Phosphorus- and water- deficiency induced morpho-physiological and yield changes in maize (Zea mays L.)

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List of abbreviations and acronyms

DAP, diammonium phosphate

DAS, days after sowing

DM, dry matter

K, potassium

LAI, Leaf area index

N, nitrogen

P, phosphorus

PAR, photosynthetically active radiation

PUE, phosphorus use efficiency

RLD, root length density (cm cm^{-3})

RP, rock phosphate

SRL, specific root length (cm g^{-1})

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1 Introduction

1.1 Phosphate fertilizer for crop production

Crop production relies heavily on the use of commercial fertilizers, which provide essential nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), that are necessary for crop growth and productivity (Stewart et al. 2005). It is estimated that 30%–50% of crop yield can be attributed to the application of commercial mineral fertilizer, highlighting the crucial role that these nutrients, particularly P, play in modern agricultural practices. The use of P-based fertilizers has significantly increased in recent decades due to the urgent need to meet the growing demand for food (Russel and Williams 1977; Zhang et al. 2017).

However, P is a non-renewable resource. The global reserves of rock phosphate (RP), from which P fertilizers are extracted, are projected to be exhausted within the next 300-400 years (Cooper et al. 2011; Jasinski 2011; Gong et al. 2022). More than three-quarters of these reserves are geographically concentrated in Morocco, with two-thirds of P production coming from China, the US, and Morocco. Within the next 100 years, most countries will have depleted their reserves. The geopolitical consequences of this depletion could be significant, potentially impacting the security of supply due to the uneven distribution of P sources (Reijnders 2014; Elser 2012). Additionally, the excessive use of P fertilizers has led to an essential component of nonpoint-source pollution and accelerated eutrophication of lakes and streams, posing threats to ecosystems and humans and causing P loss from the global economy (Daniel et al. 1998; Haque 2021; Mallin and Cahoon 2020). These issues present a serious challenge for worldwide environmentally sustainable development, resource conservation, and public health. Furthermore, the demand for P fertilizer is expected to continue rising globally.

P is an essential macronutrient for plants, which is crucial in sustaining plant growth and development. It is involved in the composition of DNA and RNA and actively participates in essential plant metabolic processes, such as respiration and photosynthesis (Uchida 2000). Two primary P sources for crop production are the inherent reserves within seeds and the acquisition of exogenous P from the environment (Nadeem et al. 2011b). Plants can absorb mainly two forms of P in soil: HPO_4^{2-} and $H_2PO_4^-$ (Blaise et al. 2014). As simulated, all P absorbed depends on transferring different forms of soil and plant P, from organic P, stable P, and active P pools to the labile P pool, and from the no-root to the root zone (Dzotsi et al. 2010). It is strongly affected by interacting biotic processes such as root exudation, rhizosphere microbial activity, turnover of soil organic P, and abiotic processes that lead to P solubilization, adsorption, fixation, and even occlusion (Hinsinger 2001). Therefore, P is highly immobile in the soil and is characterized by low plant bioavailability (Hinsinger 2001; Ao et al. 2014). Even though there is a large amount of P in the soil due to the significant increase in fertilizer application in the last decades, P is mainly fixed in soils and non-absorbable by the plant (Sattari et al. 2012; Hinsinger 2001; Solovchenko et al. 2016). Crop yield on 40%-60% of the global arable land is limited by P availability (Cordell et al. 2009). Only 10-30% of P fertilizers can be acquired by the plant in the year applied (Manske et al. 2000). Thus, P is regarded as a major limitation for crop production in many cropping systems worldwide (Fairhust 1999).

P fertilizers are commonly categorized into different categories: water-soluble P fertilizers, weakly acidic P fertilizers, insoluble P fertilizers, and miscible P fertilizers, according to the magnitude of solubility and the difficulty of crop absorption. Nowadays, there are mostly several types of water-soluble P fertilizers on the world market for crop production, including single superphosphate (SSP), triple superphosphate (TSP), calcium magnesium phosphate (CAM), mono-ammonium phosphate (MAP), and diammonium phosphate (DAP) (Gong et al. 2022). However, during P mining for the production of mineral fertilizer, more than 10% of P is lost (Wang et al. 2020). While higher solubility and efficiency of P fertilizer improve its availability to plants, they also increase the risk of P loss (Gong et al. 2022). The application of MAP and DAP improves crop production compared to others. However, the loss of P and prices are higher (Gong et al. 2022; Zhang et al. 2008).

Currently, the P demand for crop production is still increasing due to the growing needs and the demand for higher productivity per unit of arable land (Hertel 2011). Therefore, improving P use efficiency and optimizing crop growth and development in the context of low PUE, P depletion, and global initiatives for sustainable development are extremely important.

1.2 Maize production and challenges

1.2.1 Effects of P deficiency on morpho-physiological and yield changes of maize plants

Maize is one of the world's most important crops, along with rice and wheat, as one of the three most crucial staples for global food security. It is utilized as human food, animal feed, and biofuels (Shiferaw et al. 2011). As highlighted by Tanumihardjo et al. (2020), maize continues to be in high demand to address hunger, improve food security, and enhance overall nutrition.

However, the availability of P in the soil affects maize plants in different aspects and with varying magnitudes at various growth stages. The growth and development of maize plants are susceptible to P deficiency in the soil, leading to even irreversible impacts in early growth stages (Nadeem et al. 2011b; Barry and Miller 1989; Colomb et al. 2000). P deficiency generally results in a smaller leaf area, lower canopy interception rate, and reduced biomass (Zhang et al. 2022). The older leaves could be observed with purple coloration as the visible symptom of P deficiency, particularly on the underside, further limiting the accumulation of dry matter by photosynthesis in plant leaves (Jeyakumar and Balamohan 2020). Post-silking P uptake accounts for 58%-75% of total P uptake, which depends on the plant structure and nutrient uptake transport (Zhang et al. 2022).

Nutrients and water absorbed by plants from the soil are generally dependent on the capacity to develop an abundant root system of the plants, which functions as a mediator between plants and soil (Lynch 2007). There are two ways for the plants to acquire P from soil: soil exploration and mobilization of P from poorly available P pools in the rhizosphere (Zhu and Lynch 2004). Plants process morphological and physiological mechanisms to adapt to P deficiency through root growth changes. Generally, plants exhibit longer root lengths, higher root length density (RLD), and higher specific root length (SRL), all of which improve soil exploitation for P. The increase in root organic acids, which are efficient in P release, and the interaction of phytohormones contribute to P release and conversion to plant-absorbable P. There are different strategies to enhance P acquisition among species because of the existence of various root traits (Wen et al. 2019b). Root morphological specific adaptation is a critical factor in P acquisition of maize, with the plant's ability to absorb P mainly dependent on young roots penetrating unexplored soil (Lyu et al. 2016; van der Bom et al. 2023).

P deficiency mainly reduces the number of roots, especially axile roots. The pattern of modification of maize roots under P deficiency is very similar to that of root growth limited by the availability of carbohydrates (Khamis et al. 1990). P deficiency leads to reduced root biomass, lower root length, and RLD, but increased SRL (Lopez et al. 2022). Root structures with greater root length and RLD can effectively reach the soil surface and utilize nutrients within a limited soil volume. A higher SRL, demonstrating greater efficiency in resource exploration with less metabolic cost, also results in more efficient P uptake and biomass production compared to a lower SRL (Lynch 2015; Wen et al. 2022). The application of P fertilizer could elevate both soluble and total P in P-deficient soils and alter the structure and functions of the bacterial community involved in P cycling and supplying (Wu et al. 2022b). Higher P fertilizer enhances root growth and nutrient uptake, while long-term overuse of P fertilizer decreases the total bacterial diversity in low P soils, which plays a vital role during the process of P release from soils (Liu et al. 2020; Li et al. 2021a).

In addition to the amount of P fertilizer, the type of fertilizer is another critical factor affecting the availability of P. Generally, the higher the solubility of the P fertilizer, the higher its cost during the production process (Gong et al. 2022). The use efficiency of P fertilizer increases with increasing solubility of the P fertilizer types, and their availability to the plants and use efficiency are higher. A type of low plant-available P fertilizer could lead to the same influence with a low amount of P fertilizer applied, even characterized by P deficiency (You et al. 2021; Colomb et al. 2000). Additionally, soil pH, considered to be the “master variable” of the soil, affects nutrient availability depending on both soil physiochemical properties and reactions, as well as plants (Barrow and Hartemink 2023; Zhao et al. 2022). The effects of different P fertilizers vary under various soil conditions based on soil pH, e.g., alkaline, neutral, and acidic soil (Zhao et al. 2022; Wang et al. 2023). DAP led to the highest grain yield in neutral soil, while the yield of CMP was highest in acidic soil among five different P fertilizers (DAP, calcium superphosphate (CSP), CMP, MAP, and ammonium polyphosphate (APP)) (Zhao et al. 2022). Therefore, it is crucial to investigate the amount and types of P fertilizers that need to be applied based on soil conditions.

1.2.2 Effects of drought on morpho-physiological and yield changes of maize plants

The change in global climate is characterized by more frequent extreme scenarios, such as drought, flood, and global warming, and the increasing climatic variability puts significant pressure on agricultural systems and imposes more constraints on crop production (Vogel and Meyer 2018; Aggarwal 2008). Among all these changes, the spatial extent, frequency, and duration of droughts have been increasing in most parts of the world (Wang et al. 2021b). It has a wide range of impacts on agriculture, economics, ecosystems, energy, human health, recreation, and water resources (Sugg et al. 2020). Crop production needs to be improved despite the pressing challenges of hunger and food insecurity. However, drought, one of the most critical abiotic factors, restricts crop production. The effect of drought varies with crops (cereals, legumes, root/tuber crops), growth stages (vegetative phase, reproductive phase, and flowering), and stress intensity (Daryanto et al. 2017; Queiroz et al. 2019; Zhang et al. 2018). Meta-analysis has shown that drought decreases the yield of the three essential cereals by 30%, with around 40% yield reduction at 40% water reduction (Zhang et al. 2018; Daryanto et al. 2016; Daryanto et al. 2017). A study based on the AquaCrop model quantified the sensitivity of maize losses due to drought (Fawen et al. 2022). The results indicated that the maximum aboveground biomass loss due to the impact of drought at the seedling stage, booting stage, and flowering/grain-filling stage amounted to 38.06%, 56.21%, and 11.00%, respectively.

Drought impacts maize production physiologically and morphologically. Leaf water potential decreases with water availability, restricting the expansion of leaves and the growth of plants (Hsiao and Acevedo 1975). Under severe drought, intracellular abscisic acid (ABA) contents increase, and thus the drought tolerance of the plants increases (Nagatoshi et al. 2023; Yang et al. 2021). The accumulated ABA after drought stress increases the dormancy of seeds and buds to improve the drought tolerance of plants and enhances stomatal closure, reducing water evapotranspiration (Zhao et al. 2017). When water stress is severe, it disrupts cell membrane structure, resulting in cell death (Waraich et al. 2011).

