  for WS), while under submerged conditions  $\delta^{13}\text{C}$  values did not differ between both varieties. The  $\delta^{13}\text{C}$  values strongly differed among water treatments for NERICA-L-19, but not for NERICA4 (Table 3 and Figure 1). Increased P availability increased the  $\delta^{13}\text{C}$  values, especially under reduced water availability (Table 3). The responses of the relative biomass production and the corresponding  $\delta^{13}\text{C}$  values under water stress (relative to field capacity) are presented in Figure 1.

**Table 3.** Shoot, root, carbon isotope discrimination, and P uptake data from two varieties grown in a pot trial using lowland soil to which P was applied to achieve P concentrations in soil solution of <0.01 mg L<sup>-1</sup>, 0.1 mg L<sup>-1</sup>, and 2 mg L<sup>-1</sup>. Water supply was managed to achieve: water stress (WS), field capacity (FC) and submergence (Sub). Data are presented as means with (standard errors). Probabilities of F-statistics for the treatment effects are presented at the bottom of the table. (Pc = Shoot P concentration; Pup = Total P uptake by the shoot; Pup<sub>root</sub> = Shoot P uptake per unit root weight; Pup<sub>nodal</sub> = Shoot P uptake per nodal root).

Variety	Water	P	Shoot Mass	$\delta^{13}\text{C}$	Nodal Roots	Root Mass	Root-Shoot Ratio	Pc	Pup	Pup <sub>root</sub>	Pup <sub>nodal</sub>
NERICA4	Sub pF = 0	2	11.5 (1.9)	-30.99 (0.08)	146 (16.9)	2.57 (0.55)	0.22 (0.02)	4391 (127)	50.3 (8.1)	35.3 (3.0)	0.34 (0.028)
		0.1	5.1 (0.9)	-30.70 (0.25)	64 (8.3)	1.09 (0.09)	0.23 (0.03)	1782 (88)	9.2 (1.6)	11.1 (0.7)	0.15 (0.031)
		<0.01	1.5 (0.1)	-31.28 (0.09)	34 (2.3)	0.38 (0.02)	0.26 (0.01)	1127 (159)	1.6 (0.2)	4.9 (0.7)	0.05 (0.007)
	FC pF = 2	2	12.7 (0.7)	-30.72 (0.20)	98 (3.8)	2.18 (0.31)	0.17 (0.02)	4440 (483)	56.6 (7.2)	42.1 (5.9)	0.58 (0.074)
		0.1	5.9 (0.7)	-31.20 (0.13)	64 (8.0)	0.93 (0.07)	0.16 (0.01)	2154 (126)	12.8 (2.2)	19.0 (1.2)	0.22 (0.068)
		<0.01	2.4 (0.4)	-31.13 (0.07)	41 (3.2)	0.47 (0.07)	0.20 (0.01)	2085 (175)	5.1 (1.0)	12.4 (1.1)	0.12 (0.015)
	WS pF = 3–3.5	2	5.4 (0.4)	-30.25 (0.31)	37 (1.9)	0.73 (0.06)	0.14 (0.01)	3284 (243)	17.8 (2.0)	30.2 (3.4)	0.48 (0.57)
		0.1	3.8 (0.1)	-30.50 (0.42)	36 (3.4)	0.74 (0.10)	0.19 (0.03)	2478 (141)	9.5 (0.6)	15.4 (2.1)	0.27 (0.026)
		<0.01	2.4 (0.2)	-31.30 (0.19)	27 (1.5)	0.34 (0.02)	0.14 (0.01)	2332 (157)	5.7 (0.8)	19.8 (3.2)	0.22 (0.035)
NERICA-L-19	Sub pF = 0	2	21.0 (2.4)	-30.71 (0.20)	367 (24.2)	8.11 (1.61)	0.39 (0.06)	3031 (163)	62.7 (4.7)	18.5 (3.0)	0.17 (0.009)
		0.1	9.6 (2.0)	-30.46 (0.10)	191 (32.4)	3.48 (0.84)	0.35 (0.01)	1510 (134)	14.2 (2.7)	6.7 (0.6)	0.08 (0.009)
		<0.01	3.0 (0.5)	-30.92 (0.07)	88 (9.5)	0.90 (0.15)	0.31 (0.02)	1044 (44)	3.2 (0.6)	4.1 (0.4)	0.04 (0.003)
	FC pF = 2	2	18.6 (0.7)	-29.77 (0.32)	153 (5.6)	3.56 (0.48)	0.19 (0.02)	2822 (136)	55.5 (3.9)	31.0 (1.9)	0.37 (0.006)
		0.1	12.7 (0.8)	-29.64 (0.35)	130 (10.1)	2.14 (0.13)	0.17 (0.00)	1856 (115)	23.2 (0.4)	17.1 (1.7)	0.18 (0.014)
		<0.01	5.3 (0.2)	-30.67 (0.07)	108 (4.3)	1.26 (0.07)	0.24 (0.01)	1337 (94)	7.0 (0.3)	7.5 (1.1)	0.07 (0.006)
	WS pF = 3–3.5	2	7.6 (0.3)	-27.95 (0.29)	77 (6.3)	1.11 (0.13)	0.15 (0.02)	3263 (77)	25.0 (1.3)	50.4 (9.0)	0.33 (0.028)
		0.1	6.3 (0.3)	-28.64 (0.14)	60 (9.0)	1.07 (0.18)	0.17 (0.02)	1903 (201)	11.8 (0.8)	18.1 (2.4)	0.21 (0.026)
		<0.01	3.8 (0.3)	-29.36 (0.25)	37 (3.5)	0.43 (0.05)	0.11 (0.01)	2115 (271)	7.9 (0.5)	28.7 (1.4)	0.23 (0.034)
	Water		***	***	***	***	***	**	***	***	***
	P		***	***	***	***	ns	***	***	***	***
	Variety		***	***	***	***	***	***	**	ns	***
	Water × P		***	*	***	***	*	***	***	*	**
	Water × Variety		**	***	***	***	***	*	ns	***	ns
	P × Variety		**	ns	***	**	ns	*	ns	ns	**
	Water × P × Variety		ns	ns	***	**	ns	**	ns	**	ns

\* Significance in this table was based on a *p*-level of '\*\*' <0.05, '\*\*\*' <0.01 and '\*\*\*\*' <0.001; while ns = not significant.



**Figure 1.** Relative biomass production (under water stress relative to field capacity) plotted against carbon isotope discrimination ( $\delta^{13}\text{C}$ ) in two rice varieties (NERICA-L-19 and NERICA4) grown in lowland soil. P was applied to achieve P concentrations in soil solution of <math>0.01\text{ mg L}^{-1}</math>, <math>0.1\text{ mg L}^{-1}</math>, and <math>2\text{ mg L}^{-1}</math>. Error bars represent the standard error on the  $\delta^{13}\text{C}$ .

Root mass increased with increasing P availability. Under non-limiting P availability, reduced water availability (i.e., FC & WS) reduced root mass, but for NERICA4 this difference was not significant between submergence and field capacity. Water stress reduced root mass by 10 to 70% for NERICA4, and 50 to 86% for NERICA-L-19, while severe P deficiency reduced root mass by 53 to 85% for NERICA4, and 61 to 89% for NERICA-L-19.

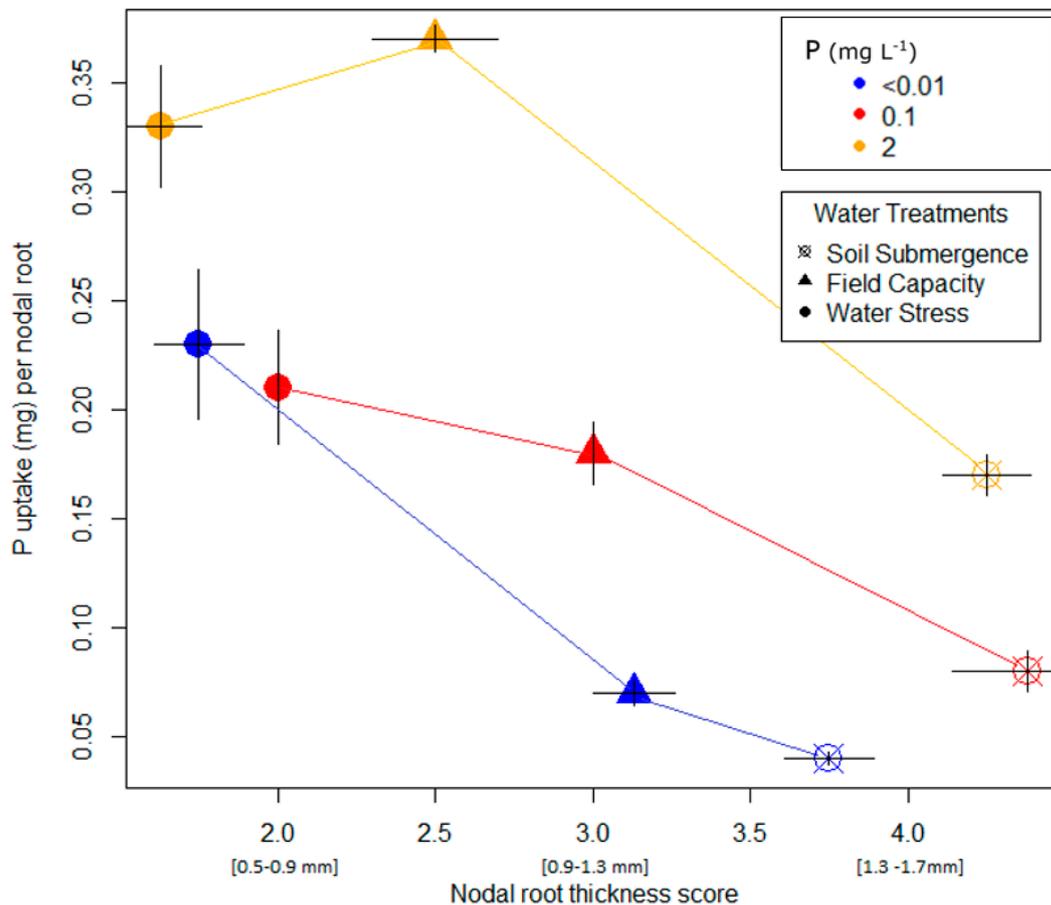
For both varieties, larger root:shoot ratios were observed with increased water availability (from WS to FC, up to Sub). Under submergence only, the root:shoot ratio of NERICA-L-19 was 48% larger than the one of NERICA4 (Table 3).

