

Article

Arbuscular Mycorrhiza Fungi Reduce Photosystem II Efficiency in Phosphorus-Deficient Maize Without Promoting Growth

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Abstract

Arbuscular mycorrhizal fungi (AMF) are widely reported to restore photosynthetic efficiency under phosphorus deficiency. Whether this holds under combined phosphorus- and-zinc deficiency in early-stage maize (*Zea mays*), and how AMF modify the relationship between photosynthetic efficiency and growth rate, remains unresolved. Maize variety SUWAN 5819 was grown under five nutrient treatments (+Zn+P, −Zn−P, +Zn−P, −Zn+P, deionized water) with and without AMF in a sand culture under a randomized complete block split-plot design with six replications. Chlorophyll fluorescence, stomatal conductance, and growth-analysis variables were measured. Lindeman–Merenda–Gold (LMG) variance partitioning and piecewise structural equation modelling (SEM) decomposed the relative growth rate (RGR) into net assimilation rate (NAR) and leaf area ratio (LAR) components. AMF reduced the quantum efficiency of photosystem II (ΦPSII) by 29.6–30.8% in phosphorus-deficient treatments. Despite this, AMF had no significant effect on NAR, LAR, or RGR across any treatment. NAR contributed 62.9–99.2% of explained RGR variance across all treatment combinations, consistently exceeding LAR. Under combined −Zn−P deficiency, stomatal conductance was significantly elevated despite reduced ΦPSII and ETR. The piecewise SEM showed that AMF caused a 1.7 times increase in the negative effect of −Zn−P on ΦPSII. This study shows that phosphorus is the main determinant of the quantum efficiency of photosystem II whilst AMF under phosphorus deficiency reduce photochemical efficiency without a compensating growth benefit in early vegetative maize.

Keywords: arbuscular mycorrhiza fungi; phosphorus deficiency; relative growth rate; net assimilation rate; leaf area ratio; photosystem II



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1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops globally, grown on over 200 million hectares with a production output exceeding 1.2 billion tons as of 2024 [1–3]. Despite this importance, maize production is severely constrained by abiotic stresses. Among the leading constraining factors is the bioavailability of zinc (Zn) and phosphorus (P) in

agricultural soils [4–7]. A substantial component of this limitation operates through impaired photosynthetic performance, as both nutrients are directly involved in the processes that determine carbon assimilation [8].

Zinc deficiency has adverse effects on the photochemical components of photosynthesis. The deficiency has been previously reported to reduce photosystem II activity by 37% in maize relative to a control [9], a finding supported by [10], who observed a comparable 23.1% reduction. A study on early vegetative maize revealed a decrease in all physiological parameters under zinc starvation, with physiological symptoms appearing before anatomical ones [11]. A study on dual deficiency of iron and zinc [12] found that iron deficiency and combined iron-and-zinc deficiency significantly reduced the quantum efficiency of PSII (Φ PSII) relative to the +Fe+Zn control at 12 days after transplanting, with no significant difference between the two deficiency treatments. The study further noted that the results in the absence of zinc alone, however, did not differ significantly from the control. A study on water stress combined with zinc stress in early maize showed lower PSII quantum efficiency under the combined stress [13]. Mechanistically, zinc stress reduces photosynthetic rate through overproduction of reactive oxygen species (ROS), which damage the light-harvesting complex, impair PSII reaction centers, and cause chloroplast membrane degradation [14].

Phosphorus deficiency similarly reduces the quantum yield of PSII [15–17]. A study conducted on early vegetative maize reported as much as an 86% reduction in PSII quantum efficiency during phosphorus starvation [18]. Low inorganic phosphate (Pi) concentration in the stroma reduces ATP synthase activity, leading to thylakoid lumen acidification and impaired electron transfer, with the consequence that Φ PSII and electron transport rate (ETR) are both reduced [19,20]. This is further compounded by reduced availability of ATP and NADP⁺ directly impairing the photochemical reactions of photosynthesis [19,21]. Zinc and phosphorus deficiency therefore converge on reductions in Φ PSII and ETR through separate mechanisms, with downstream consequences for plant growth.

Both zinc and phosphorus stress have been reported to reduce stomatal conductance, with findings being less in agreement. A study by [22] reported a decrease in stomatal conductance in zinc starvation consistent with an earlier study by [13]. However, contrasting results were reported by [12], noting no significant difference in stomatal conductance between $-Zn$ and the control for early maize. Phosphorus deficiency reduced stomatal conductance in early maize [15] and was also observed to reduce stomatal conductance in cotton [23]. A study on different rates of phosphorus in maize at the vegetative stage showed an increase in stomatal conductance with an increase in phosphorus supply for five maize hybrids [24]. Unlike in most studies, the stomatal conductance between low phosphorus treatment and the control (sufficient phosphorus) was comparable in early vegetative maize in a study by [18].

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that develop an extensive extraradical hyphal network from which they enhance host-plant uptake of immobile nutrients including phosphorus and zinc [25,26]. AMF develop hyphae beyond the rhizosphere, and due to their small diameter, can access small soil pores that are inaccessible to the roots [27]. AMF have been recorded to improve growth and development, including photosynthetic parameters. Studies by [28–31] with different levels of phosphorus and zinc showed a higher stomatal conductance with AMF maize compared to non-AMF maize.

Quantifying the physiological consequences of nutrient deficiency on plant growth requires a framework that attributes differences in biomass accumulation to their underlying determinants. The relative growth rate (RGR) analysis framework provides such a decomposition [32]. RGR is defined as the increase in biomass per unit of existing biomass per unit time and can be resolved into two primary components, $RGR = NAR \times LAR$,

where the net assimilation rate (NAR) represents the net rate of dry matter production per unit leaf area, and the leaf area ratio (LAR) represents the ratio of photosynthetically active leaf area to total plant biomass. RGR, LAR, and NAR have been used as growth indices to show the effects of abiotic stress in plants [33–36]. However, the determination of the contribution of NAR and LAR to RGR has not been explored in zinc and phosphorus deficiencies or the combination of the two in the presence of mycorrhiza. We hypothesized the following in this study: (i) AMF will improve the quantum efficiency of photosystem II and ETR for nutrient-starved treatments; and (ii) NAR's contribution to RGR will be greater than that of LAR in nutrient-deficient treatments with and without AMF.

The objectives of this study were: (i) to quantify the effects of zinc and phosphorus deficiency, with and without AMF inoculation, on stomatal conductance, Φ PSII, and ETR in maize during the early vegetative stage; and (ii) to determine the relative contribution of NAR and LAR to RGR under these treatment combinations in early vegetative maize.

2. Results

2.1. AMF Root Colonization

AMF inoculation significantly increased root colonization across all nutrient treatments relative to uninoculated controls ($p < 0.001$; Table 1), with colonization in M1 treatments being 5–12 times higher compared to M0. Neither the nutrient treatment effect nor the nutrient $\times$ AMF interaction was statistically significant, indicating that colonization levels did not differ among nutrient treatments within inoculated plots. Although the M0 treatments had detectable colonization ($<5.7\%$), the large difference (5–12 times) demonstrates that the residual colonization did not affect data interpretation.

Table 1. Effect of nutrient treatment and AMF inoculation on mycorrhizal root colonization in maize. † Colonization data log-transformed prior to analysis. Reported values are back-transformed geometric means with 95% confidence intervals in parentheses. Different letters indicate significant differences by Šidák adjustment ($p < 0.05$). A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control; M0 = without AMF; M1 = with AMF.

Treatment	AMF	Colonization (%) †
A1 (+Zn+P)	M0	4.31 (2.5–7.4) a
	M1	33.38 (19.5–57.2) b
A2 (−Zn−P)	M0	5.72 (3.3–9.8) a
	M1	31.74 (18.5–54.4) b
A3 (+Zn−P)	M0	3.53 (2.1–6.1) a
	M1	43.15 (25.2–74.0) b
A4 (−Zn+P)	M0	5.17 (3.0–8.9) a
	M1	49.59 (28.9–85.0) b
A5 (Control)	M0	2.80 (1.6–4.8) a
	M1	30.05 (17.5–51.5) b
Grand Mean		20.94
Nutrient		ns
AMF		***
Nutrient $\times$ AMF		ns

Significance codes: *** $p < 0.001$; ns $p \geq 0.10$.

2.2. Stomatal Conductance, Φ PSII, and Electron Transport Rate

Nutrient treatment significantly affected stomatal conductance, Φ PSII, and ETR (Table 2; Figure 1). The phosphorus-sufficient treatments (A1 and A4) showed the highest Φ PSII and ETR values, with AMF having no significant effect under these conditions. AMF inoculation significantly reduced both Φ PSII ($p < 0.01$) and ETR ($p < 0.001$) in the A2 (29.6% and 28.4%, respectively) and A3 (30.8% and 31.2%, respectively) treatments,

and a significant nutrient $\times$ AMF interaction was found for both variables. The $-Zn-P$ treatment (A2) showed anomalously high stomatal conductance relative to its $\Phi PSII$ and ETR values. There was no significant effect of AMF on stomatal conductance.

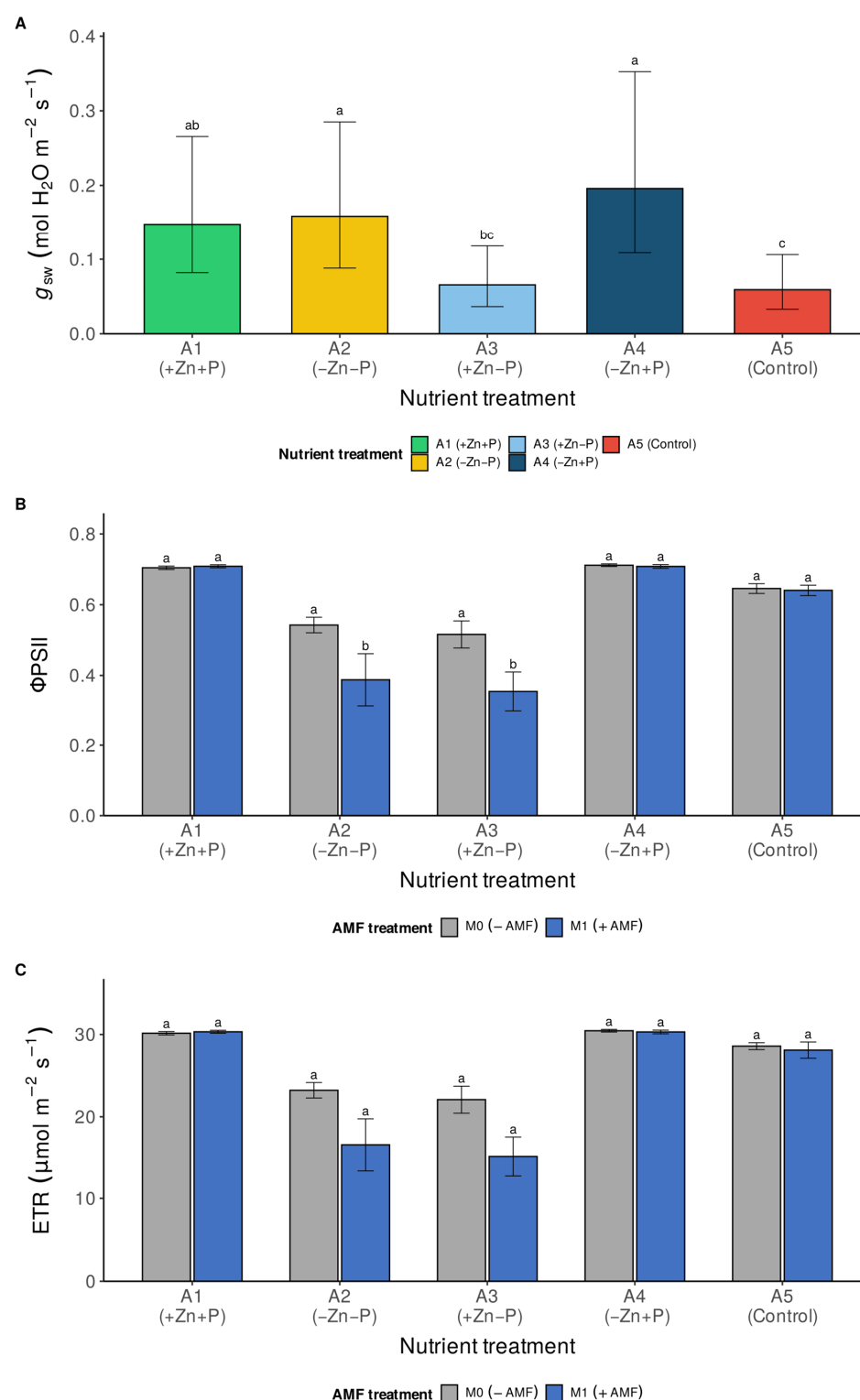


Figure 1. Effects of zinc and phosphorus nutrient treatments on (A) stomatal conductance, (B) $\Phi PSII$ with and without AMF inoculation, and (C) electron transport rate (ETR) with and without AMF inoculation. Compact letter displays indicate significant pairwise differences by Šidák adjustment at $p < 0.05$ where bars sharing a letter are not significantly different. M0 = without AMF; M1 = with AMF.