However, as drought increases, the plant first encounters problems with nutrient uptake, which is then regulated by the plant's hormone ABA (Nagatoshi et al. 2023). The uptake of nutrients by the plant via roots mainly involves three main processes: mass flow, diffusion, and root interception (McMurtrie and Näsholm 2018; Nye 1977; Flowers and Yeo 1992). The rate of transpiration influences mass flow and can reduce the probability of nutrient immobilization before reaching the root surface, which is particularly important for water-soluble nutrients (McMurtrie and Näsholm 2018). Diffusion is the rate-limiting step for the uptake of N, P, and K, especially in the growth-response concentration range (Nye 1977). Root interception involves the root intercepting ions and transporting them radially inwards (Flowers and Yeo 1992). Drought decreases soil water availability and restricts nutrient mobility and solubility, thereby presenting adverse effects on plant N, P, and K uptake (He and Dijkstra 2014; Ge et al. 2012b). Therefore, the growth and development of plants are restricted indirectly. Plant P uptake of grain sorghum was more sensitive to drought than N and K (Eck and Fanning 1961). Nagatoshi et al. (2023) reported that mild drought leads to plant P deficiency responses, reducing yield and nutrient uptake. Therefore, plants show the characteristics of nutrient deficiency, less root and shoot biomass, and reduced P uptake in the context of drought (Nagatoshi et al. 2023). Certainly, it resulted in reduced silage and grain yield (Kamara et al. 2003; Nagatoshi et al. 2023). Poudel (2023) emphasized a 70% decline in maize yield due to drought. Therefore, it is crucial to investigate the effects of drought and the mitigation measures in response to drought in maize production.

1.3 Strategies to improve P use efficiency (PUE)

To address the future depletion of P resources and associated environmental problems, crop cultivation must meet higher standards, including improved management of soil P, off-farm P inputs, and P transport processes. From the agricultural perspective, improving P use efficiency (PUE) has attracted significant attention from researchers across various fields, including bioengineering (Tian et al. 2012), biochemistry (Shen et al. 2011), plant breeding (Li et al. 2019), and botany (Wollmann et al. 2018) in recent years. The next sub-chapters will discuss potential solutions that have been developed to enhance plant PUE and enable plants to cope with P depletion and low P bioavailability.

1.3.1 Breeding

To enhance PUE in crop production, breeding for high P utilization is important (van de Wiel et al. 2016). Genotypic variation provides a great source of selecting cultivars with efficient P characteristics under specific environmental conditions; on the other hand, environmental conditions can mask the genetic variation (McKenzie and Williams 2015; Weiß et al. 2021; van de Wiel et al. 2016). Conventional breeding based on phenotypic selection is time-consuming and easily affected by environmental factors (Al-Khayri et al. 2015). Genetic engineering-related quantitative trait loci (QTL) and molecular breeding methods are proposed to introduce genes for efficient P uptake and utilization (Li et al. 2019; Miklas et al. 2006).

1.3.2 Crop rotation and intercropping

Several studies have demonstrated the potential of crop rotation and intercropping to enhance PUE. Crop rotation and intercropping, especially with legumes, are reported to be very efficient in improving PUE due to their differences in morphological and physiological responses to P deficiency in different crops (Pypers et al. 2007; Zhang et al. 2016). Organic acids secreted by crops relying on physiological responses to P deficiency, e.g., legumes, favor the release and mobilization of P from soil and make it available for uptake by crops such as maize, which depends on root morphology responses. Tang et al. (2021) reported that intercropping legumes and cereals substantially increased phosphorus uptake and reduced the need for phosphorus fertilizer.

1.3.3 Soil amendments

Soil amendments can be added to alter soil chemical and physical properties, serving as a sustainable option to enhance nutrient availability (Waddington 1992; Opala 2017). The addition of natural soil amendments, grouping organic, organic-mineral, and mineral amendments, can benefit plant growth and crop production (Garbowski et al. 2023). It provides the material basis for the mineralization of plant nutrients (e.g., N, P, and K). A meta-analysis revealed that adding a biochar soil amendment of 10 t ha^{-1} significantly enhanced soil available P and plant P uptake (Tesfaye et al. 2021). This improvement was attributed to the enhancement of soil conditions, which promote the growth of plant roots and increased soil microbial abundance. However, in alkaline soils, it is hard to exclude the possibility that the acidification of biochar before the application is the pre-requisite for improving P availability due to the difference in soil Olsen-P and plant growth by the acidified and non-acidified biochar in the post-experiment analysis (Qayyum et al. 2021). Therefore, various amendments are studied for P management in different soils (initial soil pH). The addition of soil amendments increases soil P availability due to changes in soil pH and P diffusion in the amendments, e.g., biochar and fly ash, in acidic soils (Hong et al. 2018).

1.3.4 Soil microbiome management

Many researchers reported that phosphate-solubilizing bacteria (PSB), which help to release P, can convert soil P into forms that plants can more easily take up. With PSB, the agronomic efficiency of RP can be increased, and P fertilizer efficiency can also be improved (Elhaissoufi et al. 2022). Abbasi et al. (2015) stated that the combined use of RP, poultry manure, and phosphate-solubilizing bacteria resulted in the same yield and P uptake of chili as DAP. Compared with DAP, RP inoculated with phosphate-solubilizing bacteria increased plant-available P, resulting in higher yield and P uptake in maize and wheat cropping systems (Kaur and Reddy 2015). Therefore, the losses during processing more soluble P fertilizers can be reduced or omitted.

1.3.5 Fertilizer management

Fertilizer management, as a big part of the crop production system, has been investigated intensively (Young et al. 2021; Nkebiwe et al. 2016). A 4Rs nutrient stewardship is proposed, where the application of balanced nutrients must be adapted with the **Right** product, at the **Right** rate, in the **Right** place, and at the **Right** time to improve nutrient use efficiency and reduce losses (Norton 2014). Slow-release or controlled-release fertilizers were introduced to crop production for a steady supply of P over time (Trenkel 2010). The application of slow-release/controlled-release P fertilizers benefit two main processes: 1) the nutrient availability in the system of plant and soil influenced by the interaction/competition among plant roots, soil microorganisms, and chemical reactions; 2) the release of nutrients matching with the plant needs (Shaviv and Mikkelsen 1993). The release kinetic models and mechanisms of slow-release/controlled-release fertilizers have been investigated (Lakshani et al. 2023). Slow-release P fertilizers could increase P availability for the short, mid, and long-term, and promote seed germination and growth (Glaser and Lehr 2019; Wang et al. 2021a). Plants can access nutrients at the right time, and the loss of nutrients and environmental degradation can be minimized.

Besides fertilizing with slow-release fertilizers, fertilizer placement, which refers to the precise application of fertilizers close to seeds or plant roots, might ensure a high plant availability of P (Nkebiwe et al. 2016). It is recommended as an efficient fertilizer alternative and can lead to higher PUE and yield, especially in nutrient-deficient soils, providing more concentrated nutrients for plants (Randall and Hoeft 1988; Nkebiwe et al. 2016). Fertilizer placement can increase the spatial distribution of root dry biomass and the acquisition of nutrients. Fertilizers placed near the seeds at appropriate depths prevent soil fixation and enhance early growth (Prummel 1957). As suggested by Szulc et al. (2020) and Nkebiwe et al. (2016), deep fertilizer placement has emerged as a promising agricultural practice to enhance nutrient use efficiency and crop yield.

1.4 Research gaps in improving PUE of maize production under low-P conditions and drought

Although researches and improvements have been made to increase the efficiency of P fertilizer use and reduce P losses to the environment, there are still some limitations in maize production to cope with limited resources and low plant-available soil P.

1.4.1 Cultivars selection

Maize cultivars can be classified into three categories: high-yielding P-utilizers, low-yielding P-utilizers independent of P supply, and P-sensitive utilizers (Weiß et al. 2021). Weiß et al. (2021) suggested that in a multi-location analysis, maize cultivars with high PUE can be selected without considering the P level of the soil in rich to very rich P soils. A similar result was reported with around 300 genotypes of maize, where P starter fertilizer affected plant growth and development in the early stage but did not affect grain yield at harvest in lower soil-P conditions (Roller et al. 2022). Breeding for high P utilizers can be an approach to reduce the amount of P fertilizer application. Maize production is affected by many factors, e.g., genotype, environment, and the interaction of genotype and environment (Simmonds 1981; Weiß et al. 2021). As is well known, modern maize cultivars have been selected in high-P soils due to the long history of fertilization and the presence of high residual soil P (Li et al. 2021b). Modern maize cultivars slowly lost the colonization or the changes of root organic acid anions released (Li et al. 2021b). Therefore, breeding cultivars aimed at coping with low-P environments is very critical.

1.4.2 Soil conditions

Many factors, such as soil microorganisms (Richardson 2001), soil pH (Hinsinger 2001), and soil moisture, affect the plant availability of P. Soil pH is one of the critical factors affecting P release from soil and the characteristics of P uptake by plant roots (Barrow and Hartemink 2023). P is least fixed in soil when the soil pH is around 6.5, while P fertilizer for plants is most effective at near pH 5 due to the release of the most efficient P form, $H_2PO_4^-$ (Price 2006; Hinsinger 2001). Organic acids exuded by roots play an essential role in contributing to P release in response to P deficiency (Liu 2021). It is known that the responses of maize to P deficiency depend on root morphological changes rather than physiological changes (Wen et al. 2017). The roots of maize plants under P deficiency present more fine roots and higher root length and RLD. However, all the approaches to enhancing P uptake by plants mentioned above involve soil initial pH and the modified soil pH with organic acids (Dzotsi et al., 2010). Therefore, understanding root-soil interactions under different soil pH could address the missing point regarding the mechanisms of root exudation, such as organic acids and phytohormones influencing P availability and uptake by maize plants under low-P soil conditions and the links between root exudates and root system architecture systematically.

1.4.3 Fertilizer placement

Fertilizer placement is referred to as the third R in the 4Rs nutrient stewardship: fertilizing in the Right place (Norton 2014). It could orientate to regulate plant root growth and development, and therefore, the way that plant roots exploit P from soil changed (Zhang et al. 2023). It is proposed that P fertilizer applied in bands is about 2.9 times as effective as broadcasting, 2.5 times for cereals, and the efficiency of N placed is around 1.2 for cereals, potatoes, and beets (Prummel 1957). Localized application of both P and N fertilizer significantly enhances maize growth and development compared with localized P application with N broadcast or both N and P broadcast, though stimulating root proliferation and acidifying the rhizosphere (Jing et al. 2010). Localized P fertilizer could significantly promote maize plant growth and P uptake (Wang et al. 2024). It is also proposed to be an approach to mitigate the negative consequences of increasingly frequent high temperatures and droughts that threaten food production globally.