Total P uptake by NERICA-L-19 was 25% larger than by NERICA4. Without P limitations, water stress reduced total P uptake in both varieties (compared to field capacity or submerged conditions), while at sub-optimal P availability, total P uptake increased from submerged conditions to field capacity.

$\text{Pup}_{\text{root}}$  consistently increased with decreasing water availability, although for NERICA4 this response was only significant at <math>0.01\text{ mg P L}^{-1}</math> and not observed under water stress with 0.1 and 2 mg P L<sup>-1</sup>. Under 2 mg P L<sup>-1</sup>, NERICA-L-19 had a larger  $\text{Pup}_{\text{root}}$  than NERICA4 under water stress, while the reverse was true under submerged conditions.

Within each P level, decreasing water availability significantly increased the  $\text{Pup}_{\text{nodal}}$ , although this trend was reversed going from field capacity to water stress under non-limiting P (Table 3 and Figure 2). However, the smallest  $\text{Pup}_{\text{nodal}}$  was consistently observed under submerged conditions. The negative effect of submergence versus field capacity on P uptake efficiency was highly consistent in both varieties, and it was the most prominent for NERICA-L-19. Phosphorus availability increased the  $\text{Pup}_{\text{nodal}}$ , but under water stress such effect was not significant between <math>0.01</math> and 0.1 mg P L<sup>-1</sup>. For 0.1 and 2 mg P L<sup>-1</sup> the  $\text{Pup}_{\text{nodal}}$  of NERICA4 was significantly larger than that of

NERICA-L-19. At sub-optimal P availability, an equal or larger shoot mass, larger P uptake and a larger P uptake efficiency was achieved in both varieties under field capacity compared to soil submergence (Table 3), while under field capacity a similar or even smaller root mass was observed.



**Figure 2.** P uptake (mg) per nodal root versus the nodal root thickness scores from NERICA-L-19 grown in lowland soil (pots) under combinations of P rates (<math><0.01</math>, 0.1, and 2 mg P L<sup>-1</sup>) and water levels (water stress, field capacity, and soil submergence). Standard errors of the means are presented by the error bars. With decreased water availability root thickness decreases and P acquisition efficiency ( $P_{up_{nodal}}$ ) increases. Reduced soil P availability decreases P uptake per nodal root.

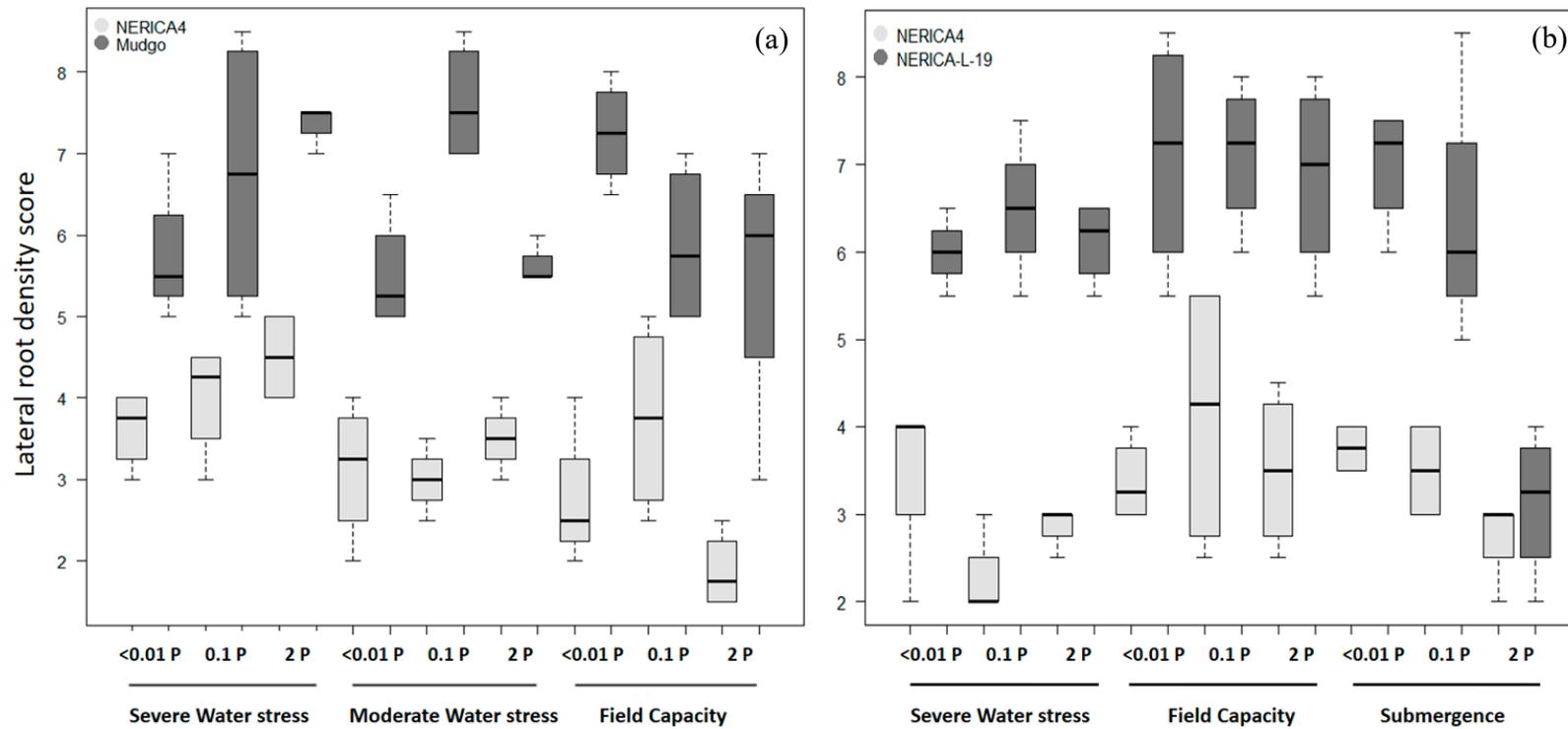
Within each P level, nodal root thickness strongly decreased under reduced water availability and this response was larger under non-limiting P (Table 4). The response of nodal root thickness to water availability was larger in NERICA-L-19 than in NERICA4. NERICA-L-19 developed significantly thicker roots under submergence compared to NERICA4. Under soil submergence, nodal root thickness significantly increased with increasing P availability from <math><0.01</math> up to 0.1 mg P L<sup>-1</sup> while under water limitations none of these P effects on nodal root thickness were significant. The response of nodal root thickness to water availability was stronger than its response to P availability, as illustrated for NERICA-L-19 in Figure S1 (Supplementary Information) and this reduced nodal thickness under reduced water availability clearly relates with an increased  $P_{up_{nodal}}$  (Figure 2).

Basal lateral root density was significantly larger for NERICA-L-19 compared to NERICA4. Except under submerged conditions with 2 mg P L<sup>-1</sup>, NERICA-L-19 showed a significantly reduced lateral root density which is not different from that of NERICA4 (Figure 3). The order of magnitude of other responses to P and water availability was much smaller compared to these differences between both varieties (Figure 3).

**Table 4.** The scores for the root architectural variables (i.e., nodal root thickness score, basal lateral root density score, and secondary branching degree) from both the lowland (left) and upland (right) pot trial. For the lowland trial two varieties, NERICA4 and NERICA-L-19, were grown under combinations of P rates (P concentration in the soil solution of <math>0.01 \text{ mg L}^{-1}</math>, <math>0.1 \text{ mg L}^{-1}</math>, and <math>2 \text{ mg L}^{-1}</math>) and water levels (water stress (WS), field capacity (FC), and submergence (Sub)). For the upland trial two varieties, NERICA4 and Mudgo, were grown under combinations of P rates (P concentration in the soil solution of <math>0.01 \text{ mg L}^{-1}</math>, <math>0.1 \text{ mg L}^{-1}</math>, and <math>2 \text{ mg L}^{-1}</math>) and water levels (severe water stress (severe WS), moderate water stress (moderate WS) and field capacity (FC)). All plants were harvested at 46 DAS. Data are presented as means with (standard errors). Probabilities of F-statistics for the effects of water, P and variety selection and their interactions on architectural the root variables in both trials are given at the bottom.