Table 2. Effects of nutrient treatment and AMF inoculation on stomatal conductance, Φ PSII, and electron transport rate (ETR) in maize. † Stomatal conductance data are log-transformed prior to analysis. Log-transformed values are geometric means ($\times / \div$ GSD). Φ PSII and ETR are arithmetic means $\pm$ SD. A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control; M0 = without AMF; M1 = with AMF. GSD = geometric standard deviation; Φ PSII = effective quantum yield of photosystem II; ETR = electron transport rate.

Treatment	AMF	Stomatal Conductance (mol m ^{−2} s ^{−1}) †	Φ PSII	Electron Transport Rate (μmol m ^{−2} s ^{−1})
		Geo ($\times / \div$ GSD)	Mean $\pm$ SD	Mean $\pm$ SD
A1 (+Zn+P)	M0	0.13 $\times / \div$ 1.12	0.70 $\pm$ 0.01	30.1 $\pm$ 0.5
	M1	0.17 $\times / \div$ 1.29	0.71 $\pm$ 0.01	30.3 $\pm$ 0.44
A2 (−Zn−P)	M0	0.26 $\times / \div$ 1.24	0.54 $\pm$ 0.05	23.2 $\pm$ 2.3
	M1	0.12 $\times / \div$ 1.08	0.38 $\pm$ 0.18	16.6 $\pm$ 7.74
A3 (+Zn−P)	M0	0.06 $\times / \div$ 1.07	0.52 $\pm$ 0.09	22.1 $\pm$ 4.02
	M1	0.07 $\times / \div$ 1.06	0.36 $\pm$ 0.13	15.2 $\pm$ 5.77
A4 (−Zn+P)	M0	0.24 $\times / \div$ 1.35	0.71 $\pm$ 0.01	30.5 $\pm$ 0.39
	M1	0.16 $\times / \div$ 1.35	0.71 $\pm$ 0.01	30.3 $\pm$ 0.55
A5 (Control)	M0	0.07 $\times / \div$ 1.06	0.65 $\pm$ 0.03	28.6 $\pm$ 1.02
	M1	0.05 $\times / \div$ 1.01	0.64 $\pm$ 0.04	28.1 $\pm$ 2.41
Grand Mean		0.18	0.59	25.50
Nutrient		*	***	***
AMF		ns	**	***
Nutrient $\times$ AMF		ns	*	**
CV/GCV (%)		65.95 †	13.52	13.34

Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns $p \geq 0.10$.

2.3. Dry Matter Production

Nutrient treatment significantly affected all dry weight parameters at both harvests: harvest 1 at 15 days after sowing (DAS) and harvest 2 at 22 days after sowing (DAS) ($p < 0.001$; Table 3). The phosphorus-sufficient treatments (A1 and A4) consistently produced the greatest leaf, stem, root, and total dry weights at both sampling dates, whereas the phosphorus-deficient treatments (A2, A3) and the deionized water control (A5) produced lower dry weights. AMF inoculation significantly affected root dry weight ($p < 0.01$) and total dry weight ($p < 0.001$) at harvest 2, and a significant nutrient $\times$ AMF interaction was found for all dry weight components at this harvest. These effects were specific to absolute biomass at the second harvest and were not detected at harvest 1, where AMF had no significant effect on any dry weight component.

Table 3. Effects of nutrient treatment and AMF inoculation on leaf, stem, root, and whole-plant dry weights at harvest 1 (subscript 1: 15 DAS) and harvest 2 (subscript 2: 22 DAS). † Root and total dry weights for harvests 1 and 2 represent data that were log-transformed prior to analysis. Values for these variables are geometric means ($\times / \div$ GSD). § Stem dry weights for first destructive sampling represent data that were Box–Cox transformed prior to analysis. Estimated marginal means are reported for this variable ($\pm$ SD). Leaf and stem dry weights are arithmetic means $\pm$ SD. LDW = leaf dry weight; SDW = stem dry weight; RDW = root dry weight; TDW = total dry weight; GSD = geometric standard deviation; DAS = days after sowing. A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control; M0 = without AMF; M1 = with AMF.

Treatment	AMF	LDW ₂ (g)	SDW ₂ (g)	RDW ₂ (g) †	TDW ₂ (g) †	LDW ₁ (g)	SDW ₁ (g) §	RDW ₁ (g) †	TDW ₁ (g) †
		Mean $\pm$ SD	Mean $\pm$ SD	Geo ($\times / \div$ GSD)	Geo ($\times / \div$ GSD)	Mean $\pm$ SD	Mean $\pm$ SD	Geo ($\times / \div$ GSD)	Geo ($\times / \div$ GSD)
A1 (+Zn+P)	M0	1.63 $\pm$ 0.20	0.79 $\pm$ 0.03	2.44 $\times / \div$ 1.54	4.91 $\times / \div$ 1.28	0.53 $\pm$ 0.09	0.24 $\pm$ 0.03	1.24 $\times / \div$ 1.62	2.05 $\times / \div$ 1.37
	M1	1.49 $\pm$ 0.18	0.64 $\pm$ 0.05	1.28 $\times / \div$ 1.38	3.45 $\times / \div$ 1.13	0.56 $\pm$ 0.05	0.23 $\pm$ 0.06	0.95 $\times / \div$ 1.94	1.81 $\times / \div$ 1.46
A2 (−Zn−P)	M0	0.50 $\pm$ 0.09	0.20 $\pm$ 0.03	1.00 $\times / \div$ 1.12	1.70 $\times / \div$ 1.09	0.34 $\pm$ 0.04	0.16 $\pm$ 0.03	1.21 $\times / \div$ 1.39	1.73 $\times / \div$ 1.29
	M1	0.48 $\pm$ 0.08	0.22 $\pm$ 0.09	0.63 $\times / \div$ 1.24	1.33 $\times / \div$ 1.19	0.30 $\pm$ 0.06	0.13 $\pm$ 0.04	0.98 $\times / \div$ 1.70	1.44 $\times / \div$ 1.50
A3 (+Zn−P)	M0	0.39 $\pm$ 0.14	0.23 $\pm$ 0.09	0.81 $\times / \div$ 1.18	1.42 $\times / \div$ 1.21	0.28 $\pm$ 0.11	0.12 $\pm$ 0.03	1.36 $\times / \div$ 1.22	1.76 $\times / \div$ 1.19
	M1	0.51 $\pm$ 0.16	0.19 $\pm$ 0.03	0.82 $\times / \div$ 1.23	1.51 $\times / \div$ 1.25	0.29 $\pm$ 0.04	0.14 $\pm$ 0.02	1.48 $\times / \div$ 1.60	1.94 $\times / \div$ 1.44
A4 (−Zn+P)	M0	1.66 $\pm$ 0.11	0.77 $\pm$ 0.06	1.66 $\times / \div$ 1.56	4.18 $\times / \div$ 1.17	0.53 $\pm$ 0.09	0.23 $\pm$ 0.04	1.18 $\times / \div$ 1.37	1.97 $\times / \div$ 1.21
	M1	1.53 $\pm$ 0.14	0.68 $\pm$ 0.09	1.67 $\times / \div$ 1.41	3.91 $\times / \div$ 1.19	0.50 $\pm$ 0.05	0.26 $\pm$ 0.19	0.83 $\times / \div$ 1.21	1.64 $\times / \div$ 1.15
A5 (Control)	M0	0.23 $\pm$ 0.06	0.09 $\pm$ 0.02	0.75 $\times / \div$ 1.36	1.09 $\times / \div$ 1.22	0.17 $\pm$ 0.05	0.07 $\pm$ 0.01	0.63 $\times / \div$ 1.42	0.88 $\times / \div$ 1.28
	M1	0.19 $\pm$ 0.08	0.10 $\pm$ 0.01	0.58 $\times / \div$ 1.38	0.87 $\times / \div$ 1.25	0.14 $\pm$ 0.06	0.08 $\pm$ 0.01	0.64 $\times / \div$ 1.77	0.89 $\times / \div$ 1.58
Grand Mean		0.86	0.39	1.16	2.44	0.37	0.18	1.13	1.68
Nutrient		***	***	***	***	***	***	*	**
AMF		ns	ns	**	***	ns	ns	ns	ns
Nutrient $\times$ AMF		*	***	*	*	ns	ns	ns	ns
CV (%)		10.70	13.15	31.20 †	16.82 †	18.80	37.90	38.50 †	27.90 †

Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns $p \geq 0.10$.

2.4. Leaf Area, Growth Rates, and Net Assimilation Rate

Nutrient treatment significantly affected leaf area, LAR, all organ-level RGRs, whole-plant RGR, and NAR ($p < 0.001$; Table 4). AMF inoculation had a significant effect on LAR ($p < 0.05$), but no significant nutrient $\times$ AMF interaction was found for any of the growth variables despite significant nutrient $\times$ AMF interaction at the second harvest for root and total dry weights. Thus, the AMF-related differences in root and total dry weights at the second harvest were not accompanied by corresponding differences in the estimated RGR.

Table 4. Effects of nutrient treatment and AMF inoculation on leaf area, leaf area ratio (LAR), leaf, stem, root, and whole-plant relative growth rate (RGR), and net assimilation rate (NAR). † Leaf area data at harvests 1 and 2 represent data that were log-transformed prior to analysis. Values for these variables are geometric means ($\times / \div$ GSD). LAR, RGRs, and NAR are arithmetic means $\pm$ SD. LA₁ = leaf area at harvest 1; LA₂ = leaf area at harvest 2; LAR = leaf area ratio; RGR = relative growth rate; NAR = net assimilation rate; GSD = geometric standard deviation. A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control; M0 = without AMF; M1 = with AMF.