Mass flow accounts for around 1-5% of a plant's P demand, while the amount intercepted by roots is only half of that (Suriyagoda et al. 2014). The remaining required P is absorbed only when reaching the root surface via diffusion, governed by soil water conditions (Suriyagoda et al. 2014). Therefore, plants could be sensitive to capture P deficiency caused by drought. The impact is not the most significant at the seedling stage of maize, however, this is the period most likely to be affected by the dual stress of P deficiency and drought (Fawen et al. 2022). Waraich et al. (2011) stated that drought tolerance could increase with the increase of P fertilizer, while based on the background of P depletion and loss, it is quite a tricky question. It is, therefore, important to investigate the response of maize seedlings to the dual stress and the potential of fertilizer placement to optimize the growth of maize under the dual stress.

1.5 Aims and Objectives

This doctoral study was performed with the support of the Sino-German International Research Training Group "Adaptation of maize-based food-feed-energy systems to limited phosphate resources" (Amaize-P project) and the China Scholarship Council (CSC). The response of different maize cultivars (Stabil and Ricardinio (both from KWS SAAT SE & Co. KGaA, Einbeck, Germany)) to P fertilizer type (RP and DAP) and soil liming (lime and no lime) was tested in a 2yr field trials. Furthermore, the impact of drought (drought stress: DS, 45% of soil water holding capacity (WHC) and well-watered: WW, 75% WHC) and fertilizer placement (mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm)) were investigated in a greenhouse experiment to evaluate how future maize production could be adapted to P depletion and drought. The effects of P deficiency on morphological and physiological changes in maize growth and development and the potential of fertilizer placement for optimizing maize plants under dual stress were investigated. The dissertation focused on the following objectives:

1. To evaluate the effects of P-fertilizer types (DAP and RP with stabilized NH_4-N) and soil liming on growth and development, P uptake, and silage yield of two maize cultivars under P-limiting field conditions.
2. To explore the effects of P-fertilizer type and soil liming on maize root morphology and physiology to delineate a potential ideal maize root system to cope with P deficiency under different soil conditions.
3. To investigate the effects of combined P-deficiency and drought stress on root growth, P uptake, and shoot development in the juvenile phase of maize.

4. To determine the impact of fertilizer placement under the dual stress of P deficiency and drought.

To fulfill the stated objectives, a two-year field trial was conducted from 2020 to 2021 at the research station ‘Oberer Lindenhof’ of Hohenheim University (Eningen unter Achalm, 72813, Germany), located in southwestern Germany. The objective was to evaluate how the shoot and root of maize plants responded to different P fertilizer types and soil pH conditions. Additionally, a greenhouse trial was carried out at the Phytotechnikum research greenhouse at the University of Hohenheim (Stuttgart, 70599, Germany), investigating the factors of drought and fertilizer placement.

The corresponding papers in chapters I, II, and III of the present dissertation provide detailed information regarding all trials.

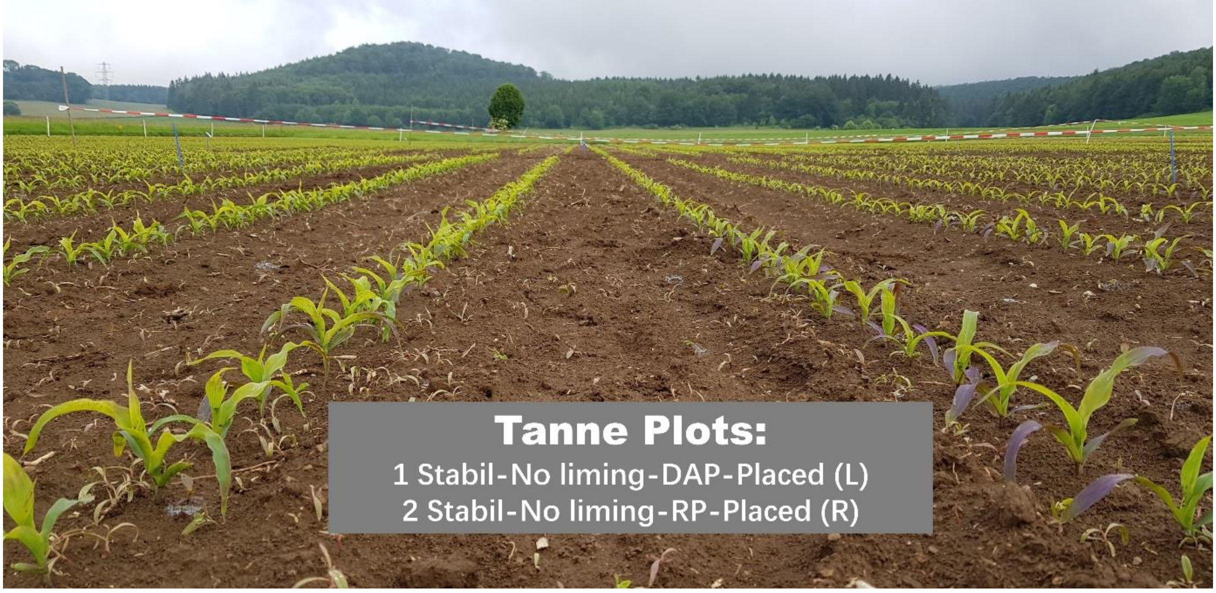

Figure 1 Maize plants with RP (left) and DAP (right) of Stabil without soil liming in the field “Tanne”.

Publication I presents the response of maize plants to different P fertilizer types (no P fertilizer as control (No-P), rock phosphate (RP), diammonium phosphate (DAP)) and different soil conditions (no liming, with liming) (Fig. 1.1). The tested cultivars, Stabil (KWS) and Ricardinio (KWS), differed in P utilization, which is an important factor in investigating the improvement of PUE. To explain the plant response, canopy characteristics, e.g., leaf area index (LAI) and light interception of the canopy, were measured, and the growth of plants and P uptake was observed at the six-leaf stage and silage stage. The results evaluated the possibility of replacing the more costly P fertilizer (DAP) with RP under different soil conditions, and different maize cultivars differ in terms of P use efficiency.

Publication II shows how the root system characteristics of the two maize cultivars were affected by different P fertilizer types and soil liming conditions. Even though plant roots show less detectable responses to the availability of P, the traits of the root system, as a mediator between plants and soil, in morphological and physiological aspects play a crucial role in P acquisition by plants. Therefore, root biomass, root length, and root exudates affected by the three factors were determined in this paper.

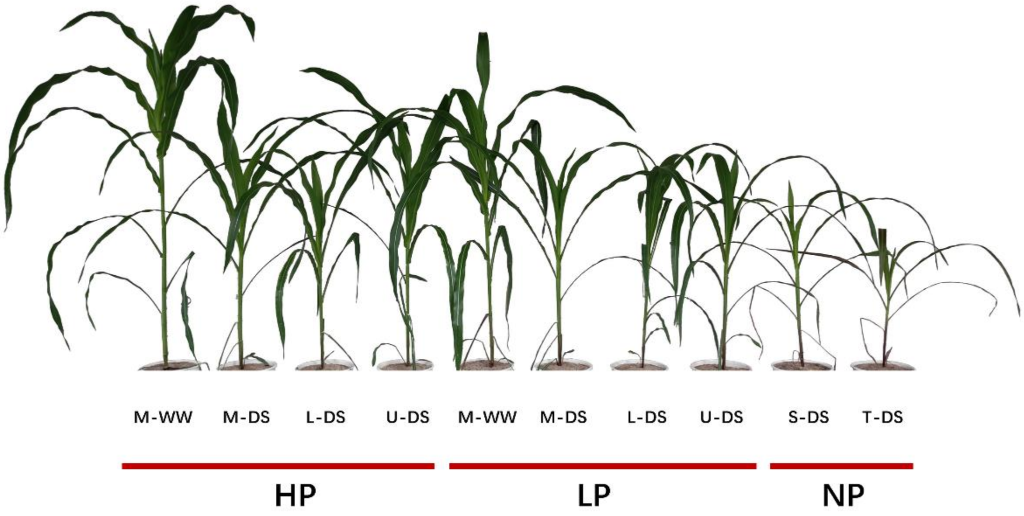

Figure 2 Schematic plant appearance in the greenhouse trial for different treatments with different P fertilizer doses and fertilizer placement at the ten-leaf stage. P fertilizer dose: No-P (NP), Low-P (LP), and High-P (HP), respectively; Fertilizer placement: mixed (M), lower (L), and upper (U) fertilizer, respectively; Water content: Drought stress (DS) and Well-watered (WW). The two factors, fertilizer placement and water content, are combined with "-."

Publication III demonstrates the response of early maize growth to different fertilizer placements and P fertilizer doses under drought stress (Fig. 1.2). Drought stress, as a more frequent natural event, can exacerbate P deficiency due to the limits of diffusion and mass flow. This study aimed to evaluate the performance of maize plants under different fertilizer placements and the dual stress of P deficiency and drought. Plant root and shoot growth and development were assessed.

2 Publications

The cumulative thesis is composed of three articles that have been published or submitted to peer-reviewed journals. To check the three articles that correspond to publications I-III in the present thesis, please use the references given below for citation.

Publication I (published, Impact Factor 3.3 2024):

Ning, F., Nkebiwe, P.M., Hartung, J., Munz, S., Huang, S., Zhou, S. and Graeff-Hönninger, S., 2023. Phosphate fertilizer type and liming affect the growth and phosphorus uptake of two maize cultivars. Agriculture, 13(9), p.1771.

DOI: 10.3390/agriculture13091771

Publication II (submitted in Journal of Plant Nutrition, Impact Factor 1.6 2024):

Ning, F., Nkebiwe, P.M., Neumann, G., Munz, S., Hartung, J., Zhou, S. and Graeff-Hönninger, S., 2024. Changes in root morphology and rhizosphere metabolites in field-grown maize with different sources of phosphorus supply.

Publication III (submitted in Journal of Plant Nutrition and Soil Science, Impact Factor 2.6 2024):

Ning, F., Nkebiwe, P.M., Munz, S., Hartung, J., Zhang, P., Huang, S. and Graeff-Hönninger, S., 2024. Effect of P fertilizer placement and amount on early growth of maize (*Zea mays* L.) under drought stress.

3 Publication I: Phosphate fertilizer type and liming affect the growth and phosphorus uptake of two maize cultivars

Publication I (published, Impact Factor 3.3):

Ning, F., Nkebiwe, P.M., Hartung, J., Munz, S., Huang, S., Zhou, S. and Graeff-Hönninger, S., 2023. Phosphate fertilizer type and liming affect the growth and phosphorus uptake of two maize cultivars. Agriculture, 13(9), p.1771.