Lowland Pot Trial (Dakawa Soil)					Upland Pot Trial (Matombo Soil)					
Variety	Water	P (mg L <sup>-1</sup> )	Nodal Root Thickness Score	Secondary Branching Degree	Variety	Water	P (mg L <sup>-1</sup> )	Nodal Root Thickness Score	Secondary Branching Degree	
NERICA4	Submerged pF = 0	2	4.13 (0.13)	2.13 (0.13)	NERICA4	FC pF = 2	2	4.63 (0.20)	2.63 (0.24)	
		0.1	3.50 (0.29)	3.13 (0.32)			0.1	4.63 (0.24)	3.75 (0.25)	
		<0.01	3.25 (0.14)	3.38 (0.24)			<0.01	3.00 (0.20)	2.63 (0.24)	
	FC pF = 2	2	2.88 (0.13)	2.75 (0.14)		NERICA4	Moderate WS pF = 2.7	2	3.13 (0.13)	3.75 (0.14)
		0.1	2.75 (0.14)	2.75 (0.25)				0.1	3.13 (0.13)	4.00 (0.20)
		<0.01	3.00 (0.00)	3.25 (0.14)				<0.01	2.50 (0.20)	3.00 (0.20)
WS pF = 3–3.5	2	2.00 (0.00)	4.50 (0.20)	NERICA4	Severe WS pF = 3–3.5	2	3.00 (0.20)	5.00 (0.00)		
	0.1	1.63 (0.13)	4.75 (0.25)			0.1	2.75 (0.14)	4.88 (0.24)		
	<0.01	2.00 (0.00)	3.63 (0.13)			<0.01	1.63 (0.24)	3.88 (0.43)		
NERICA-L-19	Submerged pF = 0	2	4.25 (0.14)	2.50 (0.00)	Mudgo	FC pF = 2	2	4.50 (0.29)	2.88 (0.43)	
		0.1	4.38 (0.24)	2.63 (0.13)			0.1	4.38 (0.24)	2.88 (0.13)	
		<0.01	3.75 (0.14)	3.25 (0.14)			<0.01	3.38 (0.43)	3.13 (0.13)	
	FC pF = 2	2	2.50 (0.20)	3.75 (0.32)		Mudgo	Moderate WS pF = 2.7	2	3.63 (0.13)	4.50 (0.20)
		0.1	3.00 (0.00)	3.75 (0.14)				0.1	3.50 (0.20)	4.38 (0.24)
		<0.01	3.13 (0.13)	3.00 (0.20)				<0.01	2.38 (0.13)	3.25 (0.14)
WS pF = 3–3.5	2	1.63 (0.13)	4.50 (0.20)	Mudgo	Severe WS pF = 3–3.5	2	3.00 (0.00)	4.88 (0.13)		
	0.1	2.00 (0.00)	5.00 (0.00)			0.1	2.75 (0.14)	5.13 (0.24)		
	<0.01	1.75 (0.14)	4.00 (0.00)			<0.01	1.75 (0.14)	3.88 (0.32)		
	Water		***	***		Water		***	***	
	P		ns	*		P		***	***	
	Variety		*	*		Variety		ns	ns	
	Water × P		***	***		Water × P		ns	**	
	Water × Variety		***	*		Water × Variety		ns	ns	
	P × Variety		***	ns		P × Variety		ns	ns	
	Water × P × Variety		ns	**		Water × P × Variety		ns	ns	

\* Significance in this table was based on a *p*-level of '\*\*' <math><0.05</math>, '\*\*\*' <math><0.01</math> and '\*\*\*\*' <math><0.001</math>; while ns = not significant.



**Figure 3.** Side-by-side boxplots of basal lateral root density scores from the roots of NERICA4 (light) and NERICA-L-19 (dark) in the lowland pot trial (right panel, **b**) and NERICA 4 (light) and Mudgo (dark) in the upland pot trial (left panel, **a**). Each soil was subjected to three P-rates (<0.01, 0.1, and 2 mg P L<sup>-1</sup>) and three different water levels. The density scores are based on the Maize ‘shovelomics board’ [43] and were determined after root harvest at 46 DAS. For each trial, NERICA4 has the smallest basal lateral root density for all treatment combinations.

Secondary branching degree strongly increased under reduced water levels for both varieties, but for NERICA4 this response to water availability was not significant under severe P deficiency nor between submergence and field capacity at  $0.1 \text{ mg P L}^{-1}$ . For NERICA-L-19 this response to water was consistently significant except between submergence and field capacity under severe P deficiency (Table 4).

### 3.2. Pot Trial with Upland Soil

Shoot responses and  $\delta^{13}\text{C}$  values (Table 5) are described and discussed in S2 (Supplementary Information). Root and shoot biomass responses to P availability were larger than the responses to water availability. NERICA4 was most sensitive to reduced P availability but less sensitive to water stress.

The root:shoot ratio of Mudgo decreased under severe water stress but not for NERICA4. Under moderate and severe water stress, the root:shoot ratio of NERICA4 was significantly larger than that of Mudgo, while there was no difference in root:shoot ratio between both varieties under field capacity. With decreasing P availability, the root:shoot ratio of both varieties consistently increased, and the more so for NERICA4 than for Mudgo under P deficiency. The root:shoot ratio increased with increasing water availability under severe P deficiency, but remained unaffected by water under non-limiting and suboptimal P (Table 5).

Pc, Pup,  $\text{Pup}_{\text{root}}$ , and  $\text{Pup}_{\text{nodal}}$  increased with increasing P availability, but the magnitude of these responses depended on both water level and variety. Under  $0.1$  and  $2 \text{ mg P L}^{-1}$  the Pup of Mudgo was larger than that of NERICA4. For Mudgo,  $\text{Pup}_{\text{root}}$  consistently increased with decreasing water availability (i.e., from FC to moderate WS and extended to severe WS), while this trend was not consistently observed for NERICA4, especially not at  $<0.01$  and  $0.1 \text{ mg P L}^{-1}$ . At  $0.1$  and  $2 \text{ mg P L}^{-1}$ , the  $\text{Pup}_{\text{root}}$  of Mudgo was generally larger by 56% compared to NERICA4.

Compared to field capacity, the  $\text{Pup}_{\text{nodal}}$  increased with decreased water availability for both varieties, but this effect was not significant for NERICA4 under severe water stress. Under severe water stress, Mudgo had a larger  $\text{Pup}_{\text{nodal}}$  than NERICA4 (factor 1.35). At  $0.1$  and  $2 \text{ mg P L}^{-1}$ , Mudgo developed a larger number of nodal roots compared to NERICA4, while the  $\text{Pup}_{\text{nodal}}$  at  $0.1$  (and  $<0.01$ )  $\text{mg P L}^{-1}$  in Mudgo was similar to that of NERICA4 and significantly larger under  $2 \text{ mg P L}^{-1}$  (Table 5).

Nodal root thickness strongly decreased under reduced water and reduced P availability, but the magnitude of the response was larger for water than for P (Table 4). Nodal root thickness remained unaffected by variety.

Basal lateral root density was strongly affected by variety only and was much larger for Mudgo than for NERICA4 (Figure 3).

Secondary branching degree strongly increased under reduced water availability, although this effect was not significant at  $<0.01 \text{ mg P L}^{-1}$  between field capacity and moderate water stress. Under moderate and severe water stress, secondary branching degree significantly decreased when going from sub-optimal P availability to severe P deficiency, but this was not observed under field capacity (Table 4).

### 3.3. Lowland Field Trial

Details on shoot responses,  $\delta^{13}\text{C}$  values, and grain yields (Table 6) are described in S2 (Supplementary Information). Growth reductions (in terms of biomass) by the absence of P fertilization were smallest for Mudgo (24%), followed by NERICA-L-19 (34%) and largest for NERICA4 (45%). Grown at field capacity, Mudgo and NERICA-L-19 displayed a slightly larger  $\delta^{13}\text{C}$  compared to NERICA4 (respectively averages of  $-29.59\text{‰}$  and  $-29.37\text{‰}$  versus  $-29.97\text{‰}$ ), while such differences under submergence were less obvious.

**Table 5.** Shoot, root, carbon isotope discrimination, and P uptake data from two varieties grown in a pot trial using the upland soil to which P was applied to achieve P concentrations in soil solution of <0.01 mg L<sup>-1</sup>, 0.1 mg L<sup>-1</sup>, and 2 mg L<sup>-1</sup>. Water supply was managed to achieve: Severe water stress (severe WS), moderate water stress (moderate WS) and field capacity (FC). Data are presented as means with (standard errors). Probabilities of F-statistics for the treatment effects are presented at the bottom of the table. (Pc = Shoot P concentration; Pup = Total P uptake by the shoot; Pup<sub>root</sub> = Shoot P uptake per unit root weight; Pup<sub>nodal</sub> = Shoot P uptake per nodal root).