Treatment	AMF	LA ₂ (cm ²) †	LA ₁ (cm ²) †	LAR (cm ² g ^{−1})	Leaf RGR (g g ^{−1} d ^{−1})	Stem RGR (g g ^{−1} d ^{−1})	Root RGR (g g ^{−1} d ^{−1})	Total RGR (g g ^{−1} d ^{−1})	NAR (g cm ^{−2} d ^{−1})
		Geo ($\times / \div$ GSD)	Geo ($\times / \div$ GSD)	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
A1 (+Zn+P)	M0	404.3 $\times / \div$ 1.06	130.6 $\times / \div$ 1.09	84.8 $\pm$ 19.3	0.16 $\pm$ 0.03	0.17 $\pm$ 0.02	0.10 $\pm$ 0.06	0.12 $\pm$ 0.04	0.0017 $\pm$ 0.0006
	M1	370.6 $\times / \div$ 1.27	131.5 $\times / \div$ 1.09	112 $\pm$ 31.5	0.14 $\pm$ 0.02	0.14 $\pm$ 0.04	0.04 $\pm$ 0.09	0.092 $\pm$ 0.05	0.0010 $\pm$ 0.0005
A2 (−Zn−P)	M0	97.6 $\times / \div$ 1.07	62.7 $\times / \div$ 1.09	57.5 $\pm$ 5.08	0.05 $\pm$ 0.04	0.03 $\pm$ 0.05	−0.03 $\pm$ 0.06	−0.002 $\pm$ 0.04	−0.00013 $\pm$ 0.0009
	M1	91.1 $\times / \div$ 1.13	58.1 $\times / \div$ 1.10	69.0 $\pm$ 8.61	0.07 $\pm$ 0.03	0.06 $\pm$ 0.07	−0.064 $\pm$ 0.07	−0.012 $\pm$ 0.05	−0.00041 $\pm$ 0.001
A3 (+Zn−P)	M0	104.5 $\times / \div$ 1.35	61.9 $\times / \div$ 1.12	75.1 $\pm$ 17.4	0.05 $\pm$ 0.09	0.08 $\pm$ 0.07	−0.07 $\pm$ 0.04	−0.031 $\pm$ 0.05	−0.00068 $\pm$ 0.0009
	M1	95.5 $\times / \div$ 1.20	59.4 $\times / \div$ 1.09	64.6 $\pm$ 14.4	0.07 $\pm$ 0.05	0.04 $\pm$ 0.03	−0.084 $\pm$ 0.04	−0.037 $\pm$ 0.04	−0.00092 $\pm$ 0.001
A4 (−Zn+P)	M0	428.8 $\times / \div$ 1.04	141.6 $\times / \div$ 1.07	103 $\pm$ 14.6	0.16 $\pm$ 0.02	0.17 $\pm$ 0.03	0.05 $\pm$ 0.06	0.11 $\pm$ 0.02	0.00012 $\pm$ 0.0003
	M1	433.5 $\times / \div$ 1.06	127.3 $\times / \div$ 1.09	113 $\pm$ 23.1	0.16 $\pm$ 0.01	0.13 $\pm$ 0.08	0.099 $\pm$ 0.06	0.12 $\pm$ 0.02	0.0013 $\pm$ 0.0004
A5 (Control)	M0	69.9 $\times / \div$ 1.28	51.6 $\times / \div$ 1.05	68.8 $\pm$ 24.9	0.05 $\pm$ 0.06	0.04 $\pm$ 0.05	0.03 $\pm$ 0.03	0.031 $\pm$ 0.03	0.00049 $\pm$ 0.0005
	M1	70.9 $\times / \div$ 1.43	54.9 $\times / \div$ 1.08	83.4 $\pm$ 19.0	0.05 $\pm$ 0.10	0.03 $\pm$ 0.02	−0.017 $\pm$ 0.07	−0.002 $\pm$ 0.06	−0.00017 $\pm$ 0.0009
Grand Mean		216.75	87.94	83.1	0.097	0.089	0.011	0.039	0.00029
Nutrient		***	***	***	***	***	***	***	***
AMF		ns	ns	*	ns	ns	ns	ns	ns
Nutrient $\times$ AMF		ns	ns	ns	ns	ns	ns	ns	ns
CV (%)		20.05 †	8.81 †	23.0	5.09	5.02	6.17	3.78	0.072

Significance codes: *** $p < 0.001$; * $p < 0.05$; ns $p \geq 0.10$.

The phosphorus-sufficient treatments (A1, A4) showed positive RGRs and NAR values. Conversely, the phosphorus-deficient treatments (A2, A3) showed near-zero or negative root and whole-plant RGRs over the seven-day inter-harvest period. Consistent with the declining RGRs in the phosphorus-deficient treatments was the negative NAR, consistent with declining photosynthetic efficiency. Whole-plant RGR most closely followed the pattern of root RGR (Figures 2 and 3).

2.5. Principal Component Analysis

Together, principal component 1 (PC1) and principal component 2 (PC2) explained 64.2% of the variance among the nine variables examined in colonization, growth (leaf, stem, root, and whole-plant RGRs, NAR, LAR), and physiology (stomatal conductance, Φ PSII) (Figure 4). PC1 (50.2%) separated treatments along a growth axis, with phosphorus-sufficient treatments clustering to the right, characterized by positive RGR and high values of NAR and Φ PSII, and phosphorus-deficient treatments clustering to the left irrespective of AMF. The acute angle between the NAR and whole-plant RGR vectors indicated a stronger association between these two variables as compared to LAR and whole-plant RGR. This observed pattern has been quantified in Section 2.6. Moreover, the proximity of root RGR to the whole-plant RGR vector indicated that root growth rate was the primary driver of whole-plant growth dynamics. The short colonization vector indicated limited overall influence of inoculation on multivariate growth patterns. PC2 (14.0%) was defined primarily by leaf morphology (stomatal conductance and LAR). The acute angle between AMF colonization and LAR suggests a greater association of AMF with leaf morphology than with stomatal physiology.

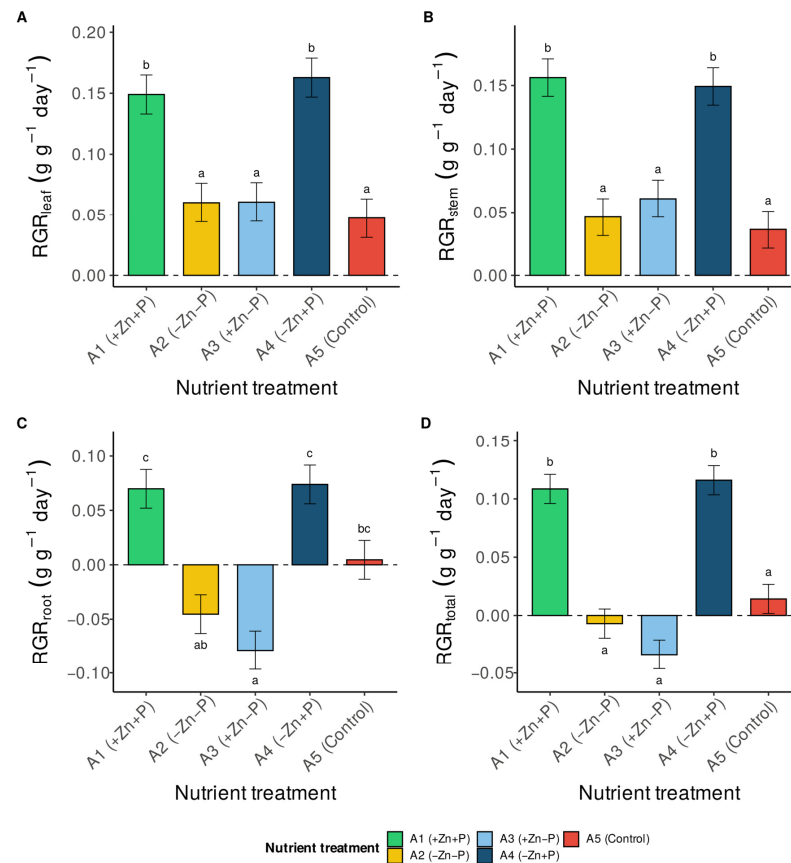


Figure 2. Relative growth rates for (A) leaf, (B) stem, (C) root, and (D) whole plant, calculated from dry weights measured at two destructive samplings seven days apart. Compact letter displays indicate significant pairwise differences by Šidák adjustment ($p < 0.05$) where bars sharing a letter are not significantly different. Treatment codes: A1 = +Zn+P; A2 = -Zn-P; A3 = +Zn-P; A4 = -Zn+P; A5 = deionized water control.

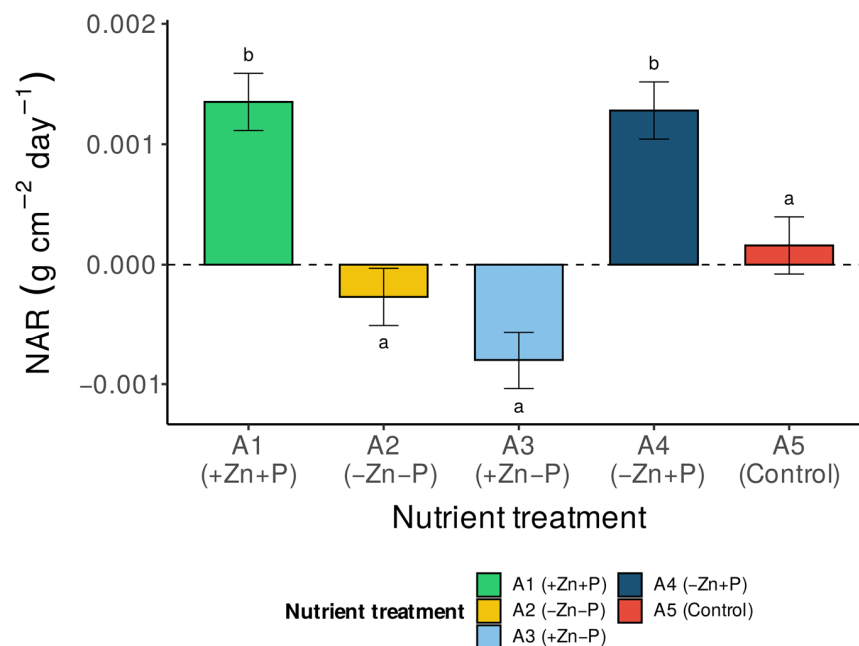


Figure 3. Net assimilation rate (NAR) for each nutrient treatment, calculated from destructive samplings seven days apart. Compact letter displays indicate significant pairwise differences by Šidák adjustment ($p < 0.05$) where bars sharing a letter are not significantly different. Treatment codes: A1 = +Zn+P; A2 = -Zn-P; A3 = +Zn-P; A4 = -Zn+P; A5 = deionized water control.

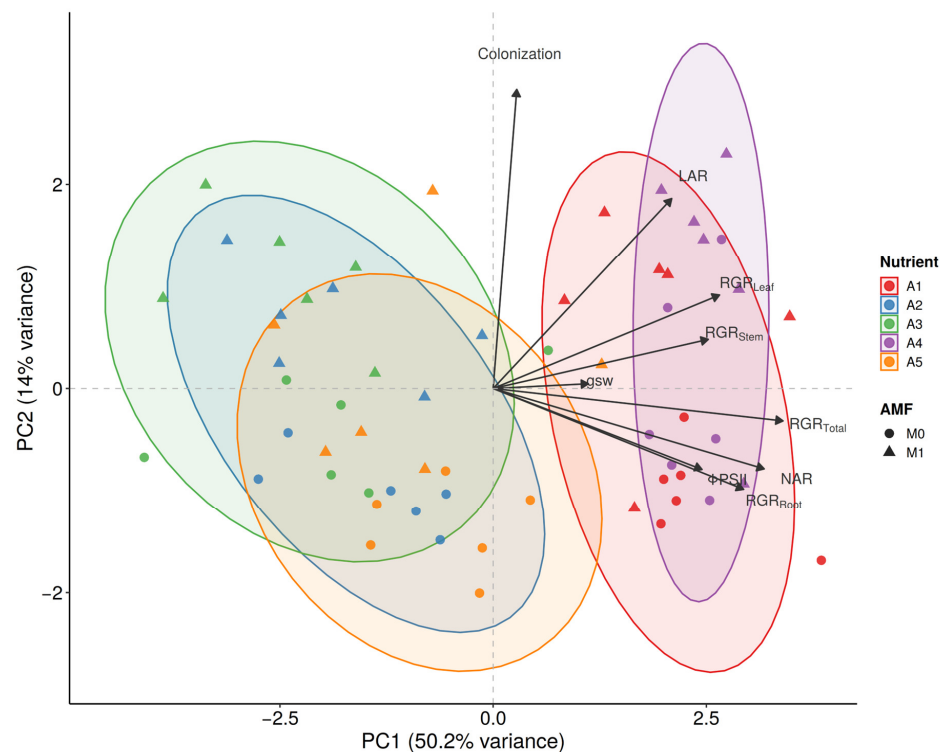


Figure 4. Principal component analysis (PCA) biplot of nine maize physiological and growth parameters showing principal component 1 (PC1) (50.2%) against principal component 2 (PC2) (14.0%). Circles = without AMF (M0); triangles = with AMF (M1). Ellipses represent 95% confidence regions for each treatment combination. LAR = leaf area ratio; RGR = relative growth rate; gsw = stomatal conductance; NAR = net assimilation rate; Φ PSII = effective quantum yield of photosystem II. A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control.