P is an essential, non-renewable macronutrient in crop production. It is projected that P will be exhausted in the next 300-400 years. The demand for P is still increasing, and P use efficiency is very low in crop production. The residues of P in soil cause severe environmental issues, such as the eutrophication of water bodies. To examine the possibility of replacing DAP with less processed (lower cost) RP, the effects of P fertilizer type and soil liming on growth and development, P uptake, and silage yield of two maize cultivars were evaluated in publication I. Publication I focuses on plant traits, shoot development, and P uptake affected by P deficiency due to different P fertilizer types and soil liming conditions.

agriculture

MDPI

Article

Phosphate Fertilizer Type and Liming Affect the Growth and Phosphorus Uptake of Two Maize Cultivars

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Abstract: In the context of phosphorus (P) exhaustion and low P use efficiency (PUE) in crop production, a field trial was designed on a low-P soil in southwestern Germany in 2020 and 2021 to investigate the effects of P fertilizer type and liming on maize growth and P uptake and PUE. The experimental factors were (i) two P fertilizer types, rock phosphate (RP) and diammonium phosphate (DAP); (ii) lime application, lime and no lime; and (iii) two maize cultivars. The results showed that RP resulted in a lower leaf area index and light interception compared with DAP, a 33% lower silage yield, and a 29% lower P content at harvest. The PUE of RP was 18%, which was 37% lower than DAP. Soil liming reduced shoot biomass and led to 35% less shoot P content at the six-leaf stage. The maize cultivar Stabil expressed higher yielding and P acquisition characteristics. In conclusion, DAP cannot be replaced by placed RP, regardless of the lime application in silage maize production in this study. Future research on the PUE of maize cultivars should also consider root characteristics in response to P fertilizer type and soil pH.

Keywords: diammonium phosphate; rock phosphate; pH; leaf area index; biomass accumulation; phosphorus-use efficiency

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1. Introduction

Phosphorus (P) is an essential macronutrient for plant growth which is non-substitutable and non-renewable. However, P is characterized by low bioavailability, and most P is fixed in soils; thus, the P use efficiency (PUE) of plants is still quite low. and P plays a limiting role in plant growth in most cropping systems [1–3]. Additionally, it is estimated that the reserves of rock phosphate (RP) from which P is extracted for fertilizer production will be exhausted during the next 300 to 400 years [4,5].

In crop production, the physical and chemical properties of the applied P-fertilizers are important factors for solubility and thus plant availability. Owing to differences in water solubility, the crop PUE of RP is low, whereas that of diammonium phosphate (DAP) is high [6–8]. During the process from the mining of RP to the production of P-rich fertilizers like DAP, around 16% of the initial P is lost as monoammonium phosphate to the environment [9]. Fertilizing with DAP may be desirable due to its high PUE and thus its potential for higher crop yields, but it may be less attractive in comparison to RP due to the larger environmental footprint associated with its use [8]. Therefore, a cropping system designed to improve the PUE of RP will not only save costs associated with a more expensive water-soluble P fertilizer like DAP, but in addition will reduce P losses in the production chain, thus reducing the environmental impact of crop production. Localized P fertilizer application could effectively enhance the effect of fertilizer on plant growth, resulting in root proliferation and higher yields of maize [10–13].

Soil pH is another factor that influences the bioavailability of soil P for plant growth, and therefore the PUE of fertilizers. It is called the "master soil variable" because it influences a large number of soil biological, chemical, and physical properties and processes, which affect the bioavailability of P from soil, and thus plant growth and biomass yield [14]. Soil liming has been used in many studies to modify soil pH, and thus plant P availability and crop P uptake [15–17]. The influence of soil pH on soil plant-available P is mainly expressed as changes in the ratio of $H_2PO_4{}^-$ to $HPO_4{}^{2-}$ in soil. The uptake rate for $HPO_4{}^{2-}$ was around one-tenth of $H_2PO_4{}^-$ when soil pH varied from 4.7 to 8.3 [18]. On the other hand, P uptake by plant roots increases with the decrease in soil pH, and is most effective at pH close to 5, where $H_2PO_4{}^-$ dominates in soil [19,20]. The utilization of sparingly soluble phosphate, e.g., rock phosphate (RP), can be improved by a soil pH of 6 or lower [15,17,19]. However, the P uptake of applied DAP is better in neutral soil (pH 7.2) than acidic soil (pH 5.0) [20]. Therefore, adjusting soil pH via the application of lime may be a potential strategy to alter the plant-availability of soil P and affect the utilization of RP for maize growth [17,18,21,22].

Maize (*Zea mays* L.) is sensitive to low plant-available soil P levels. The growth and development processes of maize can be irreversibly limited under conditions of P deficiency, especially in the early growth stages, e.g., reduced leaf area expansion, and thus lower light interception and biomass accumulation [1,23,24]. As a consequence of maize breeding over the last few decades, the roots of modern maize genotypes exude lower amounts of beneficial organic anions to mobilize soil P under limited P supply than older genotypes, resulting in lower PUE [25,26]. Several studies have shown that variations in PUE exist between cultivars, resulting from differences in root systems, biomass accumulation, and tolerance to soil P deficiency [25,27,28]. Weiß et al. [29] investigated the interaction between maize cultivars and starter-P fertilizer in a multi-location field study, and showed that the best-performing cultivar can be identified irrespective of the presence of starter-P fertilizer. Different cultivars were grouped in different categories: high yielding P utilizers, low yielding cultivars independent of P-supply, and P-sensitive cultivars. Therefore, selection of efficient P utilizers plays an important role in improving maize yields under moderate to low soil P conditions [29,30].

Therefore, this study investigated the impact of two P fertilizer types, DAP and RP, in combination with soil liming on the growth, P uptake, and silage yield of two maize cultivars in a low-P soil.

2. Materials and Methods

2.1. Experimental Site

The field experiments were conducted in southwestern Germany at the research station Oberer Lindenhof (48°28′26″ N, 9°18′12″ E) of the University of Hohenheim in 2020 and 2021. The site was selected due to its very low soil plant-available P level, defined as 0.9 mg P per 100 g^{-1} soil dry matter (DM) in the top 0–30 cm (using the calcium-acetate-lactate method (CAL)), which corresponds to the lowest class "A" (very poor), according to the classification system used in Germany [31]. During the growing season, the mean temperature and total precipitation were 14.6 °C and 356 mm in 2020 and 13.8 °C and 461 mm in 2021, respectively. The weather in 2021 in the first period was characterized by higher temperatures, and frequent and more precipitation than in 2020 (Figure 1).

The field experiments were set up in both years on the same field. The field was split into two halves, one for each year. Soil samples at three depths (0–30 cm, 30–60 cm, and 60–90 cm) were collected from the field on 8 April 2020 and 10 May 2021, and the soil properties were analyzed each year before sowing (Table 1). According to a soil texture analysis of the entire field, the soil was categorized as silty clay loam with a bulk density of 1.3–1.4 g cm^{-3}. The average soil pH in 0–30 cm depth for both years was 5. In the two years, mineral nitrogen contents (N_{min}) from 0–90 cm depth were 66 and 79 kg N ha^{-1}, respectively. Plant-available P (measured as CAL-P) was consistently within the lowest class "A".

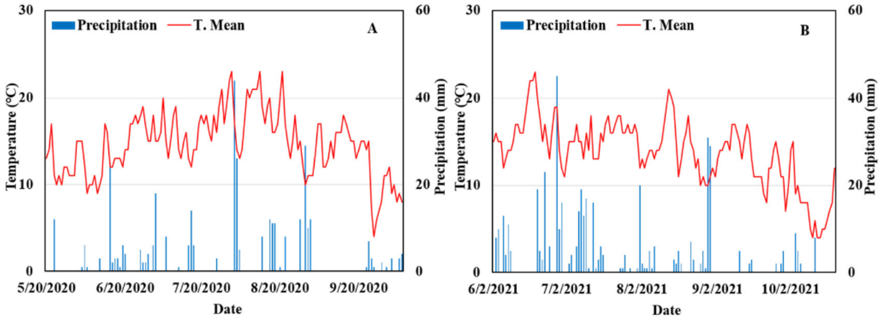

Figure 1. Daily precipitation (blue bars) and mean daily temperatures (red line) during the experimental period in 2020 (**A**) and 2021 (**B**).

Table 1. Soil properties.

Year	**Depth** cm	**Clay** %	**Silt** %	**Sand** %	**Bulk Density** g cm^{-3}	**pH**	N_{min} kg N ha^{-1}
2020	0–30	32.6	60.9	6.5	1.3	5.3	23.4
	30–60	35.3	59.3	5.4	1.3	5.5	13.1
	60–90	34.9	54.5	10.6	1.4	6.0	29.9
2021	0–30	-	-	-	-	4.7	23.9
	30–60	-	-	-	-	4.8	21.3
	60–90	-	-	-	-	4.3	33.4

2.2. *Field Experiments*

In 2020, the field trial was a 2 × 2 × 2 factorial design of eight treatments tested in four replicates. The experimental factors were (i) P fertilizer type, rock phosphate (RP, Dolophos® 26; NATURKALK, Barbing, Germany) and diammonium phosphate (DAP, 18% N–20% P, DAP; Van de Reijt Meststoffen B.V., Moerdijk, The Netherlands); (ii) maize cultivar (*Zea mays* L. cv. Stabil (high-yielding P utilizer) and Ricardinio (P-sensitive cultivar) (both from KWS SAAT SE & Co. KGaA, Einbeck, Germany); and (iii) liming, no lime application and 16,800 kg $CaCO_3$ ha^{-1} lime application (75% $CaCO_3$ and 15% $MgCO_3$, Kohlensaurer Magnesiumkalk 90; Zement- und Kalkwerke Otterbein GmbH & Co. KG, Grossenlüder-Müs, Germany). The two cultivars were chosen due to their difference in terms of yield response to P fertilizer and P uptake [29]. The change in soil pH from 0–10 cm was 4.9 for no lime treatment and 6.0 for lime treatment at the six-leaf stage. Treatments were allocated to plots according to a design comparable to a split-plot design, where the combination of lime application and maize cultivar was used as the main plot and the P fertilizer type as subplot factors. In contrast to a common split-plot design, wherein main plots are randomized according to a randomized complete block design, the main plot factor combinations were randomized as a Latin square design. The 16 main-plots were arranged in a 4 × 4 grid. Each main plot is split into two plots. In total, there were 32 plots each, with an area of 66 m^2 (11 m length × 6 m width) (Figure S1).

In 2021, control plots with no P fertilizer were included as another level of the factor P fertilizer type, giving a total of 48 plots. A total of six main plots were arranged in a randomized complete block design with complete blocks within a row. Due to space limitation, the area of the control sub-plots was 33 m^2 (11 m length × 3 m width), whereas the area of the other sub-plots was kept the same as in 2020, at 66 m^2 (Figure S2).

The application amount of P fertilizer was 75 kg P ha^{-1} in both years. RP was co-placed with stabilized ammonium sulfate fertilizer (21% N, NovaTec® Solub 21; Compo Expert, Münster, Germany) to achieve the same N quantity (68 kg N ha^{-1}) as is present in DAP. For the DAP treatment, N and P were applied as DAP. DAP and RP + stabilized NH_4-N were each locally placed at 5 cm to the side and 7 cm below the maize seed upon sowing, using a single seed sowing machine equipped for fertilizer placement (Unisem; RAU Landmaschinen GmbH, Korntal-Münchingen, Germany). In 2020, a 46 kg N ha^{-1} area was treated with urea stabilized with a urease inhibitor (46% N, Alzon® neo-N; SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany), and 34 kg N ha^{-1} in 2021, after accounting for soil N_{min} (Table 1), in order to meet a total N supply of 180 kg N ha^{-1} over the whole maize growing season in each year.