Variety	Water	P (mg L <sup>-1</sup> )	Shoot Mass (g)	δ <sup>13</sup> C (‰)	Nodal Roots (number plant <sup>-1</sup> )	Root Mass (g)	Root-Shoot Ratio	Pc (mg P kg <sup>-1</sup> )	Pup (mg P plant <sup>-1</sup> )	Pup <sub>root</sub> (mg P g <sup>-1</sup> )	Pup <sub>nodal</sub> (mg P nodal <sup>-1</sup> )
NERICA4	FC pF = 2	2	15.1 (1.3)	-31.35 (0.09)	118 (9.5)	2.63 (0.29)	0.17 (0.01)	3542 (65)	53.2 (3.8)	29.8 (1.5)	0.45 (0.014)
		0.1	9.9 (0.8)	-31.36 (0.14)	88 (8.4)	1.87 (0.28)	0.19 (0.02)	1649 (60)	16.2 (1.0)	12.2 (1.7)	0.18 (0.004)
		<0.01	0.9 (0.1)	-30.91 (0.18)	27 (2.2)	0.40 (0.05)	0.44 (0.02)	832 (49)	0.8 (0.1)	2.1 (0.1)	0.03 (0.003)
	Moderate WS	2	11.9 (0.7)	-30.57 (0.20)	82 (4.9)	2.25 (0.17)	0.19 (0.01)	3915 (155)	46.6 (3.6)	32.1 (2.1)	0.58 (0.046)
		0.1	8.5 (0.9)	-31.02 (0.27)	71 (5.0)	1.88 (0.19)	0.23 (0.02)	1758 (85)	14.8 (1.2)	9.5 (1.1)	0.21 (0.010)
		<0.01	0.9 (0.2)	-31.37 (0.07)	26 (1.1)	0.34 (0.06)	0.42 (0.06)	1127 (155)	1.1 (0.3)	3.3 (1.1)	0.04 (0.013)
	Severe WS pF =3-3.5	2	7.1 (0.9)	-29.97 (0.11)	66 (11.3)	1.33 (0.19)	0.19 (0.02)	3764 (54)	26.7 (3.1)	26.6 (2.7)	0.44 (0.083)
		0.1	6.1 (0.7)	-30.30 (0.14)	48 (4.1)	1.61 (0.25)	0.26 (0.02)	1489 (69)	9.1 (1.3)	7.4 (0.7)	0.19 (0.018)
		<0.01	1.0 (0.1)	-31.34 (0.11)	16 (2.2)	0.37 (0.05)	0.37 (0.04)	771 (21)	0.8 (0.1)	2.3 (0.2)	0.05 (0.005)
Mudgo	FC pF = 2	2	17.2 (2.3)	-30.59 (0.19)	150 (12.2)	3.02 (0.74)	0.17 (0.02)	4188 (276)	72.6 (12.5)	35.1 (3.3)	0.47 (0.047)
		0.1	14.5 (2.1)	-30.70 (0.06)	159 (16.7)	3.40 (0.63)	0.23 (0.02)	1599 (27)	23.2 (3.3)	9.9 (1.3)	0.15 (0.009)
		<0.01	1.2 (0.2)	-30.84 (0.16)	37 (2.6)	0.48 (0.09)	0.40 (0.03)	719 (36)	0.9 (0.1)	2.0 (0.1)	0.02 (0.002)
	Moderate WS	2	15.4 (0.5)	-29.33 (0.18)	127 (4.1)	2.85 (0.24)	0.19 (0.02)	4921 (469)	76.3 (9.1)	44.6 (5.6)	0.60 (0.065)
		0.1	12.2 (1.0)	-30.25 (0.40)	120 (7.9)	2.53 (0.32)	0.21 (0.01)	2054 (72)	24.9 (1.8)	13.8 (0.5)	0.21 (0.010)
		<0.01	1.0 (0.1)	-30.85 (0.06)	28 (2.3)	0.32 (0.06)	0.31 (0.04)	826 (63)	0.8 (0.1)	2.9 (0.3)	0.03 (0.006)
	Severe WS pF = 3-3.5	2	10.0 (0.6)	-28.94 (0.47)	93 (3.8)	1.40 (0.22)	0.14 (0.01)	6210 (340)	62.0 (5.1)	68.3 (7.3)	0.66 (0.042)
		0.1	9.1 (0.5)	-29.03 (0.25)	81 (6.9)	1.70 (0.19)	0.19 (0.01)	1853 (61)	16.9 (1.4)	14.3 (2.4)	0.21 (0.009)
		<0.01	1.2 (0.1)	-30.20 (0.11)	25 (2.9)	0.24 (0.03)	0.21 (0.01)	980 (101)	1.1 (0.1)	5.4 (0.6)	0.05 (0.008)
	Water		***	***	***	***	**	***	**	**	**
	P		***	***	***	***	***	***	***	***	***
	Variety		***	***	***	**	***	***	***	***	ns
	Water × P		***	***	***	*	**	***	*	**	ns
	Water × Variety		ns	*	ns	ns	**	***	ns	***	*
	P × Variety		**	ns	***	ns	**	***	***	***	*
	Water × P × Variety		ns	ns	ns	ns	ns	**	ns	***	ns

\* Significance in this table was based on a p-level of '\*' <0.05, '\*\*' <0.01 and '\*\*\*' <0.001; while ns = not significant.

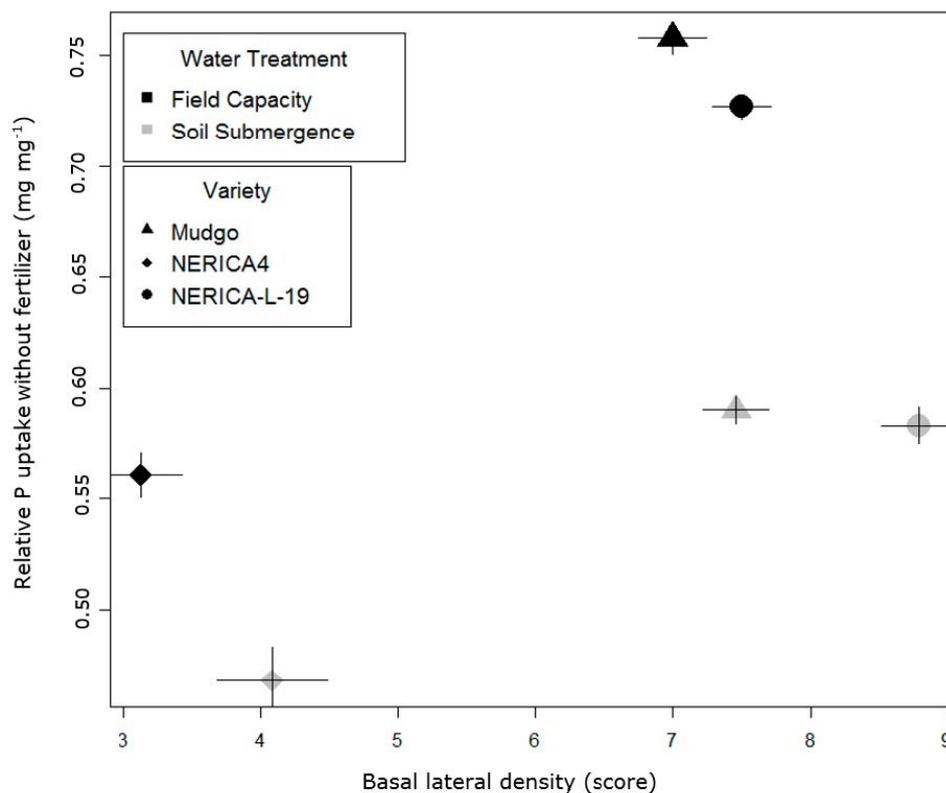
**Table 6.** Shoot, root, carbon isotope discrimination, and P uptake data from the lowland field trial. Three varieties (NERICA4, NERICA-L-19 and Mudgo) were grown in a lowland field under two different P rates (0 kg P ha<sup>-1</sup> (0P) versus 30 kg P ha<sup>-1</sup> (+P)) and two different water treatments (field capacity (FC) versus submergence (Sub)). Data were collected at 51 DAS except for grain yield which was estimated at crop maturity stage. Data are presented as means with (standard errors). Probabilities of F-statistics for the treatment effects are given at the bottom. (Pc = Shoot P concentration; Pup = Total P uptake by the shoot; Pup<sub>root</sub> = Shoot P uptake per unit root weight; Pup<sub>nodal</sub> = Shoot P uptake per nodal root).

Variety	Water	P	Shoot Mass (g)	δ <sup>13</sup> C (‰)	Nodal Roots (number plant <sup>-1</sup> )	Root Mass (g)	Root-Shoot Ratio	Pc (mg P kg <sup>-1</sup> )	Pup (mg P plant <sup>-1</sup> )	Pup <sub>root</sub> (mg P g <sup>-1</sup> )	Pup <sub>nodal</sub> (mg P nodal <sup>-1</sup> )	Grain Yield (kg ha <sup>-1</sup> )
NERICA4	Sub	+P	16.45 (0.92)	-29.75 (0.05)	164 (5.9)	3.17 (0.21)	0.19 (0.01)	2425 (93)	40.2 (3.0)	20.5 (1.4)	0.24 (0.014)	3960 (1090.2)
		0P	9.23 (0.99)	-29.66 (0.10)	120 (6.4)	1.88 (0.19)	0.21 (0.01)	1910 (143)	18.8 (3.6)	14.4 (1.8)	0.15 (0.021)	3170 (741.2)
	FC	+P	17.40 (1.08)	-29.95 (0.06)	140 (7.3)	3.23 (0.24)	0.19 (0.01)	2131 (56)	37.1 (2.6)	18.1 (1.2)	0.27 (0.016)	3947 (233.4)
		0P	9.41 (1.07)	-29.99 (0.07)	98 (6.3)	1.84 (0.20)	0.20 (0.01)	2110 (165)	20.8 (3.0)	15.8 (1.6)	0.20 (0.023)	3044 (405.9)
NERICA-L-19	Sub	+P	21.77 (1.34)	-29.67 (0.09)	406 (13.9)	7.17 (0.46)	0.33 (0.02)	2099 (89)	45.3 (2.9)	12.6 (1.0)	0.11 (0.007)	4925 (543.6)
		0P	13.91 (1.06)	-29.99 (0.11)	314 (15.8)	4.64 (0.37)	0.34 (0.02)	1847 (109)	26.4 (3.1)	10.8 (1.8)	0.08 (0.008)	4884 (435.6)
	FC	+P	21.96 (1.92)	-29.28 (0.12)	276 (14.5)	5.30 (0.42)	0.25 (0.01)	1613 (46)	35.9 (3.8)	14.6 (1.3)	0.13 (0.009)	4212 (377.6)
		0P	15.00 (0.70)	-29.45 (0.09)	274 (14.5)	3.90 (0.24)	0.26 (0.01)	1759 (42)	26.1 (1.3)	12.3 (0.8)	0.10 (0.005)	3504 (275.7)
Mudgo	Sub	+P	22.24 (1.22)	-30.04 (0.07)	313 (9.1)	4.40 (0.30)	0.20 (0.01)	2925 (67)	64.6 (3.2)	28.0 (1.2)	0.21 (0.011)	4060 (449.4)
		0P	16.95 (1.38)	-30.12 (0.06)	232 (8.0)	3.12 (0.22)	0.19 (0.01)	2245 (129)	38.1 (3.6)	19.9 (2.1)	0.16 (0.013)	3535 (937.7)
	FC	+P	21.69 (1.46)	-29.53 (0.05)	240 (16.9)	3.67 (0.28)	0.17 (0.01)	2306 (160)	50.8 (6.0)	23.4 (2.8)	0.21 (0.015)	3435 (104.5)
		0P	16.59 (1.35)	-29.64 (0.10)	196 (6.0)	2.82 (0.14)	0.18 (0.01)	2290 (82)	38.5 (3.8)	21.9 (1.7)	0.19 (0.016)	2626 (637.5)
Water			ns	ns	**	***	***	**	*	ns	ns	<i>p</i> = 0.052
P			***	*	***	***	ns	***	***	***	***	<i>p</i> = 0.052
Variety			***	***	***	***	***	***	***	***	***	*
Water × P			ns	ns	**	ns	ns	***	*	0.058	ns	ns
Water × Variety			ns	***	***	***	***	ns	ns	ns	ns	ns
P × Variety			ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Water × P × Variety			ns	ns	*	ns	ns	ns	ns	ns	ns	ns

\* Significance in this table was based on a *p*-level of '\*\*' <0.05, '\*\*\*' <0.01 and '\*\*\*\*' <0.001; while ns = not significant.