2.6. Relative Importance of NAR and LAR

The closer alignment of NAR with RGR relative to LAR observed in the PCA (Section 2.5) was quantified using Lindeman Merenda Gold (LMG) variance partitioning. The analysis identified NAR as the dominant contributor to variation in whole-plant RGR across all nutrient and AMF treatment combinations (Table 5). Contributions ranged from 62.9% (A4M1) to 99.2% (A5M1) for NAR, compared with 0.8% (A5M1) to 37.1% (A4M1) for LAR. LAR’s relative contribution was greatest in the A4 (−Zn+P) treatment with AMF inoculation.

Table 5. Relative importance (%) of net assimilation rate (NAR) and leaf area ratio (LAR) in explaining variation in whole-plant relative growth rate (RGR), estimated by the Lindeman Merenda Gold (LMG) variance partitioning method. NAR = net assimilation rate ($\text{g cm}^{-2} \text{d}^{-1}$); LAR = leaf area ratio ($\text{cm}^2 \text{g}^{-1}$). A1 = +Zn+P; A2 = −Zn−P; A3 = +Zn−P; A4 = −Zn+P; A5 = deionized water control; M0 = without AMF; M1 = with AMF.

Treatment	AMF	NAR (%)	LAR (%)
A1 (+Zn+P)	M0	80.9	19.1
	M1	78.5	21.5
A2 (−Zn−P)	M0	89.0	11.0
	M1	83.9	16.1
A3 (+Zn−P)	M0	95.7	4.3
	M1	91.8	8.2
A4 (−Zn+P)	M0	75.1	24.9
	M1	62.9	37.1
A5 (Control)	M0	95.5	4.5
	M1	99.2	0.8

2.7. Piecewise Structural Equation Model (SEM)

The piecewise SEM was used to map the causal structure of NAR and LAR contributions to RGR and to test for pathways not captured by the NAR/LAR decomposition. The model revealed three significant pathways through which phosphorus availability influenced whole-plant RGR (Table 6; Figure 5). The first and dominant pathway was via P status $\rightarrow$ NAR ($\beta = 0.573$) $\rightarrow$ RGR ($\beta = 0.768$). The second pathway was via P status $\rightarrow$ LAR ($\beta = 0.915$) $\rightarrow$ RGR ($\beta = 0.104$). The third was a direct pathway from P status to RGR ($\beta = 0.213$) independent of NAR and LAR. Phosphorus was 1.3 times less effective on Φ PSII with the presence of AMF ($\beta = 0.558 \rightarrow 0.408$). Dual deficiency had a negative impact on PSII ($\beta = -0.278$), with the effect increasing 1.7 times with the dual deficiency–AMF interaction ($\beta = -0.494$). Phosphorus interaction with zinc had a negative impact on LAR ($\beta = -0.570$), with phosphorus–zinc–AMF interaction resulting in a positive influence on LAR ($\beta = 0.473$). Zinc status had no significant direct effect on Φ PSII in the model.

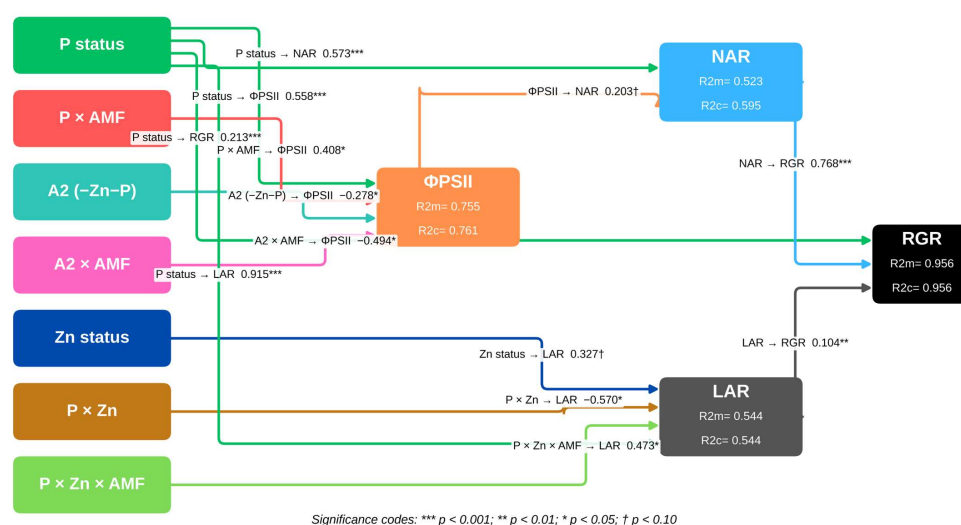


Figure 5. Piecewise structural equation model (SEM) path diagram showing the direct and indirect effects of P supply status, Zn supply status, and AMF inoculation on whole-plant relative growth rate (RGR) mediated through Φ PSII, net assimilation rate (NAR), and leaf area ratio (LAR). Arrow widths are proportional to the magnitude of standardized path coefficients (β). Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; † $p < 0.1$.

Table 6. Standardized path coefficients from the piecewise structural equation model (SEM) relating P supply, Zn supply, dual deficiency (A2), and AMF inoculation to Φ PSII, NAR, LAR, and whole-plant RGR. Models fitted with lmer (REML = FALSE) and random effects (1 | Block/Nutrient). β = standardized path coefficient. Φ PSII = quantum efficiency of photosystem II, NAR = net assimilation rate, LAR = leaf area ratio, RGR = relative growth rate.

Predictor	Response	β	Significance
P status	Φ PSII	0.558	***
Zn status	Φ PSII	−0.088	ns
P × Zn	Φ PSII	0.052	ns
AMF	Φ PSII	−0.017	ns
P × AMF	Φ PSII	0.408	*
Zn × AMF	Φ PSII	−0.017	ns
P × Zn × AMF	Φ PSII	0.028	ns
A2 (−Zn−P)	Φ PSII	−0.278	*
A2 × AMF	Φ PSII	−0.494	*
Φ PSII	NAR	0.203	.
P status	NAR	0.573	***
Φ PSII	LAR	−0.080	ns

Table 6. Cont.

Predictor	Response	β	Significance
P status	LAR	0.915	***
Zn status	LAR	0.327	.
P $\times$ Zn	LAR	−0.570	*
AMF	LAR	0.281	ns
P $\times$ AMF	LAR	0.000	ns
Zn $\times$ AMF	LAR	−0.343	ns
P $\times$ Zn $\times$ AMF	LAR	0.473	*
A2 (−Zn−P)	LAR	−0.197	ns
A2 $\times$ AMF	LAR	−0.097	ns
NAR	RGR	0.768	***
LAR	RGR	0.104	**
P status	RGR	0.213	***

R² (marginal/conditional): Φ PSII = 0.755/0.761; NAR = 0.523/0.596; LAR = 0.544/0.544; RGR = 0.956/0.956. Marginal R² reflects fixed effects only; conditional R² adds the random effects (1 | Block/Nutrient). Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.10$; ns $p \geq 0.10$.

3. Discussion

3.1. Phosphorus Deficiency Is the Primary Determinant of Φ PSII and ETR

Phosphorus deficiency, not zinc deficiency, drove the photochemical impairment recorded at 22 days after sowing. Phosphorus-deficient treatments showed reduced Φ PSII and ETR, while zinc status alone produced no comparable effect. Reduced photochemical activity has been shown to result in low stromal Pi that suppresses ATP synthase activity and acidifies the thylakoid lumen, thereby impairing electron transfer [19,21,37–40]. Moreover, low phosphorus drives membrane and chlorophyll loss through H₂O₂ accumulation [19,41], compounded by a 10–47% reduction in light-harvesting complex components [39].

Previous studies have shown zinc deficiency to reduce Φ PSII, stomatal conductance, and transpiration in maize and other species [11,14,23,42–44]. The contrast with the current study seems to be linked with the timing of zinc starvation. A rice study found no decline in Φ PSII under zinc deficiency in the early vegetative stage, while the phosphorus-deficient treatment showed a decline [8]. Moreover, a longer maize study [14] detected a Φ PSII decline under zinc deficiency after two months—well beyond the 22-day period in the current study. These results, therefore, suggest zinc-driven photochemical decline is a late-onset effect not captured in the duration of the current study. Combined −Zn−P deficiency, by contrast, damaged PSII reaction centers and the oxygen-evolving complex in the early vegetative stage in rice seedlings [8], causing a decline in Φ PSII, a pattern observed in the current study as well.

The combined −Zn−P treatment showed an unusually high stomatal conductance while Φ PSII and ETR remained low. We hypothesize that this decoupling reflects impaired carbonic anhydrase-mediated stomatal regulation. Phosphorus deficiency is understood to raise internal CO₂ concentration, thereby triggering stomatal closure [45]. A mechanism involving carbonic anhydrase 4 (CA4) in Arabidopsis has been reported to drive stomatal closure in two complementary ways: catalytically, by converting accumulated CO₂ to bicarbonate and so activating the slow anion channel-associated 1 (SLAC1) anion channel through a protein kinase cascade [46–48]; and directly, by binding and activating SLAC1 independently of the catalytic mechanism [46–48]. We hypothesize a similar mechanism in the dual deficiency where combined zinc and phosphorus deficiency could disable both mechanisms simultaneously. CA activity declines as zinc is a cofactor of the enzyme [49], while the kinase cascade needed for the catalytic route is separately impaired by low Pi [46].

The suppression of both pathways results in stomata opening despite declining Φ_{PSII} and ETR. The hypothesized mechanism is represented in Figure 6.

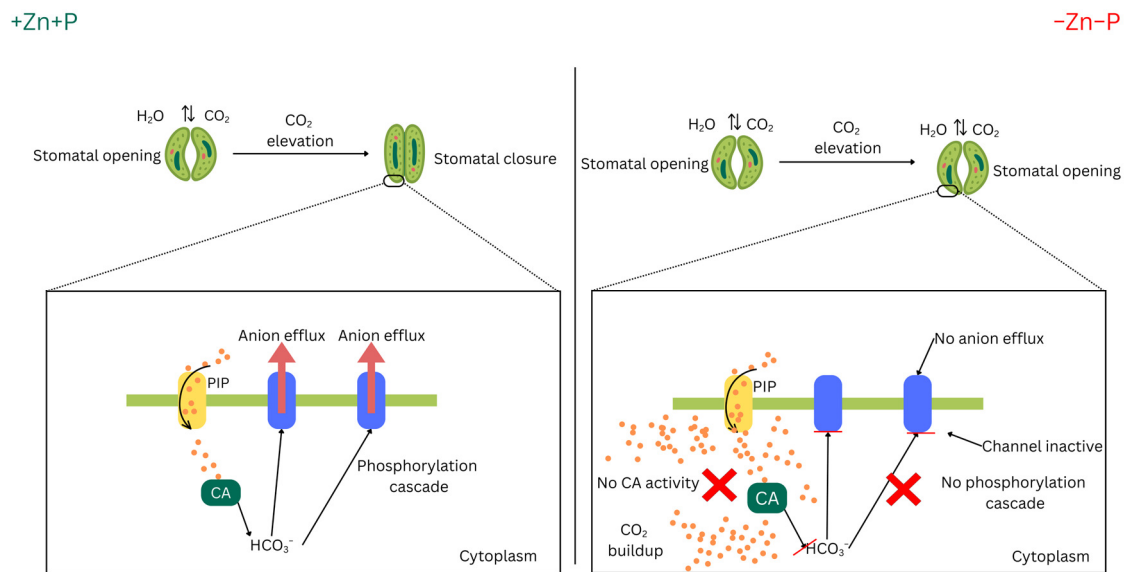


Figure 6. The hypothesized mechanism for high stomatal conductance in low Φ_{PSII} and ETR where dual zinc and phosphorus deficiency inactivates the CA-regulated stomatal closure.