After broadcast and incorporation (5 cm depth) of the stabilized urea fertilizer, maize was sown on the same day at 3 cm depth on 20 May 2020 and 2 June 2021 (incl. placement of fertilizer). The sowing density in both years was 100,000 plants ha^{-1}. The inter-row distance was 0.75 m. Silage maize was harvested at 141 days after sowing (DAS) on 7 October 2020 and 20 October 2021.

2.3. Data Collection

Three representative plants were selected from the middle rows of each plot on a bi-weekly basis after sowing in 2020 for the determination of *plant leaf area*. On the first three dates, *plant leaf area* was calculated for each plant as the sum of the leaf area of all leaves (n), according to the following:

$$Plant\ leaf\ area = \sum_{i=1}^{n} (leaf\ length_i \times maximum\ leaf\ width_i) \times 0.75, \quad (1)$$

where i refers to each single leaf of a plant.

During later samplings, leaf area was measured using a Leaf Area Meter (LI-3100; LI-COR, Lincoln, NE, USA). Leaf area index (LAI) was calculated by multiplying the plant density m^{-2} with the *plant leaf area* [32].

At the six-leaf stage (48 DAS in 2020 and 43 DAS in 2021), three selected plants of each plot were cut and dried at 60 °C (VTU 125/200; Vötsch, Borken, Germany) to a constant weight for plant dry shoot biomass. At harvest (141 DAS in 2020 and 2021), a border strip of 1 m width was discarded, all plants from the two adjacent rows in the center of each plot were harvested, and fresh silage biomass was determined using a plot combine harvester (Silager SF2000; Baural, Blois, France). A subsample of around 3000 g fresh silage biomass was weighed and also dried to a constant weight at 60 °C. The silage yield of each treatment was determined using the dry biomass concentration and total fresh silage biomass.

All dried samples were ground using a cutting mill equipped with a 0.5 mm sieve (SM 200; Retsch, Haan, Germany). After microwave digestion, the biomass P concentration was measured via inductively coupled plasma-optical emission spectrometry (5110 ICP-OES; Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). Plant P content was calculated using the P concentration and plant aboveground dry biomass.

The fraction of midday photosynthetically active radiation (PAR) intercepted by the maize canopy was measured with a ceptometer (AccuPAR LP 80; Meter Group Inc., Pullman, WA, USA) to be 65, 93, and 118 DAS in 2020, and 99 DAS in 2021. The ceptometer was placed perpendicular to the row within the center of the plots recording the mean value of PAR at four different positions. The percentage of PAR intercepted by the canopy was calculated for each plot using the formula below [33]:

$$I = [(I_0 - R - T)/I_0] \times 100 \quad (2)$$

where I is the intercepted PAR (%), I_0 the incident PAR (μmol m^{-2} s^{-1}) about 20 cm above the plant canopy, R is the reflected PAR (μmol m^{-2} s^{-1}) about 20 cm above the canopy with the sensor area facing downwards, and T is the transmitted PAR (μmol m^{-2} s^{-1}) measured below the plants at the soil surface.

2.4. Statistical Analysis

Data from each year were statistically analyzed according to the experimental design (both field plans are given in Figures S1 and S2). Both trials were randomized as split-plot designs with main plots randomized as a Latin square design in 2020 and as an RCBD in 2021. Both trials include the same three treatment factors. Data were analyzed using a mixed-model approach in SAS v9.4 (SAS Institute, Cary, NC, USA). For 2020, the model is described as follows:

$$y_{hijklm} = \mu + r_i + c_j + m_{ij} + \alpha_k + \beta_l + \gamma_m + (\alpha\beta)_{kl} + (\alpha\gamma)_{km} + (\beta\gamma)_{lm} + (\alpha\beta\gamma)_{klm} + e_{hijklm}, \quad (3)$$

where r_i, c_j, and m_{ij} represent the design and α_k, β_l, and γ_m represent the three treatment factors. In detail, y_{hijklm} is the observation of plot h in the ith row and jth column treated with the kth level of maize cultivar, the lth level of lime application and mth level of P fertilizer, μ is the intercept, and r_i and c_j are the fixed effects of the ith complete row and jth column. The term m_{ij} is the random main plot effect associated with the area of two plots in a combination of row and column, α_k, β_l, and γ_m are the fixed main effects of the kth maize cultivar, the lth lime application level and mth P fertilizer, respectively, $(\alpha\beta)_{kl}$, $(\alpha\gamma)_{km}$, $(\beta\gamma)_{lm}$, and $(\alpha\beta\gamma)_{klm}$ are the fixed two- and three-way interaction effects of the corresponding factors involved, and e_{hijklm} is the plot error of y_{hijklm}. In 2021, the experiment was slightly modified. First, two main plots were added per row as control. The main plot factor was now either a lime application level-by-P fertilizer combination or a lime application level-by-maize cultivar combination. The latter allowed us to test maize cultivar-by-lime application level combinations additionally under the no-fertilizer control treatment. Additionally, PUE can be calculated from the analysis of total P content from the treatment with P application. As the P fertilizer amount was constant at a rate of 75 kg P ha^{-1}, the treatment-to-control difference in total P harvested divided by 75 can be interpreted as the PUE. Second, as control plots were sown with four rows instead of eight rows, data were weighted by the number of rows per plot. The model is similar to [3], but dropping the fixed-column effect and adding a weight statement.

For both statistical analyses, residuals were checked graphically for normal distribution and homogeneous variances. Afterwards, global F tests were performed to test for differences between the levels of main effects and their interaction effects. In cases of significant differences, a Fisher's LSD test was performed at $\alpha = 0.05$. The results of the LSD test were presented using a letter display [34]. Additionally, simple means for cultivar-by-lime application-by-P fertilizer combinations were calculated for presentation purposes (Tables S1 and S2 in the Supplementary Materials).

3. Results

3.1. Leaf Area Index and Light Interception

During 2020, leaf area index (LAI) was mainly influenced by P fertilizer (Table S3). From 34 DAS onwards, maize plants supplied with DAP had a higher LAI than those supplied with RP (Figure 2A). Fertilization with DAP resulted in a 30% higher maximum LAI (4.2) compared with RP plots (3.2).

In 2020 and 2021, P fertilizer significantly influenced intercepted midday photosynthetically active radiation (PAR) measured at different growth stages (Table S4). At 65, 93, and 118 DAS in 2020, the intercepted PAR for DAP was 45%, 89%, and 93%, compared with 28%, 77%, and 89% for RP, respectively (Figure 2B). Therefore, DAP contributed to 60%, 17%, and 4% higher intercepted PAR at 65, 93, and 118 DAS. In 2021, the intercepted PAR in the DAP treatment was significantly higher, with 93%, compared to similar values of No-P and RP with around 84%, as measured at 99 DAS. No significant difference in intercepted PAR was found between No-P and RP.

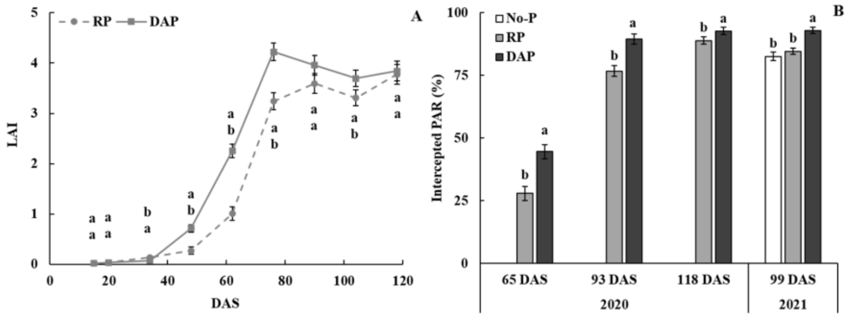

Figure 2. Effect of P fertilizer on LAI (**A**) in 2020 and intercepted PAR (%) (**B**) in 2021 at different days after sowing (DAS). No-P, no P fertilizer; RP, rock phosphate; DAP, diammonium phosphate. For each DAS, bars headed by at least one identical letter did not differ significantly according to Fisher's LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

3.2. Biomass Accumulation

The factor P fertilizer was significant for shoot biomass accumulation at the six-leaf stage and the harvest of silage maize in 2020 (Table 2). In 2021, the cultivar factors and the interaction of P fertilizer and lime application were significant.

Table 2. ANOVA for the effects of P fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on shoot biomass at the six-leaf stage and silage yield at harvest in 2020 and 2021. ns: not significant at $\alpha = 0.05$.

Trait	**Shoot Biomass at Six-Leaf Stage**		**Silage Yield at Harvest**	
Unit	**kg DM ha^{-1}**			
Year	**2020**	**2021**	**2020**	**2021**
Pf	<0.001	<0.001	<0.001	<0.001
Cul	ns	0.035	ns	<0.001
Lime	ns	0.002	ns	<0.001
Pf × Cul	ns	ns	ns	ns
Pf × Lime	ns	0.033	ns	0.002
Cul × Lime	ns	ns	ns	ns
Pf × Cul × Lime	ns	ns	ns	ns

In 2020, shoot biomass at the six-leaf stage was 2.4 times higher in plots fertilized with DAP compared with RP (Figure 3A). Results were in general similar in 2021; however, within the DAP treatments, shoot biomass was 37% higher without liming compared with the treatment with lime application (Figure 3B). Shoot biomass was around two times higher in plots with No-P*No lime compared with RP and No-P*Lime, both being lower than treatments fertilized with DAP. Significant differences were found between cultivars, with a 30% higher shoot biomass for Stabil compared with Ricardinio across treatments (Figure 3C).

Application of DAP led to a 38% higher silage yield compared with the application of RP in 2020 (Figure 3D). These results were confirmed in 2021 with a higher silage yield with application of DAP in comparison to plots of RP and also No-P (Figure 3E). Lime application resulted in a 15% lower silage yield, indicating the influence of the change in soil pH. Among the combinations of P fertilizer and lime application, No-P*No lime showed a 22% higher silage yield than RP and No-P*Lime, all being lower compared with the application of DAP. At harvest, Stabil produced a 21% higher silage yield than Ricardinio (Figure 3F).