The root:shoot ratio decreased going from submergence to field capacity for NERICA-L-19, while this response was not significant for Mudgo and NERICA4. Root:shoot ratios were larger for NERICA-L-19 compared to those of Mudgo and NERICA4 (Table 6).

Pc increased with P fertilization under submergence but not under field capacity. The Pc was largest for Mudgo, followed by NERICA4, and was smallest for NERICA-L-19. Pup increased under submerged conditions compared to field capacity, only when P was applied and Pup was largest for Mudgo and smallest for NERICA4. The differences in relative P uptake by the contrasting varieties (indicator for low P tolerance) correspond with strong differences in basal lateral density, and this tolerance is enhanced under reduced water availability (Figure 4). The latter response is not related with a response in basal lateral density, but rather related with a response in nodal root thickness and secondary branching degree (Table 7).



**Figure 4.** Relative P uptake of different rice varieties (i.e., the P uptake without P fertilization relative to the P uptake when P is sufficiently available) in relation to the observed basal lateral density (analyzed at 51 DAS). Three varieties (NERICA4, Mudgo, and NERICA-L-19) were grown on a lowland rice field with ( $30 \text{ kg P ha}^{-1}$ ) and without ( $0 \text{ kg P ha}^{-1}$ ) P fertilization and they were subjected to two water treatments (soil submergence versus field capacity). The higher basal lateral branching density relates with the low P tolerance of contrasting varieties and reduced water availability additionally enhances this relative P uptake.

The  $\text{Pup}_{\text{root}}$  significantly increased with P fertilization and  $\text{Pup}_{\text{root}}$  was larger for Mudgo compared to NERICA4, for which it was larger than for NERICA-L-19. The  $\text{Pup}_{\text{nodal}}$  increased with P fertilization for both NERICA4 and Mudgo, but not in NERICA-L-19. The  $\text{Pup}_{\text{nodal}}$  of NERICA-L-19 was smaller than that of Mudgo and NERICA4 (both with and without P fertilization), while the  $\text{Pup}_{\text{nodal}}$  of Mudgo was larger than that of NERICA4 with P fertilization only (Table 6).

**Table 7.** The scores for root architectural traits (i.e., nodal root thickness score, basal lateral root density score, and secondary branching degree) of three varieties (NERICA4, NERICA-L-19 and Mudgo) grown in two field sites in Tanzania. For the lowland field, plants were grown under two different P rates (0 kg P ha<sup>-1</sup> (0P) versus 30 kg P ha<sup>-1</sup> (+P)) and two different water treatments (field capacity (FC) versus submergence (Sub)). For the upland field, plants were grown under the same two different P rates (0 kg P ha<sup>-1</sup> (0P) versus 30 kg P ha<sup>-1</sup> (+P)) and two different water treatments (drought versus field capacity (FC)). Roots were harvested and analyzed at 51 DAS. Data are presented as means with (standard errors). Probabilities of F-statistics for the treatment effects are given at the bottom.

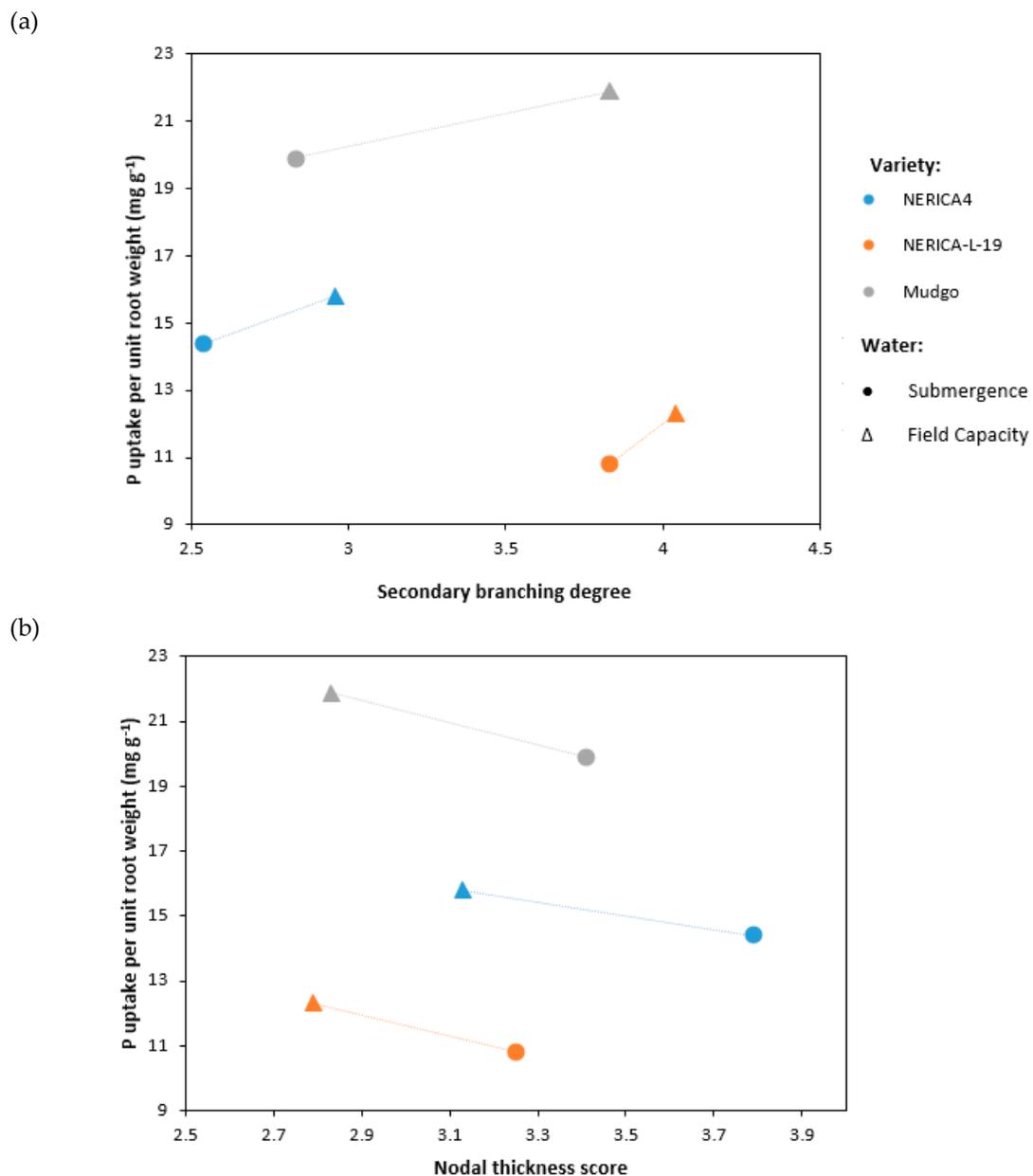
Variety	Lowland Field Trial (Ruvu)					Upland Field Trial (Morogoro)					
	Water	P	Nodal Root Thickness Score	Basal Lateral Root Density Score	Secondary Branching Degree	Water	P	Nodal Root Thickness Score	Basal Lateral Root Density Score	Secondary Branching Degree	
NERICA4	Sub	+P	4.08 (0.08)	3.71 (0.29)	2.42 (0.15)	FC	+P	3.0 (0.20)	2.7 (0.40)	2.4 (0.14)	
		0P	3.79 (0.10)	4.08 (0.40)	2.54 (0.13)		0P	2.6 (0.18)	2.1 (0.22)	2.1 (0.08)	
NERICA4	FC	+P	3.33 (0.07)	2.46 (0.23)	3.08 (0.13)	drought	+P	2.7 (0.12)	3.5 (0.30)	2.8 (0.17)	
		0P	3.13 (0.07)	3.13 (0.30)	2.96 (0.07)		0P	2.4 (0.16)	2.7 (0.29)	2.4 (0.14)	
NERICA-L-19	Sub	+P	3.50 (0.11)	7.00 (0.21)	3.75 (0.13)	FC	+P	2.4 (0.13)	4.7 (0.37)	3.0 (0.13)	
		0P	3.25 (0.10)	8.79 (0.27)	3.83 (0.18)		0P	2.3 (0.10)	3.9 (0.34)	2.8 (0.11)	
NERICA-L-19	FC	+P	2.92 (0.08)	7.21 (0.22)	4.17 (0.07)	drought	+P	2.3 (0.14)	3.5 (0.52)	2.5 (0.20)	
		0P	2.79 (0.07)	7.50 (0.21)	4.04 (0.07)		0P	2.3 (0.12)	4.7 (0.46)	3.0 (0.21)	
Mudgo	Sub	+P	3.50 (0.11)	6.69 (0.23)	2.83 (0.13)	FC	+P	2.8 (0.13)	4.1 (0.65)	2.6 (0.26)	
		0P	3.41 (0.12)	7.46 (0.24)	2.83 (0.18)		0P	2.7 (0.14)	3.5 (0.63)	2.8 (0.19)	
Mudgo	FC	+P	3.00 (0.06)	6.71 (0.32)	3.92 (0.10)	drought	+P	2.6 (0.15)	4.8 (0.38)	3.0 (0.12)	
		0P	2.83 (0.07)	7.00 (0.25)	3.83 (0.13)		0P	2.4 (0.11)	4.3 (0.46)	2.8 (0.18)	
<b>Water</b>			***	ns	ns	<b>Water</b>			*	ns	ns
<b>P</b>			ns	ns	ns	<b>P</b>			*	ns	ns
<b>Variety</b>			ns	***	**	<b>Variety</b>			***	***	***
<b>Water × P</b>			ns	ns	ns	<b>Water × P</b>			ns	ns	ns
<b>Water × Variety</b>			ns	ns	**	<b>Water × Variety</b>			ns	ns	ns
<b>P × Variety</b>			ns	ns	ns	<b>P × Variety</b>			ns	ns	ns
<b>Water × P × Variety</b>			ns	ns	ns	<b>Water × P × Variety</b>			ns	ns	*

\* Significance in this table was based on a *p*-level of \*\* <0.05, \*\*\* <0.01, and \*\*\*\* <0.001; while ns = not significant.