3.2. NAR Is the Dominant Contributor to RGR Under Both Sufficiency and Deficiency

Three independent methods—PCA, LMG variance partitioning, and piecewise SEM—converged on the dominance of NAR over LAR in determining variation in whole-plant RGR across every treatment combination tested. These results are supported by previous studies on early vegetative growth, where NAR outweighed LAR in contributing towards RGR [50,51]. A wild-barley study under nutrient limitation reported the same NAR dominance across deficiency levels [52], reinforcing these observations across species and stress combinations. A study on field-grown maize under phosphorus deficiency [33] had contrasting results to the current study where leaf canopy rather than NAR was identified as the main driver of RGR. This divergence could be rooted in the difference in growth stages and differences in the sand-culture system under greenhouse conditions versus field conditions.

The piecewise SEM quantified the magnitude of NAR's influence on RGR ($\beta = 0.768$) and showed that phosphorus availability influenced RGR predominantly through its effect on NAR ($\beta = 0.573$), which is in line with the role of phosphorus in photosynthetic electron transport and the Calvin cycle [19,53]. The SEM also identified a smaller but significant contribution from LAR ($\beta = 0.104$), consistent with the established role of phosphorus in leaf area expansion [54–56].

3.3. A Direct P-to-RGR Pathway Independent of NAR and LAR

A significant direct path from P status to RGR ($\beta = 0.213$) was identified in the piecewise SEM after accounting for mediation through NAR and LAR. This indicates that phosphorus availability influences plant growth through processes not fully captured by the leaf area-based growth components. One plausible mechanism is the specific absorption rate of phosphorus by roots (SAR[P]), defined as the amount of phosphorus absorbed per unit root dry mass per unit time. A positive relationship between SAR[P] and RGR has been documented in *Lactuca sativa*, where both NAR and SAR[P] were identified as principal determinants of RGR [57]. Therefore, we hypothesize that SAR[P]

may represent a root-mediated pathway through which phosphorus availability influenced RGR independently of the measured leaf-level components.

The SEM also notably identified a significant interaction effect between phosphorus and zinc sufficiency on LAR ($P \times Zn$, $\beta = -0.570$). This pattern was significantly attenuated in AMF-inoculated plants ($P \times Zn \times AMF$, $\beta = 0.473$, $p < 0.05$), consistent with the substantially larger AMF-associated LAR increase observed specifically in A1 (84.8 to 112 cm² g^{−1}; Table 4) relative to the other treatments.

The piecewise SEM explained a substantial proportion of variance across all four response variables. Marginal R^2 ranged from 0.523 for NAR to 0.956 for RGR. The high marginal R^2 (0.956) for RGR reflects the arithmetic relationship between RGR and its components NAR and LAR in the classical growth-analysis framework. The R^2 for $\Phi PSII$ (0.755), on the other hand, indicates that the phosphorus, zinc, and AMF treatment structure accounts for the large majority of variance in photochemical efficiency. The comparatively lower R^2 for NAR (0.523) reflects effects on NAR not captured by the model, possibly related to cellular respiration, a component of NAR not measured in the study.

The SEM also has some notable limitations. It failed to capture two relationships that are theoretically backed and have been empirically established by the results of this study: the positive effect of AMF on LAR under phosphorus-sufficient conditions (represented here by a non-significant path coefficient of 0.281), and the pathway from $\Phi PSII$ to NAR. The well-established coupling between $\Phi PSII$, ETR, and photosynthetic carbon assimilation [58,59] suggests that the non-significance of the $\Phi PSII \rightarrow NAR$ path may instead reflect a model structural limitation. However, the ($p < 0.10$) could reflect a trend that could possibly be captured by a longer experimental duration.

3.4. AMF Reduces $\Phi PSII$ in Phosphorus-Deficient Maize

In contrast to studies reporting AMF-mediated restoration of $\Phi PSII$ and ETR under phosphorus deficiency [60,61], the present study found that AMF inoculation significantly reduced $\Phi PSII$ in phosphorus-deficient treatments. The SEM's $P \times AMF$ interaction ($\beta = 0.408$) implies a weaker phosphorus– $\Phi PSII$ association under AMF inoculation as compared to phosphorus– $\Phi PSII$ alone ($\beta = 0.558$). Moreover, the A2 (dual deficiency) treatment carried a significant negative effect on $\Phi PSII$ beyond what the main phosphorus and zinc effects alone predict ($\beta = -0.278$), and this reduction was roughly 1.8 times larger under AMF inoculation ($\beta = -0.494$). This pattern is directly corroborated by the observed treatment means, where AMF significantly reduced $\Phi PSII$ by 29.6–30.8% in A2 and A3 ($p < 0.01$; Table 2). This is consistent with AMF imposing a photochemical cost specifically under combined nutrient deficiency, plausibly related to carbon diversion toward fungal structures during active colonization. During the symbiosis, host photosynthates are drawn towards the AMF [62]. Moreover, the periarbuscular membrane formed at the site of nutrient exchange between the AMF and host has been established to be host-derived [63,64]. As AMF colonization was high (31–43%), corresponding to a metabolically active symbiosis, we hypothesize that some carbon was directed towards the AMF and periarbuscular membrane. This carbon diversion could have reduced the carbon available for maintaining the photosynthetic apparatus and could plausibly account for the observed $\Phi PSII$ /ETR suppression. However, this mechanism was not directly measured and cannot be confirmed from the present data.

The high $\Phi PSII$ and ETR observed in the deionized water control (A5), alongside low stomatal conductance and NAR, indicate a decoupling of electron transport from CO₂ assimilation. This pattern is consistent with electron flow to alternative acceptors, notably molecular oxygen, generating reactive oxygen species (ROS) [65]. This phenomenon has

been documented in maize under cold stress [66,67], heat stress [68,69], and nutrient stress [70].

3.5. Limitations

The study contains some methodological and analytical limitations. The experiment captured plant responses only during the first 22 days after sowing, corresponding to the early vegetative stage. Physiological and growth responses to AMF and nutrient deficiency during later vegetative development, during the reproductive stage, or at final yield cannot be inferred from these data. Moreover, plants were grown in sand culture under controlled greenhouse conditions rather than in natural agricultural soils. The medium notably lacks native microbial and AMF communities as well as the physical heterogeneity of field soils. These conditions contribute to nutrient availability and plant–AMF interactions under real growing conditions. Furthermore, only one genotype, SUWAN 5819, was evaluated. AMF responsiveness and nutrient-acquisition strategies are known to vary substantially among maize genotypes, meaning the magnitude and direction of the effects reported here may not generalize across maize germplasm. Another limitation involved not using sterilized, AMF-free inoculum carrier to isolate AMF-specific effects from those attributable to the carrier substrate itself. However, the variability in M1 treatments over M0 is unlikely to be attributed to carrier material.

One of the limitations of this study was potassium not being compensated for when KH_2PO_4 was removed from the $-P$ treatments. This reduced the total solution potassium from 6 mM to 5 mM, representing a 16.7% reduction. This reduction is smaller than levels shown to affect ΦPSII and ETR in maize. A 60% reduction in solution potassium produced only a 5% decline in stomatal conductance [71]. Significant declines in net photosynthetic rate (31–35%), ΦPSII (46%), and ETR (29%) required reductions of 95% or greater in potassium [71]. A separate maize study similarly required a 97.5% reduction in solution potassium to significantly reduce net photosynthetic rate and stomatal conductance [72]. The 16.7% reduction applied here therefore falls well below these thresholds. However, the influence of potassium reduction in ΦPSII and ETR in the $-P$ treatments cannot be fully ignored as tissue potassium was not measured.

Another limitation of the study was that zinc and phosphorus concentrations in leaf, stem, and root tissue were not directly measured. This limits the strength of mechanistic explanations linking pathways governed by internal nutrient status. Tissue nutrient analysis in future studies would allow more direct mechanistic attribution. Furthermore, relative growth rate and net assimilation rate were calculated from destructive harvests taken seven days apart (15 and 22 DAS) rather than from repeated non-destructive measurements across the growth period. The reported RGR and NAR values therefore represent average growth and assimilation rates integrated over that interval, not a continuously varying trajectory of growth and carbon gain within it.

4. Materials and Methods

4.1. Experimental Location

Maize seedlings were grown for 22 days at the Central Laboratory greenhouse, Bangkhen campus, Kasetsart University, Bangkok, Thailand ($13^\circ 51' 11.5''$ N, $100^\circ 34' 20.4''$ E). Greenhouse conditions were monitored continuously throughout the experimental period. The mean daily air temperature was 31.0°C (daily maxima 37.1 – 41.7°C ; minima 25.5 – 29.8°C) and the mean relative humidity was 73.2%. Plants were grown under a natural photoperiod (~ 12.4 h). Solar light intensity was recorded in lux and converted to photosynthetic photon flux density (PPFD) using the standard sunlight conversion factor

of $0.0185 \mu\text{mol m}^{-2} \text{s}^{-1} \text{lux}^{-1}$ [73], yielding a mean daytime PPFD of $253 \mu\text{mol m}^{-2} \text{s}^{-1}$ (daily means: $102\text{--}435 \mu\text{mol m}^{-2} \text{s}^{-1}$; mean daily maximum $1322 \mu\text{mol m}^{-2} \text{s}^{-1}$).

4.2. Plant Material and Seed Sterilization

The maize variety used was SUWAN 5819, which is a single-cross hybrid of Ki64 and Ki60, provided by the Department of Agronomy, Faculty of Agriculture Kamphaeng Saen Campus, and National Corn and Sorghum Research Center, Kasetsart University, Bangkok, Thailand. Prior to use, a modified protocol by [74] was used to surface sterilize the seeds. In brief, the seeds were soaked in 10% analytical-grade sodium hypochlorite (*v/v*) for 15 min and rinsed 3 times with deionized water. They were then placed on autoclaved germination paper (10 seeds per sheet) and incubated for 3 days at 25°C and 80% relative humidity. After germination, the seedlings were transplanted into plastic pots containing 1 kg of acid-washed quartz sand. The inside of each pot was lined with a $30 \mu\text{m}$ cloth mesh to prevent sand from falling out.

4.3. Sand Preparation

Sand washing was performed per the modified protocol of [75]. The sand was first washed with tap water to reduce dust until the water appeared clear upon settling. Then, the sand was immersed for 2 h in 10% solution of commercial bleach (6% sodium hypochlorite) (2:1 sand:solution). After this, the sand was washed 5 times with tap water and soaked in 0.1 M HCl for 24 h. The sand was then rinsed 4 times with tap water followed by two times with reverse osmosis (RO) water, and another two times with deionized water to pH 7. After this, the sand was dried for 3 days, sieved ($<1 \text{ mm}$), and autoclaved at 121°C for 20 min.