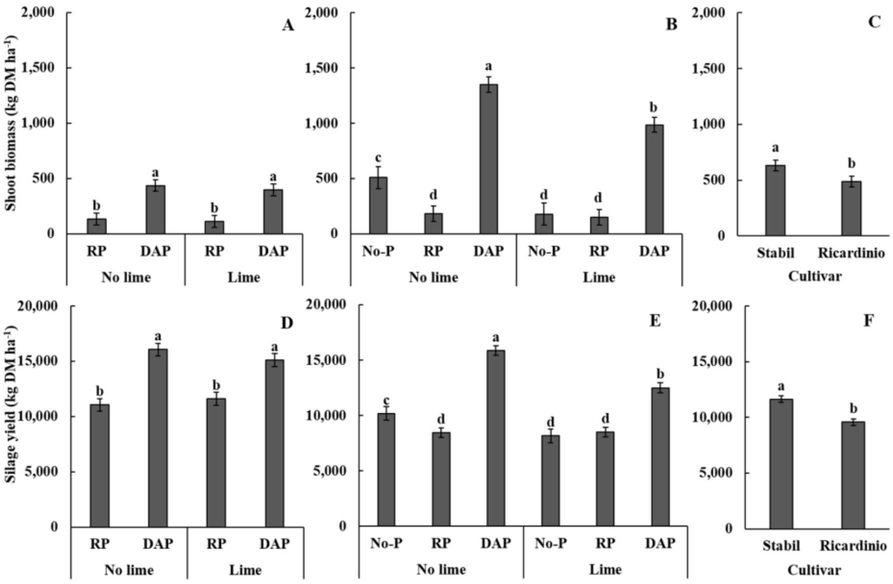

Figure 3. Effects of P fertilizer (No-P, RP, DAP), cultivar (Stabil, Ricardinio), and lime application (No lime, Lime) on shoot biomass at the six-leaf stage in 2020 (**A**) and 2021 (**B**,**C**) and silage yield at harvest in 2020 (**D**) and 2021 (**E**,**F**). No-P, no P fertilizer; RP, rock phosphate; DAP, diammonium phosphate; with (Lime) and without (No lime) lime application. Bars headed by at least one identical letter did not differ significantly according to Fisher's LSD test, $p < 0.05$. In case of non-significant interactions, letters from marginal mean comparisons were presented. Error bars indicate the standard error of the least-squares means.

3.3. P Concentration, P Content, and PUE

In both years, P fertilizer significantly affected shoot P concentration at the six-leaf stage, but not at harvest (Table 3). At the six-leaf stage, shoot P content was influenced by P fertilizer in 2020, and by the interaction of P fertilizer*lime application in 2021, respectively. At harvest in 2020, the interactions of P fertilizer*cultivar and P fertilizer*lime application were significant for shoot P content, whereas P fertilizer and cultivar were significant in 2021. At the six-leaf stage, PUE in 2021 was affected by the interaction of P fertilizer*cultivar*lime application, and P fertilizer and cultivar were significant for PUE at harvest.

The application of DAP resulted in a significantly higher shoot P concentration of 3.37 g P kg^{-1} in comparison with 2.53 g P kg^{-1} for RP in 2020, and 4.11 g P kg^{-1} compared with 2.15 g P kg^{-1} for No-P and 2.05 g P kg^{-1} for RP in 2021, respectively (Table 4).

The application of DAP resulted in higher shoot P content at the six-leaf stage, compared to No-P and RP, while No-P applied with No-lime caused a 2.5 times higher shoot P content than the RP and No-P*Lime plots (Figure 4A). At harvest, shoot P content was around 61% higher with the application of DAP compared with No-P and RP (Figure 4B). The cultivar Stabil had a 18% higher shoot P content than Ricardinio (Figure 4C).

PUE analyzed for the year 2021 showed a higher PUE with the application of DAP (5.2–7.2%) compared with RP (0.4–0.5%) at the six-leaf stage (Figure 5A). At harvest in 2021, the application of DAP resulted in a PUE of 29.4%, which was 60% higher than that found when applying RP (Figure 5B). The cultivar Stabil led to a 16% higher PUE (23.8%), compared with Ricardinio (Figure 5C).

Table 3. ANOVA for the effects of P fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on plant shoot P concentration, and P content and PUE at the six-leaf stage and harvest in 2020 and 2021. ns: not significant at α = 0.05.

	Six-Leaf Stage					**Harvest**				
Trait	**P Concentration**		**P Content**		**PUE**	**P Concentration**		**P Content**		**PUE**
Unit	**g P kg^{-1}**		**mg P Plant^{-1}**		**%**	**g P kg^{-1}**		**mg P Plant^{-1}**		**%**
Year	**2020**	**2021**	**2020**	**2021**	**2021**	**2020**	**2021**	**2020**	**2021**	**2021**
Pf	<0.001	<0.001	<0.001	<0.001	<0.001	ns	ns	<0.001	<0.001	<0.001
Cul	ns	ns	ns	ns	ns	ns	ns	ns	0.025	0.016
Lime	ns	ns	ns	<0.001	0.003	ns	ns	ns	ns	ns
Pf × Cul	ns	ns	ns	ns	0.003	ns	ns	0.010	ns	ns
Pf × Lime	ns	ns	ns	0.004	0.018	ns	ns	0.026	ns	ns
Cul × Lime	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pf × Cul × Lime	ns	ns	ns	ns	0.004	ns	ns	ns	ns	ns

Table 4. Mean values for the effects of P fertilizer (rock phosphate, RP; diammonium phosphate, DAP) and the control without P fertilizer (No-P) on shoot P concentration at the six-leaf stage in 2020 and 2021. Within each column, means followed by at least one identical letter did not differ significantly according to Fisher's LSD test, $p < 0.05$.

P Fertilizer	**P Concentration**	
	g P kg^{-1}	
	2020	**2021**
No-P	-	2.15 ^{b}
RP	2.53 ^{b}	2.05 ^{b}
DAP	3.37 ^{a}	4.11 ^{a}

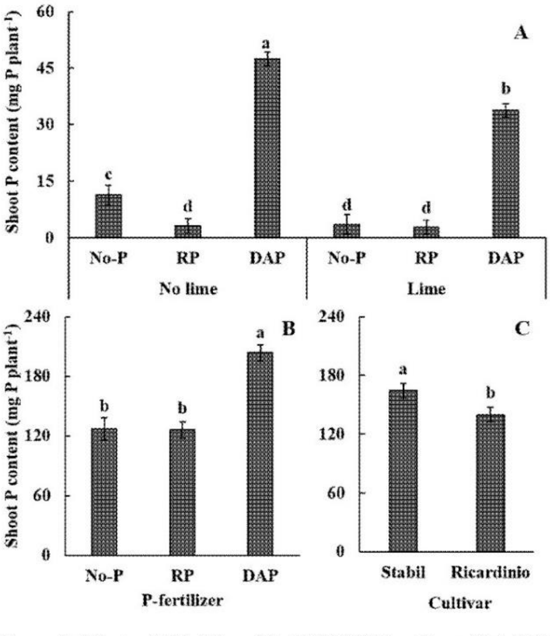

Figure 4. Effects of P fertilizer (No-P, RP, DAP), cultivar (Stabil, Ricardinio), and lime application (No lime, Lime) on shoot P content (mg P plant^{-1}) at the six-leaf stage (**A**) and harvest (**B**,**C**) in 2021. No-P,

no P fertilizer applied; RP, rock phosphate; DAP, diammonium phosphate; with (Lime) and without (No lime) lime application. Bars headed by at least one identical letter did not differ significantly according to Fisher's LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

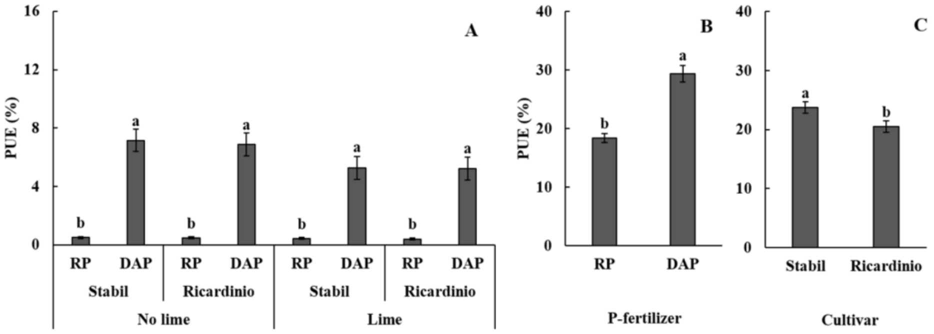

Figure 5. Effects of P fertilizer (RP, DAP), cultivar (Stabil, Ricardinio), and lime application (No lime, Lime) on phosphorus use efficiency (PUE, %) at the six-leaf stage (**A**) and harvest (**B**,**C**) in 2021. RP, rock phosphate; DAP, diammonium phosphate; with (Lime) and without (No lime) lime application. Bars headed by at least one identical letter did not differ significantly according to Fisher's LSD test, $p < 0.05$. In (**A**), letters compare P fertilizers within each combination of cultivar and lime application. Error bars indicate the standard error of the least-squares means.

4. Discussion

4.1. Effect of Phosphate Fertilizer Type on the Growth and P Uptake of Maize

P fertilization plays an important role in the growth, development, and nutrient uptake of maize plants, while differences in the solubility of P fertilizers result in a wide variation in the P availability for plants and thus the effectiveness of different fertilizers [8]. The results of this study are consistent with previous observations that the expansion and the maximum leaf area decreased when the P supply was limited. Thus, with the application of DAP—with a higher water-solubility than RP—a 30% higher canopy LAI was reached in a relatively shorter period, while lower LAI occurred in RP treatments. Lower LAI caused by P deficiency is attributed to two factors: lower leaf appearance rate and slower leaf expansion [1]. Canopy-intercepted PAR is closely associated with LAI [1,35]. The temporal difference in intercepted PAR between maize canopies under different soil plant-available P levels became smaller at later growth stages, which may be attributed to the delayed leaf area expansion process at earlier growth stages [1].

Plant shoot biomass is influenced by P availability due to its effects on LAI, absorbed PAR, and the conversion of PAR into biomass [1,35,36]. Maize biomass accumulation is affected by P fertilizer type, which also determines the plant-availability of P fertilizer [8,37]. As a water-soluble P fertilizer, DAP is highly plant-available, resulting in no P limitation and thus a higher LAI, which increases intercepted PAR and finally plant biomass. Our results showed that the application of DAP resulted in more than a 37% higher shoot biomass at the six-leaf stage, and a 38–67% higher silage yield than RP, respectively. In 2021, shoot biomass with the application of DAP was 1.8 times higher than in 2020, which could be attributed to the higher temperature and greater precipitation during the early stages of 2021. This difference in shoot biomass between years was not found for RP, indicating that P deficiency was the most limiting factor, rather than an influence of water and temperature, as was apparent for DAP.

As well as shoot biomass, differences in P concentration and P content in shoots and in PUE were also found for different P fertilizer types [29,37]. P fertilizers with a higher water-solubility, e.g., DAP and monoammonium phosphate, can contribute to a higher plant P concentration and P acquisition efficiency. Our results clearly show that the application of DAP resulted in at least a 33% higher shoot P concentration besides higher LAI, PAR interception, and shoot biomass, in comparison to RP at the six-leaf stage. As shown in the results of Li et al. [26], the shoot P concentration of maize ranged from around 0.1–0.4 mg g^{-1} at the six-leaf stage under low (CAL-P: 9.8 mg kg^{-1}) to high (CAL-P: 61.8 mg kg^{-1}) levels of plant-available P in the soil. In our study, shoot P content was around 12 times higher with the application of DAP compared with RP at the six-leaf stage, and 61% higher at harvest, respectively. The difference in shoot P content became smaller over time, as also shown for LAI and biomass accumulation. Thus, for the same amount of P acquired by the plants, more RP fertilizer is needed compared with DAP. However, the relative effectiveness of rock phosphate was proven to decline with the increase in the amount of RP applied [7]. Therefore, even though P loss to the environment during processes from the mining of RP to DAP is higher than for other P fertilizer types, the high efficiency of DAP is unsubstituted [8]. The inoculation of RP with phosphate-solubilizing bacteria could be a another way to increase the P-availability of RP, as shown by the 55% higher shoot biomass of maize compared with the application of DAP [38,39]. The addition of phosphate-solubilizing bacteria decreased the soil pH (8.2 to 7.7) and increased plant-available P (74%) mainly by acid and alkaline phosphatase and dehydrogenase enzyme activities [38,40,41]. The effectiveness of phosphate-solubilizing bacteria is, however, still difficult to generalize, given the effect of local site conditions and the limited number of studies conducted under field conditions [42]. Therefore, although some studies have also been published on the use of soil amendments to increase P availability (e.g., organic acids [43], biochar [44]), improving P use efficiency and reducing P losses in crop production remains a challenge to be further investigated.