Nodal root thickness decreased with reduced water levels but did not differ among the varieties (Table 7).

The basal lateral root density was not affected by P or water but was significantly larger for NERICA-L-19 and Mudgo compared to NERICA4 (Table 7).

All varieties showed a significantly larger secondary branching degree under field capacity compared to submergence, but the response was largest in NERICA-L-19. The secondary branching degree of NERICA4 was lower than that of the other varieties, and only under submergence, the secondary branching degree of Mudgo was smaller than that of NERICA-L-19 (Table 7). Without P fertilization, the responses in nodal thickness and secondary branching to reduced water availability relate with an increased P uptake efficiency (Figure 5).



**Figure 5.** The P uptake (mg) per unit root mass (g) versus the secondary branching degree (a), and nodal thickness (b) in response to reduced water availability (Soil Submergence versus Field Capacity). Data are presented for three varieties grown on a lowland field without P application.

### 3.4. Upland Field Trial

For this field trial, data on shoot and root mass were not collected. The remaining plant data (tiller number, plant height,  $\delta^{13}\text{C}$ , number of nodal roots, P concentration in the shoot tissue, grain yield, and harvest index) are given in Table S1 (Supplementary Information).

Nodal root thickness significantly reduced under drought and significantly increased with P fertilization. Nodal roots of NERICA4 and Mudgo were thicker than nodal roots of NERICA-L-19. No significant factor interactions were observed for nodal root thickness (Table 7).

Basal lateral root density was significantly affected by variety only. NERICA4 had a significantly lower basal lateral root density than both NERICA-L-19 and Mudgo (Table 7).

In general, drought increased the secondary branching degree, although differences were not consistently significant and for NERICA-L-19 under P fertilization a reversed response was observed (Table 7). The secondary branching degree of NERICA4 was generally, while not consistently, smaller than that of NERICA-L-19 and Mudgo (Table 7).

## 4. Discussion

### 4.1. Shoot and Grain Yield Responses of Contrasting Rice Varieties to Combinations of P and Water Availability

Under both pot and field conditions, P availability limited growth and grain yield more strongly than water availability. With severe P deficiency in the pot trials, shoot growth of each variety was extremely limited precluding observations of additional growth reductions by water stress. NERICA-L-19 and Mudgo had a larger growth potential (in terms of biomass and yield) and demonstrated a larger total P uptake compared to NERICA4, as earlier reported by [37,38] for Mudgo and NERICA4. NERICA-L-19 and Mudgo are thus less sensitive to low and especially to sub-optimal P availability compared to NERICA4.

In contrast, it was confirmed that NERICA4 is more tolerant to water stress compared to the other varieties. As a result of their larger growth potential, NERICA-L-19 and Mudgo had larger shoot biomass under water stress, but this trend would change after stronger and longer periods of water stress. Additionally, larger and faster growing genotypes generally have larger water requirements and would therefore show greater susceptibility to water stress. The observed differences in performance under reduced water availability were expected for NERICA4 and NERICA-L-19 as the former is a typical upland variety, known for its drought tolerance [45,46], while the latter is a typical lowland variety with large water requirements [47].

### 4.2. Architectural Responses of Rice Roots to P and Water Availability

The degree of secondary branching increased under reduced water levels (i.e., field capacity and water stress), which is in agreement with the findings of [48,49], who reported a similar increase under field capacity compared to soil submergence. Nodal root thickness strongly decreased with decreasing water availability, as also observed by [50,51]. P availability had only a minor influence on nodal thickness which can be considered as an indirect result of reduced growth under P deficiency, whereby less developed and smaller plants have thinner roots. Increased root branching and reduced nodal thickness increase the soil volume explored, improve the root-soil contact and—especially in deeper soil layers—improves water acquisition and tolerance under reduced water availability [24,52]. Our results demonstrate that rice roots respond plastically to reduced water availability by reducing nodal diameter and developing more higher order branches. First order branching density (S-type roots [33]) at the nodal root base, however, was mainly determined by variety and remained largely unaffected by water and P availability.

In general, the root:shoot ratio of crops is inversely proportional to both nutrient and water availability, as plants allocate relatively more photosynthates to their roots under sub-optimal and limiting conditions to increase soil exploration [28,53–58]. Additionally an ontogenetic effect is in place where smaller and less developed plants generally have larger root:shoot ratios [18]. Hence it

is remarkable that the root:shoot ratio of rice generally decreased with reduced water availability (especially going from submergence to field capacity, but also in response to water stress). For rice, a decrease of root:shoot ratio in response to drought was also observed by [34], and this is in line with our observed decrease in nodal root thickness, which resulted in a more efficient conversion of biomass to root length. An additional contribution to a reduced root:shoot ratio under drought can be the increased formation of aerenchyma (as stress response) [59,60]. This negative response of the root:shoot ratio to reduced water availability in the lowland pot trial was much larger for NERICA-L-19 than for NERICA4 where it was less obvious. The ability of NERICA4 to maintain a high root:shoot ratio under drought could either be the cause of its reduced sensitivity to drought, or be an indirect result of its reduced sensitivity. For NERICA4, the increased rooting depth recently observed under reduced water availability is in line with these findings [39].

In conclusion, while P availability was perceived as the most determinant factor for growth and yield, root architecture (i.e., nodal root thickness, secondary branching degree, and root:shoot ratio) was primarily modified by water availability, and not or only to a minor extent by P availability. The responses of the basal lateral root density to changing water (or P) availability were less obvious compared to responses of nodal root thickness or secondary branching.

#### 4.3. Comparing Root Architecture among Varieties in Relation to Stress (Low P & Drought) Tolerance

In contrast to nodal root thickness and secondary branching degree, basal lateral root density was a more robust and less plastic root trait (related to P and water availability), only slightly responsive to P and water availability and strongly determined by variety. The much higher basal lateral root density in roots of NERICA-L-19 and Mudgo compared to roots of NERICA4 can explain the larger growth rate and larger P uptake capacity as suggested by [12]. High basal lateral root density thus relates with low P tolerance of rice and is a relatively stable trait (not responsive to P and water availability), presenting good opportunities for breeding towards low P tolerant rice varieties.

In contrast, first order basal lateral roots (S-type) are more sensitive to drought, and therefore drought tolerant varieties rather avoid investing in such risky laterals and invest more photosynthates in deep rooting. The reduced drought sensitivity of NERICA4 was in practice related to a smaller basal lateral root density, which theoretically enhances drought tolerance by enabling root growth into deeper layers [13,23]. Basal lateral root density hence shows antagonistic effects on drought and low P tolerance [28], which should be taken into account when selecting varieties for specific environments.

In addition, the roots of NERICA4 grown in the field trials were slightly thicker and more stiff. Root performance (e.g., nodal root thickness or root:shoot ratio) of this drought tolerant variety (NERICA4) was more robust and less responsive to its environment compared to the other varieties. Additionally, the reduced transpiration requirements would contribute to these findings. A larger and more stable relative investment to roots under drought may increase its chances of survival under longer drought periods. NERICA4 can hence be qualified as a stable and robust variety in terms of growth, plant performance and root architecture [61,62] partly explaining its wide adoption by farmers [63].

The relatively large root biomass of NERICA-L-19 under soil submergence enhanced its total P uptake capacity, but resulted in a relatively low P uptake efficiency ( $P_{up_{root}}$  and  $P_{up_{nodal}}$ ). Perhaps the large number of nodal roots under field capacity and submergence, combined with the high basal lateral root density increased the inter-root competition for P acquisition within each plant [64–66]. Hence, the number of nodal roots and total root investments of NERICA-L-19 may have exceeded a critical point, whereby the increase in P uptake by each additional nodal root or additional unit root mass decreased due to increased inter-root competition.

Mudgo had the most efficient root system in terms of P uptake (i.e., P uptake per nodal root and per unit root mass). With fewer nodal roots, smaller root mass, and similar basal root density, Mudgo was able to acquire a larger quantity of P compared to NERICA-L-19 (in the field), suggesting another P uptake strategy of Mudgo not addressed in this study (e.g., an up-regulation of high affinity P

transporters, the release of phosphatases, or the release of protons or other organic compounds to mobilize inorganic P in the rhizosphere [9]). Future research would thus benefit from unraveling this enhanced P uptake mechanism of Mudgo, as it could potentially be exploited by breeding.

#### 4.4. Implications of the Root Responses to Reduced Water Availability for P Acquisition and Agronomic Measures

Root traits such as secondary branching degree and nodal root diameter responded largely to water availability, with large implications for P uptake due to the importance of fine roots for P acquisition [67,68]. In all trials, larger values of P uptake efficiency (i.e.,  $P_{up_{root}}$  and  $P_{up_{nodal}}$ ) were observed under reduced water availability, especially under limiting P, while the total P uptake was often observed to increase with reduced water levels. However, with decreasing water availability in a soil, from a certain point the reduced P diffusion will overrule the beneficial effects of an improved root system on total P uptake.