4.4. Experimental Design and Treatments

The experiment was a randomized complete block split-plot design with six replications. Five levels of nutrient treatments were assigned to main plots, and two levels of AMF inoculation were assigned to sub-plots. Nutrient treatments were based on Hoagland solution with the following composition: 5 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 5 mM KNO_3 ; 1 mM KH_2PO_4 ; 2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 mM Fe-EDTA; $46 \mu\text{M}$ H_3BO_3 ; $9 \mu\text{M}$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; $0.32 \mu\text{M}$ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; $0.11 \mu\text{M}$ $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; $0.75 \mu\text{M}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Table 1). The $-P$ treatments were modified by the removal of KH_2PO_4 , and $-Zn$ treatments were modified by the removal of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. Potassium was not compensated for in the $-P$ treatments (see Section 3). Plants supplied with deionized water constituted the control treatment. The pH of the Hoagland solution was maintained and adjusted at 5.8–6.2 using drops of 4 M NaOH.

Nutrient treatments started at 8 days after sowing (DAS). An automated irrigation timer was used to maintain soil moisture at 70% water-holding capacity. An amount of 1 kg of dried sand was initially soaked with deionized water to full capacity to determine the weight at 100% WHC. The pots were then supplied with 65 mL daily to maintain 70% WHC, a volume determined prior to the experiment. The five nutrient treatments were: A1 (+Zn+P), A2 ($-Zn-P$), A3 (+Zn $-P$), A4 ($-Zn+P$), and A5 (deionized water control). AMF-inoculated sub-plots were designated M1 and non-inoculated were designated M0.

The AMF inoculant consisted of a sterilized 1:1 (*w/w*) mixture of sand and soil containing *Rhizophagus irregularis* at a propagule density of 25 spores g^{-1} , supplied by the Soil Microbiology Research Group, Department of Agriculture, Bangkok, Thailand. An amount of 30 g of inoculant was used in the M1 treatments 7 DAS. Plants in M0 treatments did not receive any inoculant material.

4.5. Sampling and Measurements

Destructive sampling was conducted twice, at 15 DAS (harvest 1) and 22 DAS (harvest 2). Growth parameters (dry weights and leaf area) were measured and calculated from both harvests, while physiological measurements were taken at the second harvest. Leaf, stem, and root dry weights were obtained by drying fresh samples in a hot-air oven at 70 °C for 48 h followed by weighing on an electric balance. Chlorophyll content was measured using a Konica Minolta SPAD-502Plus Chlorophyll Meter manufactured by Konica Minolta Sensing, Inc., Osaka, Japan. Each value is a mean of three readings taken from the third fully expanded leaf from the apex at 09:00 h [76]. Stomatal conductance (gsw), Φ PSII, and ETR were measured between 11:00 and 12:00 h using LI-600 porometer/fluorometer manufactured by LI-COR Biosciences, Lincoln, NE, USA, software version 3.0.1, with each data point averaged across three readings per plant. Leaf area was estimated using the formula proposed by [77]: leaf area = leaf length $\times$ leaf width $\times$ 0.75. The correction factor used has been previously validated for SUWAN 5819 [78] and was applied uniformly across all treatment groups.

4.6. Derived Growth Parameters

Leaf area ratio (LAR) was calculated as described by [79]. Relative growth rate (RGR) and net assimilation rate (NAR) were calculated using the following equations [80]:

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_1 and W_2 are dry weights of the leaf, stem, root, or total plant at times t_1 and t_2 , respectively.

$$\text{NAR} = [(W_2 - W_1) / (t_2 - t_1)] \times [(\ln L_2 - \ln L_1) / (L_2 - L_1)]$$

where L_1 and L_2 are total leaf areas at times t_1 and t_2 , respectively.

4.7. AMF Colonization Assessment

Harvested root samples were heated in 10% KOH for 5–7 min, washed, and stained with trypan blue following the method of [81]. Colonization was quantified using the gridline intersection method at 45 $\times$ magnification with 6 replications [82].

4.8. Piecewise Structural Equation Modelling

Piecewise structural equation modelling (piecewise SEM) was used to quantify the direct and indirect pathways through which phosphorus supply, zinc supply, and AMF inoculation influence whole-plant RGR. Four endogenous variables were specified: Φ PSII, NAR, LAR, and whole-plant RGR. Exogenous predictors were binary treatment indicators for phosphorus supply status (P status: 1 for A1 and A4; 0 otherwise), zinc supply status (Zn status: 1 for A1 and A3; 0 otherwise), AMF inoculation (AMF: 1 for M1; 0 for M0), and their two-way interactions P $\times$ AMF and Zn $\times$ AMF. Treatment A5 served as the reference intercept.

Each endogenous variable was modelled using a linear mixed-effects model fitted with the lmer function from the lme4 package v2.0.1 [83] with maximum likelihood estimation (REML = FALSE). Continuous endogenous variables were z-score standardized prior to model fitting. The hypothesized path structure was: P status, Zn status, AMF, P $\times$ AMF, and Zn $\times$ AMF as predictors of Φ PSII; Φ PSII and P status as predictors of NAR; Φ PSII, P status, AMF, P $\times$ AMF, and Zn $\times$ AMF as predictors of LAR; and NAR, LAR, and P status as predictors of RGR. The four component models were assembled using the psem function

from the piecewiseSEM package v2.3.1 [84]. Marginal and conditional R^2 values were calculated following [85] using the MuMIn package v1.48.19 [86].

4.9. Relative Importance Analysis

The LMG method [87] via the relaimpo package v2.2.7 was used to estimate the relative importance of NAR and LAR in explaining variation in RGR [88].

4.10. Statistical Analysis

All variables were analyzed using two-way ANOVA ($p < 0.05$) via linear mixed-effects models fitted with lmer (lme4 package v2.0.1) or lme (nlme package v3.1.169). Nutrient treatment (five levels), AMF inoculation (two levels), and their interaction were specified as fixed effects and the block (six replications) as a random effect. Normality of residuals was confirmed using the Shapiro–Wilk test ($p > 0.05$), and homogeneity of variance was assessed using Levene’s test ($p > 0.05$). Where normality assumptions were violated, log transformation was applied. For log-transformed parameters, back-transformed geometric means with geometric standard deviations (GSDs) are reported and indicated by a dagger (†) in the tables, except for Table 1 where 95% confidence intervals in parentheses are reported instead. The stem dry weight of the first destructive sampling was Box–Cox transformed as log transformation failed to restore normality; estimated marginal means are reported for this variable. Stem dry weight at harvest 2 exhibited heterogeneous variance and instead was analyzed using the lme function with a varIdent weighting structure. Φ PSII and ETR were analyzed using aligned-ranks transformation ANOVA (ART-ANOVA) via the ARTool package v0.11.2, for which raw means are reported. SS and MS are not given for ART-ANOVA as the analysis involves aligned and ranked data. Therefore, conventional sums of squares (SS) and mean squares (MS) are not directly interpretable and are typically not reported. Full ANOVA tables for physiological data (stomatal conductance, Φ PSII, and ETR) and growth parameters (dry weights and leaf area for harvests 1 and 2, LAR, and RGR for leaf, stem, root, and whole plant) have been provided in the Supplementary Materials as Table S1 and Table S2, respectively.

Post hoc pairwise comparisons of estimated marginal means were performed by Šidák adjustment [89]. Principal component analysis (PCA) was used to explore multivariate patterns among colonization, growth, and physiological variables, using the FactoMineR v2.14 and factoextra v2.0.0 packages. The number of components retained was determined by the scree plot [90] together with the Kaiser criterion [91]. All visualizations were generated using ggplot2 v4.0.3 [92]. GenAI Claude (Sonnet 4.5, Anthropic, San Francisco, CA, USA) was used for assistance in data analysis and R code generation. Each line of code was manually validated by the authors for statistical appropriateness and reproducibility. All statistical analyses were conducted in R (RStudio version 2026.04.0+526).

5. Conclusions

Across every analysis in this study, phosphorus status—not zinc status—was the variable that mattered for early-stage maize photochemistry and growth. Zinc deficiency alone produced no detectable effect on Φ PSII or ETR at this developmental stage, while phosphorus deficiency suppressed both consistently. Combined zinc and phosphorus deficiency produced a decoupling of stomatal aperture from photosynthetic performance, hypothesized to result from the convergent failure of CA-mediated stomatal closure. On the growth side, NAR rather than LAR accounted for most of the variance in whole-plant RGR across every treatment combination, and the piecewise SEM located a further direct phosphorus-to-RGR path independent of either. The third path is hypothesized to be root-level phosphorus absorption capacity rather than anything visible at the leaf level.

Unlike in previous studies, AMF inoculation significantly reduced Φ PSII under phosphorus deficiency. These results show a system in which phosphorus availability, not zinc, sets the photosynthetic and growth ceiling in early vegetative maize in sand culture under the conditions tested. Moreover, AMF were shown to impose a cost in plant photochemistry without improved RGR in the tested conditions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/plants15142215/s1>: Table S1: Analysis of variance of physiological parameters for zinc and phosphorus deficiency with and without mycorrhiza; Table S2: Analysis of variance of biomass and leaf area parameters for harvests 1 and 2, and RGR for leaf, stem, root, and whole plant for zinc and phosphorus deficiency with and without mycorrhiza.

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Abbreviations

The following abbreviations are used in this manuscript:

AMF	Arbuscular Mycorrhiza Fungi
PSII	Photosystem II
ETR	Electron Transport Rate
RGR	Relative Growth Rate
NAR	Net Assimilation Rate
LAR	Leaf Area Ratio
CA	Carbonic Anhydrase
DAS	Days After Sowing
SEM	Structural Equation Model
SAR[P]	Specific Absorption Rate of Phosphorus
SLAC	Slow Anion Channel-Associated

ATP	Adenosine Triphosphate
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
RO	Reverse Osmosis
DI	Deionized
PCA	Principal Component Analysis
LMG	Lindeman–Merenda–Gold
Pi	Inorganic Phosphate
ΦPSII	Quantum Efficiency of Photosystem II
gsw	Stomatal Conductance
ART-ANOVA	Aligned-Ranks Transformation Analysis of Variance
PPFD	Photosynthetic Photon Flux Density