The localized application of P fertilizer has shown in general positive effects on biomass accumulation, silage and grain yield, and P uptake, mainly due to the reduction in P deficiency during early growth stages [10,11]. In our study, however, placement of RP did not increase P uptake compared with no P fertilizer application. Thus, considering maize yield and PUE, the applied RP fertilizer could not replace DAP, at least under the environmental conditions in our study.

4.2. *Effect of Soil Liming on Growth and P Uptake of Maize*

Recent studies have shown that soil pH affects the performance of P fertilizers and P uptake by plants [20,45]. In neutral soil (pH 7.2), DAP showed a 23% and 40% higher yield and plant P uptake, respectively, and thus higher PUE than in acid soil (pH 5.0). Soil liming affects the soil's chemical properties and is used to increase the soil pH, which can influence P availability [15,18,46]. Alemu et al. [46] reported around a 20% increase in maize grain and silage yield with lime application (4000 kg ha^{-1} lime), in which the soil pH was initially 5. Appropriate liming could lower the amount of P fertilizer for maize grown in low-P soils [47]. The authors showed that maize accumulated at least 17% more biomass at low rate of lime application combined with moderate amounts of P, compared with lower and higher rates of P fertilizer with and without lime application, as measured six weeks after sowing. In our study, on the contrary, lime application caused around 15% less dry biomass at harvest and lower P uptake. This might be related to a decrease in water-extractable P and P diffusion in the soil due to a lower distance of movement from the point of lime application and adsorption of P onto the clay mineral surfaces, caused by lime [48]. These results agrees that at a soil pH near 5.0, plant roots take up P more effectively [45].

4.3. *Maize Cultivar and PUE*

The plant effect is strong in the process of P uptake, and even stronger than the soil effect [45]. Plants of different maize cultivars differ in biomass accumulation during vegetative and reproductive stages, and during final P utilization [25,29,30]. The improvement of the PUE in cultivars is an important factor in maize production, and high PUE cultivars may be selected without taking P fertilizer into consideration [29]. In our study, differences in shoot biomass and P uptake were found between the cultivars Stabil and Ricardinio, which is consistent with a study about the reaction of modern maize cultivars to starter P fertilizer [29]. The cultivar Stabil led to a 30% and 21% higher shoot biomass at the six-leaf stage and harvest compared with Ricardinio, respectively. At harvest, the P content of Stabil was 18% higher, resulting in an increase in PUE of around 16%. Based on the assumption that P-efficient cultivars can be selected regardless of soil P-status, Stabil could be regarded as a high-yielding P utilizer, and tested in further research. The different expressions in biomass accumulation and P uptake of Stabil and Ricardinio might be attributed to differences in root architecture [49]. Maize cultivars can differ in their roots' morphological and physiological aspects, e.g., the release of organic acid anions mobilizing soil P, the accessibility of nutrients, and their metabolic cost [26,49].

5. Conclusions

This study has shown that under the local environmental conditions, DAP could not be substituted by RP in a silage maize cropping system under P-limiting conditions. The cultivar differences in PUE found in this study suggest that future research on the PUE of cultivars should take root morphology into account, in response to P fertilizer type and soil pH.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13091771/s1, Figure S1: Experimental design in 2020; Figure S2: Experimental design in 2021; Table S1: Simple means for interactions of P-fertilizer (Pf), cultivar (Cul), lime application (Lime) on different traits in 2020 and at different stages; Table S2: Simple means for interactions of P-fertilizer (Pf), cultivar (Cul), lime application (Lime) on different traits in 2021 and at different stages; Table S3: ANOVA for the effects of P-fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on LAI at different DAS in 2020; Table S4: ANOVA for the effects of P-fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on the percentage of canopy absorbed PAR (%) at 65, 93, and 118 DAS in 2020, and at 99 DAS in 2021.

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See appendix for supplementary material.

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4 Publication II: Changes in root morphology and rhizosphere metabolites in field-grown maize with different sources of phosphorus supply.

Publication II (submitted in Journal of Plant Nutrition):

Ning, F., Nkebiwe, P.M., Neumann, G., Munz, S., Hartung, J. and Graeff-Hönninger, S., 2024. Changes in root morphology and rhizosphere metabolites in field-grown maize with different sources of phosphorus supply.

Roots, which are the hidden part of plants, play a crucial role in mediating the responses of plants to P stress. Maize, in particular, exhibits a high dependence on root morphology to enhance P acquisition rather than root physiology. However, it is important to note that the morphological response is closely connected to root physiology. Publication I evaluated the effects of P deficiency, caused by different types of P fertilizers and soil liming on canopy characteristics, biomass accumulation, and P acquisition. This publication presents the effects of different P fertilizers in low-P fields on changes in root structure and physiology, including root growth and rhizosphere metabolites in different zones of the root system. This study offers a new perspective on integrating root spatial adaptations (root growth effects) and modifications in rhizosphere chemistry under P-deficient conditions in maize.

Changes in root morphology and rhizosphere metabolites in field-grown maize with different sources of phosphorus supply

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Abstract:

Background

Plant root systems are crucial in addressing phosphorus (P) deficiency, as they mediate the interaction between plants and soil.

Aim

This study investigated the mechanisms of acquisition of P fertilizers with different solubility in maize under field conditions, concerning spatial adaptations and modifications in rhizosphere chemistry.

Methods

A field trial was conducted in southwestern Germany in 2020 and 2021. Three factors were evaluated: (i) types of P fertilizer, rock phosphate (RP) and diammonium phosphate (DAP); (ii) maize cultivar, namely ‘Stabil’ and ‘Ricardinio’; and (iii) liming, with and without lime application, to assess their impacts on root growth and carboxylate release.

Results

At the sixth-leaf stage, RP significantly inhibited root dry biomass, root length, and root length density (RLD) in plant roots compared to DAP. Stabil showed significantly higher root dry biomass, root length, and RLD than Ricardinio at the grain-filling stage, however no effect was found for P fertilizer type. However, RP resulted in higher root length, fine root length, and RLD of Ricardinio in P2 (Root cores in the middle of two plants) than DAP. The release of carboxylates and phytohormones showed no difference for P fertilizer type, maize cultivar, and lime application, while the releases of carboxylates varied with different zones of the root system.

Conclusion:

This finding provides a perspective to integrate different aspects of root growth effects and modifications in rhizosphere solution.

Keywords: P deficiency; maize; root morphology; rhizosphere metabolites

1. Introduction

Plant P uptake generally depends on the plant root system, which acts as a mediator between plants and soil. The variation in plant-available P triggers morphological and physiological changes in roots, allowing them to adapt to the soil environment. Notably, a deficiency in P has a negative impact on the growth of new roots (Mollier and Pellerin 1999). Root biomass, total root length, and root length density (RLD: root length per unit soil volume, cm^{-3}) typically decrease under severe P deficiency (Lopez et al. 2022). At the same time, the plant root system undergoes changes, characterized by increased length and fineness, enabling the plant to reach a larger soil volume and absorb P at a lower metabolic cost (Lynch 2013; Lynch 2015; Postma et al. 2017). When fertilizer is applied locally, root proliferation is stimulated, and RLD and fine root length are increased, especially in the nutrient-rich zone (Zhang et al. 2023). In addition, plant root physiology responds to P deficiency. Physiologically, root exudates are important for maintaining root-soil contact (Walker et al. 2003). Plant-available P can be altered by root exudates, such as organic acid anions, and organic acid anions, predominantly malate and citrate, released by crop roots to cope with P deficiency (Gaume 2000; Jones 1998). Increased exudation of citrate in the rhizosphere enhances the mobilization and availability of P for maize growth, leading to increased P uptake and PUE (Zhang et al. 2016). The type of P fertilizers varies in terms of their availability to the plants, affecting plant growth, biomass accumulation, and nutrient uptake (Gong et al. 2022; Ning et al. 2023). The variation is primarily ascribed to the diverse degrees of dissolution of P fertilizers (Bolland and Gilkes 1990). RP showed very poor agronomic performance, low yield, and P uptake compared to DAP and superphosphate (Bolland and Gilkes 1990). The low availability of RP restricts plant growth under conditions where P is deficient. It is necessary to understand how P fertilizer affects root morphological and, especially, physiological changes. It helps enhance the performance of RP and other economical but sparingly soluble P fertilizers.

Maize cultivars respond differently to P fertilizers and can be categorized as P-efficient, P-inefficient, and P-sensitive cultivars (Weiß et al. 2021). These differences could be attributed to different root morphological and physiological traits (Liu et al. 2004; Weiß et al. 2021; Wen et al. 2019). Root formation and acid phosphatase were more affected by P deficiency in P-efficient cultivars than in P-sensitive ones (Gaume et al. 2001). P-efficient cultivars are associated with larger root systems, e.g. greater root length, which improves P exploration in a larger soil volume, and the root with reduced metabolic costs of soil exploration compared to P-inefficient cultivars (Gaume et al. 2001; Lynch 2015). In general, a higher RLD and a higher proportion of fine root length can be observed under P deficiency. Li et al. (2021) showed that lower P use and PUE are associated with smaller root systems, reduced ability to uptake external P, lower rhizosphere pH and shorter root hairs. Less P-efficient maize cultivars do not acidify the rhizosphere soil. The higher concentration of citric acid in root exudates may also enhance plant P uptake, functioning as the alteration in root morphological and physiological traits of different maize cultivars in response to P deficiency at similar rhizosphere pH (Tang et al. 2020).

Additionally, soil pH affects nutrient availability and uptake, depending on both soil and plant (Barrow and Hartemink 2023). Most P is fixed by iron in soils where the soil pH is below 4.5, whereas it is fixed by calcium where the soil pH is above 6.5 (Price 2006). $H_2PO_4^-$, the main form of P uptake by plants, dominates in soil when soil pH is between 4.5 and 6.5 (Chen and Barber 1990; Hinsinger 2001). Barrow and Hartemink (2023) demonstrated that the plant effect shows a stronger response for P uptake than the soil effect, and generally, P fertilizer is more effective for plants around soil pH = 5 due to less soil sorption. Soil pH in the rhizosphere affects soil P release, while root exudates might, counteract changes in soil pH (Liu et al. 2004; Zhang et al. 2016). The plant's ability to acidify the rhizosphere, or to release organic acids, is an important trait to enhance P mobilization and cope with P deficiency.