The benefits of a more optimized root system (in terms of root architecture and acquisition efficiency) on P uptake would theoretically be largest under sub-optimal P availability. The largest increase in shoot biomass and P uptake in response to reduced water availability (i.e., field capacity versus soil submergence) was effectively observed for NERICA-L-19 under sub-optimal P availability (Table 3, for  $0.01 \text{ mg P L}^{-1}$ ). A generally smaller increase in P uptake efficiency in response to reduced water availability in NERICA4 corresponded with a lower degree of root plasticity in response to such lower water levels.

These findings imply that P uptake and yield in low P environments could be enhanced by moderating water supply to the crop. Architectural root responses to water availability have strong implications for the selection and breeding of low P tolerant rice varieties based on the expression of root characteristics (e.g., [32,69–71]), as beneficial root traits for P uptake (e.g., nodal root thickness or degree of second order branching) could so easily be modified by water availability. These results highlight the importance of understanding the responses of root traits to multiple environmental factors and their plasticity before exploiting them in breeding.

## 5. Conclusions

This study is the first report on rice root responses to combined stresses (here P and water availability) of the genotypes selected, and the first report on many of the root traits presented (i.e., nodal root thickness, basal lateral root density, secondary branching degree). We demonstrated that the different performance of contrasting rice varieties under conditions of P and water stress are related to specific root architectural traits and root responses. Moreover, root architectural traits of rice respond much more to water than to P availability and a change in water availability alters the P uptake efficiency. It follows that reduced water availability could enhance P uptake efficiency under low P conditions as it modifies the root system, while it can also enhance the formation of more drought resilient roots. Future research on rice root architecture and nutrient uptake efficiency should take into account the large effects of water availability on root development, and breeding programs should consider the plasticity of architectural root traits in response to multiple environmental factors.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/1/11/s1>. S1: Details on the set-up and maintenance of the lowland field experiment; S2: Detailed Result description of the growth responses to P and water availability for (a) the pot trial with upland soil (b) the lowland field trial; Table S1: Additional data from the upland field trial; Figure S1: Response plane of the nodal root thickness of NERICA-L-19 grown in lowland soil under combinations of P rates and water levels.

**Author Contributions:** P.D.B., A.L., and L.M. conducted the experiments (with assistance as described in the acknowledgments), P.D.B. analyzed the data and wrote the manuscript. E.V., K.S., and R.M. conceived the research and revised the manuscript.

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## References

1. Chauhan, B.S.; Jabran, K.; Mahajan, G. (Eds.) *Rice Production Worldwide*; Springer International Publishing: Cham, Switzerland, 2017; ISBN 978-3-319-47514-1.
2. Seck, P.A.; Tollens, E.; Wopereis, M.C.S.; Diagne, A.; Bamba, I. Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. *Food Policy* **2010**, *35*, 403–411. [[CrossRef](#)]
3. van Oort, P.A.J.; Saito, K.; Tanaka, A.; Amovin-Assagba, E.; Van Bussel, L.G.J.; van Wart, J.; de Groot, H.; van Ittersum, M.K.; Cassman, K.G.; Wopereis, M.C.S. Assessment of rice self-sufficiency in 2025 in eight African countries. *Glob. Food Secur.* **2015**, *5*, 39–49. [[CrossRef](#)]
4. Diagne, A.; Alia, D.Y.; Amovin-Assagba, E.; Wopereis, M.C.S.; Saito, K.; Nakelse, T. Farmer perceptions of the biophysical constraints to rice production in sub-Saharan Africa, and potential impact of research. In *Realizing Africa's Rice Promise*; CABI: Wallingford, UK, 2013; pp. 46–68.
5. Nziguheba, G.; Zingore, S.; Kihara, J.; Merckx, R.; Njoroge, S.; Otinga, A.; Vandamme, E.; Vanlauwe, B. Phosphorus in smallholder farming systems of sub-Saharan Africa: Implications for agricultural intensification. *Nutr. Cycl. Agroecosystems* **2016**, *104*, 321–340. [[CrossRef](#)]
6. Sahrawat, K.L.; Jones, M.P.; Diatta, S. Plant phosphorus and rice yield in an Ultisol of the humid forest zone in West Africa. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 997–1005. [[CrossRef](#)]
7. Saito, K.; Nelson, A.; Zwart, S.J.; Niang, A.; Sow, A.; Yoshida, H.; Wopereis, M.C.S. Towards a better understanding of biophysical determinants of yield gaps and the potential for expansion of the rice area in Africa. In *Realizing Africa's Rice Promise*; CABI: Wallingford, UK, 2013; pp. 188–203.
8. Serraj, R.; McNally, K.L.; Slamet-Loedin, I.; Kohli, A.; Haefele, S.M.; Atlin, G.; Kumar, A. Drought Resistance Improvement in Rice: An Integrated Genetic and Resource Management Strategy. *Plant Prod. Sci.* **2011**, *14*, 1–14. [[CrossRef](#)]
9. Vandamme, E.; Rose, T.; Saito, K.; Jeong, K.; Wissuwa, M. Integration of P acquisition efficiency, P utilization efficiency and low grain P concentrations into P-efficient rice genotypes for specific target environments. *Nutr. Cycl. Agroecosystems* **2016**, *104*, 413–427. [[CrossRef](#)]
10. Bishopp, A.; Lynch, J.P. The hidden half of crop yields. *Nat. Plants* **2015**, *1*, 15117. [[CrossRef](#)]
11. Niu, Y.F.; Chai, R.S.; Jin, G.L.; Wang, H.; Tang, C.X.; Zhang, Y.S. Responses of root architecture development to low phosphorus availability: A review. *Ann. Bot.* **2013**, *112*, 391–408. [[CrossRef](#)]
12. Ramaekers, L.; Remans, R.; Rao, I.M.; Blair, M.W.; Vanderleyden, J. Strategies for improving phosphorus acquisition efficiency of crop plants. *Field Crops Res.* **2010**, *117*, 169–176. [[CrossRef](#)]
13. Zhan, A.; Schneider, H.; Lynch, J.P. Reduced Lateral Root Branching Density Improves Drought Tolerance in Maize. *Plant Physiol.* **2015**, *168*, 1603–1615. [[CrossRef](#)]
14. Kano, M.; Inukai, Y.; Kitano, H.; Yamauchi, A. Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil* **2011**, *342*, 117–128. [[CrossRef](#)]
15. Koevoets, I.T.; Venema, J.H.; Elzenga, J.T.M.; Testerink, C. Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. *Front. Plant Sci.* **2016**, *7*, 1335. [[CrossRef](#)] [[PubMed](#)]
16. Lynch, J. Root Architecture and Plant Productivity. *Plant Physiol.* **1995**, *109*, 7–13. [[CrossRef](#)]
17. Lynch, J.P. Root Architecture and Nutrient Acquisition. In *Nutrient Acquisition by Plants*; Springer: Berlin/Heidelberg, Germany, 2005; pp. 147–183.
18. Lambers, H.; Shane, M.W.; Cramer, M.D.; Pearse, S.J.; Veneklaas, E.J. Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Ann. Bot.* **2006**, *98*, 693–713. [[CrossRef](#)] [[PubMed](#)]
19. Rose, T.J.; Impa, S.M.; Rose, M.T.; Pariasca-Tanaka, J.; Mori, A.; Heuer, S.; Johnson-Beebout, S.E.; Wissuwa, M. Enhancing phosphorus and zinc acquisition efficiency in rice: A critical review of root traits and their potential utility in rice breeding. *Ann. Bot.* **2013**, *112*, 331–345. [[CrossRef](#)] [[PubMed](#)]