References

1. FAO. *Agricultural Production Statistics–2010–2024*; FAO: Rome, Italy, 2025.
2. FAO. Crops and Livestock Products. Available online: <https://www.fao.org/faostat/en/#data/QCL> (accessed on 27 April 2026).
3. Tanumihardjo, S.A.; McCulley, L.; Roh, R.; Lopez-Ridaura, S.; Palacios-Rojas, N.; Gunaratna, N.S. Maize agro-food systems to ensure food and nutrition security in reference to the Sustainable Development Goals. *Glob. Food Secur.* **2020**, *25*, 100327. [\[CrossRef\]](#)
4. Nadeem, F.; Abbas, S.; Waseem, F.; Ali, N.; Mahmood, R.; Bibi, S.; Deng, L.; Wang, R.; Zhong, Y.; Li, X. Phosphorus (P) and Zinc (Zn) nutrition constraints: A perspective of linking soil application with plant regulations. *Environ. Exp. Bot.* **2024**, *226*, 105875. [\[CrossRef\]](#)
5. Ringeval, B.; Demay, J.; Helfenstein, J.; Kvakić, M.; Mollier, A.; Seghouani, M.; Nesme, T.; Gerber, J.S.; Mueller, N.D.; Pellerin, S. Limitation of Maize Potential Yield by Phosphorus at the Global Scale. *Glob. Change Biol.* **2025**, *31*, e70485. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Sánchez-Rodríguez, A.R.; Rey, M.-D.; Nechate-Drif, H.; Castillejo, M.Á.; Jorrín-Novo, J.V.; Torrent, J.; del Campillo, M.C.; Sacristán, D. Combining P and Zn fertilization to enhance yield and grain quality in maize grown on Mediterranean soils. *Sci. Rep.* **2021**, *11*, 7427. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Salika, R.; Riffat, J. Abiotic stress responses in maize: A review. *Acta Physiol. Plant.* **2021**, *43*, 130. [\[CrossRef\]](#)
8. Gao, D.; Ran, C.; Dang, K.; Wang, X.; Zhang, Y.; Geng, Y.; Liu, S.; Guan, Z.; Guo, L.; Shao, X. Effect of Phosphorus, Iron, Zinc, and Their Combined Deficiencies on Photosynthetic Characteristics of Rice (*Oryza sativa* L.) Seedlings. *Agronomy* **2023**, *13*, 1657. [\[CrossRef\]](#)
9. Salama, Z.A.; El-Fouly, M.M.; Lazova, G.; Popova, L.P. Carboxylating enzymes and carbonic anhydrase functions were suppressed by zinc deficiency in maize and chickpea plants. *Acta Physiol. Plant.* **2006**, *28*, 445–451. [\[CrossRef\]](#)
10. Jócsák, I.; Csima, F.; Somfalvi-Tóth, K. Alterations of Photosynthetic and Oxidative Processes Influenced by the Presence of Different Zinc and Cadmium Concentrations in Maize Seedlings: Transition from Essential to Toxic Functions. *Plants* **2024**, *13*, 1150. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Mattiello, E.M.; Ruiz, H.A.; Neves, J.C.; Ventrella, M.C.; Araújo, W.L. Zinc deficiency affects physiological and anatomical characteristics in maize leaves. *J. Plant Physiol.* **2015**, *183*, 138–143. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Mallikarjuna, M.G.; Thirunavukkarasu, N.; Sharma, R.; Shiriga, K.; Hossain, F.; Bhat, J.S.; Mithra, A.C.; Marla, S.S.; Manjaiah, K.M.; Rao, A.; et al. Comparative Transcriptome Analysis of Iron and Zinc Deficiency in Maize (*Zea mays* L.). *Plants* **2020**, *9*, 1812. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Wang, H.; Liu, R.L.; Jin, J.Y. Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. *Biol. Plant.* **2009**, *53*, 191–194. [\[CrossRef\]](#)
14. Wang, H.; Jin, J.Y. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. *Photosynthetica* **2005**, *43*, 591–596. [\[CrossRef\]](#)
15. Zhang, K.; Liu, H.; Tao, P.; Chen, H. Comparative Proteomic Analyses Provide New Insights into Low Phosphorus Stress Responses in Maize Leaves. *PLoS ONE* **2014**, *9*, e98215. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Xia, Z.; Zhang, S.; Wang, Q.; Zhang, G.; Fu, Y.; Lu, H. Effects of Root Zone Warming on Maize Seedling Growth and Photosynthetic Characteristics Under Different Phosphorus Levels. *Front. Plant Sci.* **2021**, *12*, 746152. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Kalaji, H.M.; Oukarroum, A.; Alexandrov, V.; Kouzmanova, M.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Al-lakhverdiev, S.I.; Goltsev, V. Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. *Plant Physiol. Biochem.* **2014**, *81*, 16–25. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Schlüter, U.; Colmsee, C.; Scholz, U.; Bräutigam, A.; Weber, A.P.; Zellerhoff, N.; Bucher, M.; Fahnenstich, H.; Sonnewald, U. Adaptation of maize source leaf metabolism to stress related disturbances in carbon, nitrogen and phosphorus balance. *BMC Genom.* **2013**, *14*, 442. [\[CrossRef\]](#) [\[PubMed\]](#)

19. Carstensen, A.; Herdean, A.; Schmidt, S.B.; Sharma, A.; Spetea, C.; Pribil, M.; Husted, S. The Impacts of Phosphorus Deficiency on the Photosynthetic Electron Transport Chain. *Plant Physiol.* **2018**, *177*, 271–284. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Jaisue, P.; Daengngam, C.; Pengphorm, P.; Nutthapornnitchakul, S.; Pinit, S.; Klinnawee, L. Enhanced Chlorophyll Accumulation is Early Response of Rice to Phosphorus Deficiency. *Rice Sci.* **2025**, *32*, 831–844. [\[CrossRef\]](#)
21. Kiddee, S.; Daengngam, C.; Wongarrayapanich, S.; Lau, J.Y.; Cheng, A.; Klinnawee, L. Detection of Phosphorus Deficiency Using Hyperspectral Imaging for Early Characterization of Asymptomatic Growth and Photosynthetic Symptoms in Maize. *Agronomy* **2026**, *16*, 772. [\[CrossRef\]](#)
22. Liu, H.; Gan, W.; Rengel, Z.; Zhao, P. Effects of zinc fertilizer rate and application method on photosynthetic characteristics and grain yield of summer maize. *J. Soil Sci. Plant Nutr.* **2016**, *16*, 550–562. [\[CrossRef\]](#)
23. Santos, E.F.; Pongrac, P.; Reis, A.R.; Rabêlo, F.H.S.; Azevedo, R.A.; White, P.J.; Lavres, J. Unravelling homeostasis effects of phosphorus and zinc nutrition by leaf photochemistry and metabolic adjustment in cotton plants. *Sci. Rep.* **2021**, *11*, 13746. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Rodrigues, R.E.; Neto, A.R.; Jesus, L.S.; Guedes, L.T.M.; Santos, L.S.; Xavier, M.N.; Souza, L.A. Different patterns of maize hybrids responses under variable phosphorus availability. *Acta Physiol. Plant.* **2023**, *45*, 113. [\[CrossRef\]](#)
25. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. [\[CrossRef\]](#) [\[PubMed\]](#)
26. López-Ráez, J.; Pozo, M. *Chemical Signalling in the Arbuscular Mycorrhizal Symbiosis: Biotechnological Applications*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 215–232.
27. Ma, X.; Li, X.; Ludewig, U. Arbuscular mycorrhizal colonization outcompetes root hairs in maize under low phosphorus availability. *Ann. Bot.* **2021**, *127*, 155–166. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Saboor, A.; Ali, M.A.; Husain, S.; Tahir, M.S.; Irfan, M.; Bilal, M.; Baig, K.S.; Datta, R.; Ahmed, N.; Danish, S.; et al. Regulation of Phosphorus and Zinc Uptake in Relation to Arbuscular Mycorrhizal Fungi for Better Maize Growth. *Agronomy* **2021**, *11*, 2322. [\[CrossRef\]](#)
29. Saboor, A.; Ali, M.A.; Danish, S.; Ahmed, N.; Fahad, S.; Datta, R.; Ansari, M.J.; Nasif, O.; Rahman, M.H.U.; Glick, B.R. Effect of arbuscular mycorrhizal fungi on the physiological functioning of maize under zinc-deficient soils. *Sci. Rep.* **2021**, *11*, 18468. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Bi, Y.; Wang, K.; Ma, S.; Zhou, Y.; He, Y. Role of common arbuscular mycorrhizal networks in crop phosphorus uptake and biomass in a maize–soybean intercropping system. *Arch. Microbiol.* **2026**, *208*, 286. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Saboor, A.; Ali, M.A.; Ahmed, N.; Skalicky, M.; Danish, S.; Fahad, S.; Hassan, F.; Hassan, M.M.; Brestic, M.; El Sabagh, A.; et al. Biofertilizer-Based Zinc Application Enhances Maize Growth, Gas Exchange Attributes, and Yield in Zinc-Deficient Soil. *Agriculture* **2021**, *11*, 310. [\[CrossRef\]](#)
32. Evans, G.C. *The Quantitative Analysis of Plant Growth*; Univ of California Press: Oakland, CA, USA, 1972; Volume 1.
33. Plénet, D.; Mollier, A.; Pellerin, S. Growth analysis of maize field crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. *Plant Soil* **2000**, *224*, 259–272. [\[CrossRef\]](#)
34. Mubushar, M.; El-Hendawy, S.; Dewir, Y.H.; Al-Suhaibani, N. Ability of Different Growth Indicators to Detect Salt Tolerance of Advanced Spring Wheat Lines Grown in Real Field Conditions. *Plants* **2024**, *13*, 882. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Calero Hurtado, A.; Peña Calzada, K.; Fasoli, J.V.B.; Jiménez, J.; Sánchez López, L. Synergic Effects of the Microbial Consortium and Amino Acid-Based Growth Promoter in Sunflower Productivity Under Water-Deficit Conditions. *Water* **2025**, *17*, 1365. [\[CrossRef\]](#)
36. Gómez-Cabezas, M.; España, Á. Exogenous Sucrose Enhances Growth and Physiological Performance of Tomato Seedlings Under Suboptimal Light Conditions in Passive Greenhouses. *Horticulturae* **2024**, *10*, 1337. [\[CrossRef\]](#)
37. El-Mejjaouy, Y.; Belmrhar, L.; Zeroual, Y.; Dumont, B.; Mercatoris, B.; Oukarroum, A. PCA-based detection of phosphorous deficiency in wheat plants using prompt fluorescence and 820 nm modulated reflection signals. *PLoS ONE* **2023**, *18*, e0286046. [\[CrossRef\]](#) [\[PubMed\]](#)
38. El-Mejjaouy, Y.; Lahrir, M.; Naciri, R.; Zeroual, Y.; Mercatoris, B.; Dumont, B.; Oukarroum, A. How far can chlorophyll a fluorescence detect phosphorus status in wheat leaves (*Triticum durum* L.). *Environ. Exp. Bot.* **2022**, *194*, 104762. [\[CrossRef\]](#)
39. He, M.; Li, X.; Mang, M.; Li, Z.; Ludewig, U.; Schulze, W.X. A systems-biology approach identifies co-expression modules in response to low phosphate supply in maize lines of different breeding history. *Plant J.* **2022**, *109*, 1249–1270. [\[PubMed\]](#)
40. Jacob, J.; Lawlor, D.W. In vivo photosynthetic electron transport does not limit photosynthetic capacity in phosphate-deficient sunflower and maize leaves. *Plant Cell Environ.* **1993**, *16*, 785–795. [\[CrossRef\]](#)
41. Kaya, C.; Şenbayram, M.; Akram, N.A.; Ashraf, M.; Alyemeni, M.N.; Ahmad, P. Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (*Zea mays* L.). *Sci. Rep.* **2020**, *10*, 6432. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Fu, C.; Li, M.; Zhang, Y.; Zhang, Y.; Yan, Y.; Wang, Y.a. Morphology, photosynthesis, and internal structure alterations in field apple leaves under hidden and acute zinc deficiency. *Sci. Hort.* **2015**, *193*, 47–54. [\[CrossRef\]](#)