20. Zhu, J.; Lynch, J.P. The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings. *Funct. Plant Biol.* **2004**, *31*, 949–958. [[CrossRef](#)]
21. Heppell, J.; Talboys, P.; Payvandi, S.; Zygalkakis, K.C.; Fliege, J.; Withers, P.J.A.; Jones, D.L.; Roose, T. How changing root system architecture can help tackle a reduction in soil phosphate (P) levels for better plant P acquisition. *Plant Cell Environ.* **2015**, *38*, 118–128. [[CrossRef](#)]
22. Gao, Y.; Lynch, J.P. Reduced crown root number improves water acquisition under water deficit stress in maize (*Zea mays* L.). *J. Exp. Bot.* **2016**, *67*, 4545–4557. [[CrossRef](#)]
23. Comas, L.H.; Becker, S.R.; Cruz, V.M.V.; Byrne, P.F.; Dierig, D. Root traits contributing to plant productivity under drought. *Front. Plant Sci.* **2013**, *4*, 442. [[CrossRef](#)]
24. White, R.G.; Kirkegaard, J.A. The distribution and abundance of wheat roots in a dense, structured subsoil and implications for water uptake. *Plant Cell Environ.* **2010**, *33*, 133–148. [[CrossRef](#)]
25. Kirk, G.J.D.; Yu, T.R.; Choudhury, F.A. Phosphorus chemistry in relation to water regime. In *Phosphorus Requirements for Sustainable Agriculture in Asia and Oceania. Proceedings of a Symposium, 6–10 March 1989*; International Rice Research Institute: Los Baños, Philippines, 1990; pp. 211–223.
26. Bünemann, E.; Oberson, A.; Frossard, E. (Eds.) *Phosphorus in Action*; Soil Biology; Springer: Berlin/Heidelberg, Germany, 2011; Volume 26, ISBN 978-3-642-15270-2.
27. Lal, R.; Stewart, B.A. *Soil Phosphorus*; CRC Press: Boca Raton, FL, USA, 2016; ISBN 9781482257847.
28. Ho, M.D.; Rosas, J.C.; Brown, K.M.; Lynch, J.P. Root architectural tradeoffs for water and phosphorus acquisition. *Funct. Plant Biol.* **2005**, *32*, 737. [[CrossRef](#)]
29. Ahmadi, N.; Audebert, A.; Bennett, M.J.; Bishopp, A.; de Oliveira, A.C.; Courtois, B.; Diedhiou, A.; Diévar, A.; Gantet, P.; Ghesquière, A.; et al. The roots of future rice harvests. *Rice* **2014**, *7*, 29. [[CrossRef](#)] [[PubMed](#)]
30. Donald, C.M. The breeding of crop ideotypes. *Euphytica* **1968**, *17*, 385–403. [[CrossRef](#)]
31. Mori, A.; Fukuda, T.; Vejchasarn, P.; Nestler, J.; Pariasca-Tanaka, J.; Wissuwa, M. The role of root size versus root efficiency in phosphorus acquisition in rice. *J. Exp. Bot.* **2016**, *67*, 1179–1189. [[CrossRef](#)]
32. Vejchasarn, P.; Lynch, J.P.; Brown, K.M. Genetic Variability in Phosphorus Responses of Rice Root Phenotypes. *Rice* **2016**, *9*, 29. [[CrossRef](#)] [[PubMed](#)]
33. Nestler, J.; Keyes, S.D.; Wissuwa, M. Root hair formation in rice (*Oryza sativa* L.) differs between root types and is altered in artificial growth conditions. *J. Exp. Bot.* **2016**, *67*, 3699–3708. [[CrossRef](#)] [[PubMed](#)]
34. Asch, F.; Dingkuhn, M.; Sow, A.; Audebert, A. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. *Field Crops Res.* **2005**, *93*, 223–236. [[CrossRef](#)]
35. Henry, A. IRRI's drought stress research in rice with emphasis on roots: Accomplishments over the last 50 years. *Plant Root* **2013**, *7*, 92–106. [[CrossRef](#)]
36. Farooq, M.; Wahid, A.; Lee, D.-J.; Ito, O.; Siddique, K.H.M. Advances in Drought Resistance of Rice. *CRC Crit. Rev. Plant Sci.* **2009**, *28*, 199–217. [[CrossRef](#)]
37. Saito, K.; Vandamme, E.; Segda, Z.; Fofana, M.; Ahouanton, K. A Screening Protocol for Vegetative-stage Tolerance to Phosphorus Deficiency in Upland Rice. *Crop Sci.* **2015**, *55*, 1223–1229. [[CrossRef](#)]
38. Vandamme, E.; Wissuwa, M.; Rose, T.; Dieng, I.; Drame, K.N.; Fofana, M.; Senthilkumar, K.; Venuprasad, R.; Jallow, D.; Segda, Z.; et al. Genotypic Variation in Grain P Loading across Diverse Rice Growing Environments and Implications for Field P Balances. *Front. Plant Sci.* **2016**, *7*, 1435. [[CrossRef](#)] [[PubMed](#)]
39. Menge, D.M.; Kameoka, E.; Kano-Nakata, M.; Yamauchi, A.; Asanuma, S.; Asai, H.; Kikuta, M.; Suralta, R.R.; Koyama, T.; Tran, T.T.; et al. Drought-induced root plasticity of two upland NERICA varieties under conditions with contrasting soil depth characteristics. *Plant Prod. Sci.* **2016**, *19*, 389–400. [[CrossRef](#)]
40. Farquhar, G.D.; Ehleringer, J.R.; Hubick, K.T. Carbon Isotope Discrimination and Photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 503–537. [[CrossRef](#)]
41. Farquhar, G.D.; Hubick, K.T.; Condon, A.G.; Richards, R.A. *Carbon Isotope Fractionation and Plant Water-Use Efficiency*; Springer: New York, NY, USA, 1989; pp. 21–40.
42. Muccio, Z.; Jackson, G.P. Isotope ratio mass spectrometry. *Analyst* **2009**, *134*, 213–222. [[CrossRef](#)] [[PubMed](#)]
43. Trachsel, S.; Kaeppeler, S.M.; Brown, K.M.; Lynch, J.P. Shovelomics: High throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* **2011**, *341*, 75–87. [[CrossRef](#)]
44. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2012.

45. Matsumoto, S.; Tsuboi, T.; Asea, G.; Maruyama, A.; Kikuchi, M.; Takagaki, M. Water Response of Upland Rice Varieties Adopted in Sub-Saharan Africa: A Water Application Experiment. *J. Rice Res.* **2014**, *2*, 121. [[CrossRef](#)]
46. Diagne, A.; Gnonna Midingoyi, S.-K.; Wopereis, M.; Akintayo, I. *The NERICA Success Story: Development, Achievements and Lessons Learned*; Africa Rice Center: Cotonou, Benin, 2010.
47. Somado, E.A. *NERICA®: The New Rice for Africa: A Compendium*; Africa Rice Center: Bouaké, Ivory Coast, 2008.
48. Vallino, M.; Fiorilli, V.; Bonfante, P. Rice flooding negatively impacts root branching and arbuscular mycorrhizal colonization, but not fungal viability. *Plant. Cell Environ.* **2014**, *37*, 557–572. [[CrossRef](#)]
49. Mitchell, J.; Owusu, M.; Fukai, S. Root development of rice under flooded and aerobic conditions. In Proceedings of the 16th Agronomy Conference, Armidale, Australia, 4–18 October 2012.
50. Henry, A.; Cal, A.J.; Batoto, T.C.; Torres, R.O.; Serraj, R. Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. *J. Exp. Bot.* **2012**, *63*, 4751–4763. [[CrossRef](#)]
51. Kadam, N.N.; Yin, X.; Bindraban, P.S.; Struik, P.C.; Jagadish, K.S.V. Does Morphological and Anatomical Plasticity during the Vegetative Stage Make Wheat More Tolerant of Water Deficit Stress Than Rice? *Plant Physiol.* **2015**, *167*, 1389–1401. [[CrossRef](#)]
52. Bao, Y.; Aggarwal, P.; Robbins, N.E.; Sturrock, C.J.; Thompson, M.C.; Tan, H.Q.; Tham, C.; Duan, L.; Rodriguez, P.L.; Vernoux, T.; et al. Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 9319–9324. [[CrossRef](#)]
53. De Kroon, H.; Visser, E.J.W. *Root Ecology*; Springer: Berlin/Heidelberg, Germany, 2003; ISBN 9783662097847.
54. Poorter, H.; Nagel, O.; Poorter, H.; Nagel, O. The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: A quantitative review. *Aust. J. Plant Physiol.* **2000**, *27*, 1191. [[CrossRef](#)]
55. Gregory, P.J. (Ed.) *Plant Roots*; Blackwell Publishing Ltd: Oxford, UK, 2006; ISBN 9780470995563.
56. Marschner, P. *Marschner's Mineral Nutrition of Higher Plants*; Academic Press: Cambridge, MA, USA, 2012; ISBN 9780123849052.
57. Borch, K.; Bouma, T.J.; Lynch, J.P.; Brown, K.M. Ethylene: A regulator of root architectural responses to soil phosphorus availability. *Plant Cell Environ.* **1999**, *22*, 425–431. [[CrossRef](#)]
58. Nielsen, K.L.; Eshel, A.; Lynch, J.P. The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. *J. Exp. Bot.* **2001**, *52*, 329–339. [[CrossRef](#)] [[PubMed](#)]
59. Evans, D.E. Aerenchyma formation. *New Phytol.* **2003**, *161*, 35–49. [[CrossRef](#)]
60. Postma, J.A.; Lynch, J.P. Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiol.* **2011**, *156*, 1190–1201. [[CrossRef](#)]
61. Kamara, A.Y.; Ekeleme, F.; Omoigui, L.O.; Oikeh, S.O.; Chikoye, D.; Tegbaru, A. Response of Upland Rice Cultivars to Nitrogen Fertilizer in the Savannas of Nigeria. *Agron. J.* **2010**, *102*, 333–339. [[CrossRef](#)]
62. Oikeh, S.O.; Nwilene, F.; Diatta, S.; Osiname, O.; Touré, A.; Okeleye, K.A. Responses of Upland NERICA Rice to Nitrogen and Phosphorus in Forest Agroecosystems. *Agron. J.* **2008**, *100*, 735–741. [[CrossRef](#)]
63. Kaneda, C. Breeding and Dissemination Efforts of “NERICA”. *Jpn. J. Trop.* **2007**, *51*, 1–4.
64. Nielsen, K.L.; Lynch, J.P.; Jabllokow, A.G.; Curtis, P.S. Carbon cost of root systems: An architectural approach. In *Belowground Responses to Rising Atmospheric CO<sub>2</sub>: Implications for Plants, Soil Biota, and Ecosystem Processes*; Springer: Dordrecht, The Netherlands, 1994; pp. 161–169.
65. Berntson, G.M. Modelling root architecture: Are there tradeoffs between efficiency and potential of resource acquisition? *New Phytol.* **1994**, *127*, 483–493. [[CrossRef](#)]
66. Ge, Z.; Rubio, G.; Lynch, J.P. The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: Results from a geometric simulation model. *Plant Soil* **2000**, *218*, 159–171. [[CrossRef](#)]
67. Föhse, D.; Claassen, N.; Jungk, A. Phosphorus efficiency of plants. *Plant Soil* **1991**, *132*, 261–272. [[CrossRef](#)]
68. Hammond, J.P.; Broadley, M.R.; White, P.J.; King, G.J.; Bowen, H.C.; Hayden, R.; Meacham, M.C.; Mead, A.; Overs, T.; Spracklen, W.P.; et al. Shoot yield drives phosphorus use efficiency in Brassica oleracea and correlates with root architecture traits. *J. Exp. Bot.* **2009**, *60*, 1953–1968. [[CrossRef](#)] [[PubMed](#)]
69. Wissuwa, M.; Ae, N. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breed.* **2001**, *120*, 43–48. [[CrossRef](#)]

70. Wissuwa, M.; Ae, N. Further characterization of two QTLs that increase phosphorus uptake of rice (*Oryza sativa* L.) under phosphorus deficiency. *Plant Soil* **2001**, *237*, 275–286. [[CrossRef](#)]
71. Wissuwa, M. How do plants achieve tolerance to phosphorus deficiency? Small causes with big effects. *Plant Physiol.* **2003**, *133*, 1947–1958. [[CrossRef](#)] [[PubMed](#)]



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