43. Ivanov, K.; Vassilev, A.; Mitkov, A.; Nguyen, N.; Tonev, T. Application of Zn-containing foliar fertilisers for recovery of the grain productivity potential of Zn-deficient maize plants. *Ital. J. Agron.* **2021**, *16*, 1759. [\[CrossRef\]](#)
44. Zhao, K.; Wu, Y. Effects of Zn deficiency and bicarbonate on the growth and photosynthetic characteristics of four plant species. *PLoS ONE* **2017**, *12*, e0169812. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Li, F.; He, C.; Chang, Z.; Ma, C.; Yu, J.; Liu, L.; Zhang, Y.; Hao, L. Effects of elevated carbon dioxide on plant growth and leaf photosynthesis of annual ryegrass along a phosphorus deficiency gradient. *Front. Plant Sci.* **2023**, *14*, 1271262. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Engineer, C.B.; Hashimoto-Sugimoto, M.; Negi, J.; Israelsson-Nordström, M.; Azoulay-Shemer, T.; Rappel, W.-J.; Iba, K.; Schroeder, J.I. CO₂ Sensing and CO₂ Regulation of Stomatal Conductance: Advances and Open Questions. *Trends Plant Sci.* **2016**, *21*, 16–30. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Wang, C.; Hu, H.; Qin, X.; Zeise, B.; Xu, D.; Rappel, W.J.; Boron, W.F.; Schroeder, J.I. Reconstitution of CO₂ Regulation of SLAC1 Anion Channel and Function of CO₂-Permeable PIP2;1 Aquaporin as Carbonic Anhydrase4 Interactor. *Plant Cell* **2016**, *28*, 568–582. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Xia, L.; Alvim, J.C.; Nguyen, T.-H.; Lefoulon, C.; Silva-Alvim, F.A.L.; Yu, Z.; Klejchova, M.; Farami, S.; Waghmare, S.; Karnik, R.; et al. A guard cell carbonic anhydrase binds and regulates SLAC1 separate from its catalytic activity. *Nat. Commun.* **2026**, *17*, 3911. [\[CrossRef\]](#) [\[PubMed\]](#)
49. DiMario, R.J.; Clayton, H.; Mukherjee, A.; Ludwig, M.; Moroney, J.V. Plant Carbonic Anhydrases: Structures, Locations, Evolution, and Physiological Roles. *Mol. Plant* **2017**, *10*, 30–46. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Liu, H.; Fiorani, F.; Jäck, O.; Colombi, T.; Nagel, K.A.; Weih, M. Shoot and Root Traits Underlying Genotypic Variation in Early Vigor and Nutrient Accumulation in Spring Wheat Grown in High-Latitude Light Conditions. *Plants* **2021**, *10*, 174. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Villar, R.; Marañón, T.; Quero, J.L.; Panadero, P.; Arenas, F.; Lambers, H. Variation in relative growth rate of 20 *Aegilops* species (*Poaceae*) in the field: The importance of net assimilation rate or specific leaf area depends on the time scale. *Plant Soil* **2005**, *272*, 11–27. [\[CrossRef\]](#)
52. Elberse, I.A.M.; Van Damme, J.M.M.; Van Tienderen, P.H. Plasticity of growth characteristics in wild barley (*Hordeum spontaneum*) in response to nutrient limitation. *J. Ecol.* **2003**, *91*, 371–382. [\[CrossRef\]](#)
53. Khan, F.; Siddique, A.B.; Shabala, S.; Zhou, M.; Zhao, C. Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants* **2023**, *12*, 2861. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Cai, H.; Chu, Q.; Yuan, L.; Liu, J.; Chen, X.; Chen, F.; Mi, G.; Zhang, F. Identification of quantitative trait loci for leaf area and chlorophyll content in maize (*Zea mays*) under low nitrogen and low phosphorus supply. *Mol. Breed.* **2012**, *30*, 251–266. [\[CrossRef\]](#)
55. Colomb, B.; Kiniry, J.R.; Debaeke, P. Effect of soil phosphorus on leaf development and senescence dynamics of field-grown maize. *Agron. J.* **2000**, *92*, 428–435. [\[CrossRef\]](#)
56. Usandivaras, L.M.A.; Gutiérrez-Boem, F.H.; Salvagiotti, F. Contrasting Effects of Phosphorus and Potassium Deficiencies on Leaf Area Development in Maize. *Crop Sci.* **2018**, *58*, 2099–2109. [\[CrossRef\]](#)
57. Ikkonen, E.; Yurkevich, M. Relative Growth Rate and Specific Absorption Rate of Nutrients in *Lactuca sativa* L. Under Secondary Paper Sludge Application and Soil Contamination with Lead. *Agriculture* **2025**, *15*, 1541. [\[CrossRef\]](#)
58. Edwards, G.E.; Baker, N.R. Can CO₂ assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynth. Res.* **1993**, *37*, 89–102. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Genty, B.; Briantais, J.-M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta (BBA) Gen. Subj.* **1989**, *990*, 87–92. [\[CrossRef\]](#)
60. Chen, S.; Zhao, H.; Zou, C.; Li, Y.; Chen, Y.; Wang, Z.; Jiang, Y.; Liu, A.; Zhao, P.; Wang, M.; et al. Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings. *Front. Microbiol.* **2017**, *8*, 2516. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Lazarević, B.; Carović-Stanko, K.; Šatović, Z. Physiological Responses of Basil (*Ocimum basilicum* L.) Cultivars to *Rhizopagus irregularis* Inoculation under Low Phosphorus Availability. *Plants* **2019**, *9*, 14. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Keymer, A.; Gutjahr, C. Cross-kingdom lipid transfer in arbuscular mycorrhiza symbiosis and beyond. *Curr. Opin. Plant Biol.* **2018**, *44*, 137–144. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Ivanov, S.; Austin, J., 2nd; Berg, R.H.; Harrison, M.J. Extensive membrane systems at the host-arbuscular mycorrhizal fungus interface. *Nat. Plants* **2019**, *5*, 194–203. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Pumpkin, N.; Harrison, M.J. Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. *Plant Physiol.* **2009**, *151*, 809–819. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Fryer, M.J.; Andrews, J.R.; Oxborough, K.; Blowers, D.A.; Baker, N.R. Relationship between CO₂ Assimilation, Photosynthetic Electron Transport, and Active O₂ Metabolism in Leaves of Maize in the Field during Periods of Low Temperature. *Plant Physiol.* **1998**, *116*, 571–580. [\[CrossRef\]](#) [\[PubMed\]](#)

66. Fryer, M.J.; Oxborough, K.; Martin, B.; Ort, D.R.; Baker, N.R. Factors Associated with Depression of Photosynthetic Quantum Efficiency in Maize at Low Growth Temperature. *Plant Physiol.* **1995**, *108*, 761–767. [[CrossRef](#)] [[PubMed](#)]
67. Massacci, A.; Lannelli, M.A.; Pietrini, F.; Loreto, F. The effect of growth at low temperature on photosynthetic characteristics and mechanisms of photoprotection of maize leaves. *J. Exp. Bot.* **1995**, *46*, 119–127. [[CrossRef](#)]
68. Massacci, A.; Battistelli, A.; Loreto, F. Effect of drought stress on photosynthetic characteristics, growth and sugar accumulation of field-grown sweet sorghum. *Aust. J. Plant Physiol.* **1996**, *23*, 331–340. [[CrossRef](#)]
69. Cheeseman, J.M. *Depressions of photosynthesis in mangrove canopies. Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field*; Bios Scientific Publishers: Oxford, UK, 1994; pp. 377–389.
70. Perera-Castro, A.V.; Flexas, J. The ratio of electron transport to assimilation (ETR/A(N)): Underutilized but essential for assessing both equipment's proper performance and plant status. *Planta* **2023**, *257*, 29. [[CrossRef](#)] [[PubMed](#)]
71. Thenveetil, N.; Reddy, K.N.; Reddy, K.R. Effects of Potassium Nutrition on Corn (*Zea mays* L.) Physiology and Growth for Modeling. *Agriculture* **2024**, *14*, 968. [[CrossRef](#)]
72. Xiong, W.; Wang, Y.; Guo, Y.; Tang, W.; Zhao, Y.; Yang, G.; Pei, Y.; Chen, J.; Song, X.; Sun, J. Transcriptional and Metabolic Responses of Maize Shoots to Long-Term Potassium Deficiency. *Front. Plant Sci.* **2022**, *13*, 922581. [[CrossRef](#)] [[PubMed](#)]
73. Thimijan, R.W.; Heins, R.D. Photometric, radiometric, and quantum light units of measure: A review of procedures for interconversion. *HortScience* **1983**, *18*, 818–822. [[CrossRef](#)]
74. Ortiz-Ramírez, C.; Arevalo, E.D.; Xu, X.; Jackson, D.P.; Birnbaum, K.D. An Efficient Cell Sorting Protocol for Maize Protoplasts. *Curr. Protoc. Plant Biol.* **2018**, *3*, e20072. [[CrossRef](#)] [[PubMed](#)]
75. Maitra, P.; Al-Rashid, J.; Barman, N.C.; Khan, M.N.M.; Mandal, D.; Rasul, N.M.; Chowdhury, A.; El-Sappah, A.H.; Li, J. Sand Particle Size and Phosphorus Amount Affect *Rhizophagus irregularis* Spore Production Using In Vitro Propagated Spore as a Starter Inoculum in Rhizosphere of Maize (*Zea mays*) Plantlets. *J. Fungi* **2021**, *7*, 846. [[CrossRef](#)]
76. Shivashankar, K.; Potdar, M.P.; Gawdiya, S.; Golshetti, A.; Kanade, A.K.; Balol, G.; Biradar, D.P.; Math, K.K.; Al-Ansari, N.; El-Hendawy, S.; et al. SPAD dynamics in maize crop with precision nitrogen management under rain-fed and irrigated conditions. *Sci. Rep.* **2025**, *15*, 22842. [[CrossRef](#)] [[PubMed](#)]
77. Montgomery, E.G. *Correlation Studies in Corn*; University of Nebraska: Lincoln, NE, USA, 1911; Volume 24, pp. 108–159.
78. Mao, R.; Wongkaew, A.; Khongchui, P.; Nakasathien, S. Agronomic Characteristics of maize grown in paddy Soil, along with root zone soil chemical properties and soil bacterial numbers. *Trends Sci.* **2023**, *20*, 6972. [[CrossRef](#)]
79. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytol.* **2012**, *193*, 30–50. [[CrossRef](#)] [[PubMed](#)]
80. Pandey, R.; Khetarpal, S.; Jain, V.; Kushwaha, S. Phosphorus fertilization improves growth analysis traits and reduces anthesis-to-silking interval leading to increased grain yield in maize. *Indian J. Plant Physiol.* **2015**, *20*, 385–390. [[CrossRef](#)]
81. Phillips, J.M.; Hayman, D. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [[CrossRef](#)]
82. Giovannetti, M.; Mosse, B. AN Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. *New Phytol.* **1980**, *84*, 489–500. [[CrossRef](#)]
83. Pinheiro, J.C.; Bates, D.M. *Mixed-Effects Models in S and S-PLUS*; Springer: Berlin/Heidelberg, Germany, 2000.
84. Lefcheck, J.S. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.* **2016**, *7*, 573–579.
85. Nakagawa, S.; Schielzeth, H. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods Ecol. Evol.* **2013**, *4*, 133–142. [[CrossRef](#)]
86. Bartoň, K. *R Package*, version 1.46. 0. MuMIn: Multi-Model Inference. The R Foundation for Statistical Computing Location: Vienna, Austria, 2022.
87. Lindeman, R.H.; Merenda, P.F.; Gold, R.Z. *Introduction to Bivariate and Multivariate Analysis*; Scott, Foresman and Company: Glenview, IL, USA, 1980; Volume 4.
88. Grömping, U. Relative importance for linear regression in R: The package relaimpo. *J. Stat. Softw.* **2007**, *17*, 1–27.
89. Russell, L. *R Package*, version 1.5.0. Emmeans: Estimated Marginal Means, aka Least-Squares Means; The Comprehensive R Archive Network (CRAN). The R Foundation for Statistical Computing Location: Vienna, Austria, 2020.
90. Cattell, R.B. The Scree Test For The Number Of Factors. *Multivar. Behav. Res.* **1966**, *1*, 245–276. [[CrossRef](#)] [[PubMed](#)]
91. Goretzko, D.; Böhner, M. Factor Retention Using Machine Learning With Ordinal Data. *Appl. Psychol. Meas.* **2022**, *46*, 406–421. [[CrossRef](#)] [[PubMed](#)]
92. Wickham, H. *Elegant Graphics for Data Analysis*; Springer: Berlin/Heidelberg, Germany, 2016.

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