This study aimed to explore the effects of different P fertilizers, maize cultivars, and soil lime application on root morphology and physiological characteristics. We hypothesized that, under P-limited conditions, the morphology of field-grown maize roots and the composition of rhizosphere metabolites are modulated in response to different P sources to optimize P acquisition. Specifically, we proposed that P sources influence the spatial distribution of root biomass and length, as well as the exudation of rhizosphere metabolites at distinct root positions.

2. Materials and methods

2.1. Experimental design

The field experiment was conducted at the University of Hohenheim's Oberer Lindenhof research station (48° 28′ 26″ N, 9° 18′ 12″ E) in Stuttgart, Germany, during 2020 and 2021. The description of the field design can also be found in Ning et al. (2023), but were added to the current document for clarity.

The field experiments were conducted on the same field for both years. In 2020, half of the field was used, while the other half was used in 2021. Soil samples were taken at depths of 0-30 cm, 30-60 cm, and 60-90 cm on April 8, 2020 and May 10, 2021, respectively. The detailed soil information is presented in Table 1. Soil CAL-P belonged to class “A”, and the pH of the soil at a depth of 0-30 cm was around 5.0 for the two years. Soil N_{min} was 66 in 2020 and 79 kg N ha^{-1} in 2021.

Table 1. Soil properties (Ning et al. 2023).

Year	Depth cm	Bulk density g cm^{-3}	pH	Nmin kg N ha^{-1}	CAL-P mg P 100 g^{-1} soil
2020	0-30	1.3	5.3	23.4	0.9
	30-60	1.3	5.5	13.1	-
	60-90	1.4	6.0	29.9	-
2021	0-30	-	4.7	23.9	0.7
	30-60	-	4.8	21.3	-
	60-90	-	4.3	33.4	-

In 2020, the study included three experimental factors: (i) types of P-fertilizer, rock phosphate (RP, 11% P, Dolophos® 26; NATURKALK, Barbing, Germany) and diammonium phosphate (DAP, 18% N – 20% P, DAP; Van de Reijt Meststoffen B.V., Moerdijk, Netherlands); (ii) maize cultivars, Stabil (a high-yielding P utilizer) and Ricardinio (a P-sensitive cultivar) (both from KWS SAAT SE & Co. KGaA, Einbeck, Germany); and (iii) liming practices: no lime application and 16800 kg $CaCO_3$ ha^{-1} lime application (75% $CaCO_3$ and 15% $MgCO_3$, Kohlensaurer Magnesiumkalk 90; Zement- und Kalkwerke Otterbein GmbH & Co. KG, Grossenlüder-Müs, Germany). At the six-leaf stage, the soil pH at a depth of 0-10 cm was 4.9 in the no lime treatment and 6.0 with lime treatment. The field trial was designed as a 2 × 2 × 2 factorial experiment, resulting in eight treatments each replicated four times. Plots were arranged in a split-plot design, with the combination of maize cultivar and lime application as the main-plot factor, and P-fertilizer type as the subplot factor. A Latin square design was used for the main plots, creating a 4 × 4 grid with 16 main plots. Each main plot was divided into two subplots, totaling 32 plots, each measuring 66 m^2 (11 m long × 6 m wide) (Ning et al. 2023). In 2021, additional control plots without P application were introduced, grouped into the factor P-fertilizer type as another level, bringing the total to 48 plots. A total of six main-plot factor levels were arranged in a randomized complete block design with complete blocks within a row. The six levels of the main plot factor were no P with and without lime, and the four main plot factor levels were used in 2021. Due to space limitations, the control sub-plots covered an area of 33 m^2 (11 m long × 3 m wide), while the other subplots maintained the original area of 66 m² from 2020 (Ning et al. 2023).

In the two years, a total of 75 kg P ha^{-1} was applied with RP and DAP. To ensure that the N quantity (68 kg N ha^{-1}), N-fertilizer type, and fertilizer application method were the same as in the DAP treatment, RP was applied in combination with stabilized ammonium sulfate fertilizer (21% N, NovaTec® Solub 21; Compo Expert, Münster, Germany). Both DAP and a combination of RP with stabilized NH_4-N were applied locally as starter fertilizer at sowing, positioned 5 cm to the side and 7 cm below the maize seed, using a single seed sowing machine equipped for starter fertilizer placement (Unisem; RAU Landmaschinen GmbH, Korntal-Münchingen, Germany). In 2020, 46 kg N ha^{-1} of urea, stabilized with a urease inhibitor (46% N, Alzon® neo-N; SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany), was broadcast and incorporated to a depth of 5 cm, considering soil N_{min} levels (Table 1). This brought the total N supply to 180 kg N ha^{-1} for the whole maize growing season. 34 kg N ha^{-1} was applied in 2021.

Maize was sown at 3 cm depth on 20 May 2020 and 2 June 2021, with the starter fertilizer applied simultaneously. The sowing density was 100,000 plants ha^{-1} in the two years, with an inter-row spacing of 75 cm. Root and rhizosphere soil solutions were collected on different days after sowing (DAS) in 2020 and 2021.

2.2. Sampling

2.2.1. Root sampling for root scanning and dry biomass

Root dry biomass: At the seedling stage, the whole root samples were collected with a soil volume of 37.5 cm width and length, and 30 cm depth with the plant shoot in the center. This sampling was conducted at 15, 22, and 34 DAS, in 2020. Roots were picked from the soil samples, cleaned gently, and then dried at 60 °C to constant weight.

Root length: In addition, root cores were collected at 48 DAS (sixth-leaf stage) and 122 DAS (grain-filling stage) with three replicates (Figure 1). The polyethylene tube for root cores, 2.5 cm diameter and 30 cm length, was placed into the drill, and then the soil cores were taken out with the drill. Root samples were taken from three positions per plot as illustrated in Figure 1: below the plant (P1), between two plants within the row (P2), and next to the plant where the P fertilizer depot was placed (P3). Roots were picked from soil cores, cleaned gently, and scanned using a root scanner (Epson Expression 10000Xl, Epson). The length of each root sample was analyzed with WinRhizo software (Regent Instruments Inc., Quebec, Canada). RLD and specific root length (SRL, the ratio of root length and root dry biomass, cm g^{-1}) were calculated based on the root length analyzed according to the following formulas (1 and 2) (Kuchenbuch et al. 2009):

$$RLD = \frac{Root\ length}{\pi r^2 \times h}, (1)$$

$$SRL = \frac{Root\ length}{Root\ dry\ biomass}, (2)$$

where *r* and *h* refer to the radius and height of the plastic bucket, respectively.

After scanning, root samples from the two harvests were dried at 60 °C for 3d until constant weight.

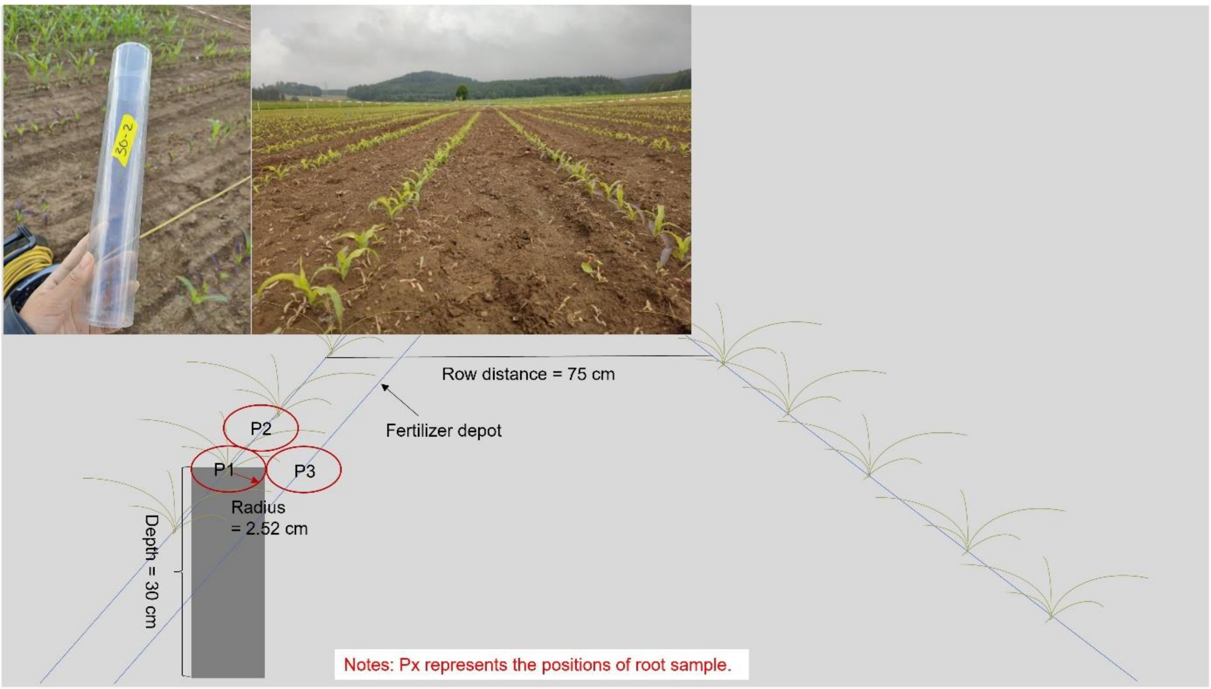

Figure 1 Root core sampling. P1, P2, and P3 represent the sampling positions, 1, 2, and 3, respectively. P1, below the plant; P2, between two plants within the row; P3, next to the plant where the P fertilizer depot was placed.

2.2.2. The installation of root windows

In 2020, root windows were installed for the collection of soil solution in the rhizosphere at 36 DAS within four out of eight treatments. These treatments were defined by P fertilizer type (RP and DAP) and cultivar (cv. 'Stabil' and 'Ricardinio') under conditions of no soil liming. In 2021, six treatments were chosen, and root windows were installed at 43 DAS. Treatments were derived from combinations of P fertilizer type (No-P, RP, and DAP) and soil liming (No lime and lime) with the cultivar 'Ricardinio'.

The installation of root windows (Figure 2), the exact location where the fertilizer was applied, was modified as shown in Behr et al. (2024). Rhizosphere soil solution, including moderately or highly soluble low molecular weight compounds in the rhizosphere, was collected at 93 DAS in 2020 and 99 DAS in 2021 through root windows, respectively. They were collected from 1 cm segments of different zones of the root system (basal, seminal (shoot-borne crown roots growing rapidly into deeper soil layers), tip (the 1 cm apical root zones of lateral roots)) and bulk soil by application of sorption filter papers (Figure 3) according to the method described by Behr et al. (2024). There were five points for each part, and the collection period was 4 h. The sorption filter papers from each part were pooled. Please find the link for details: https://dicontrol.igzev.de/root-window-2/.

Figure 2 Installation of the root window

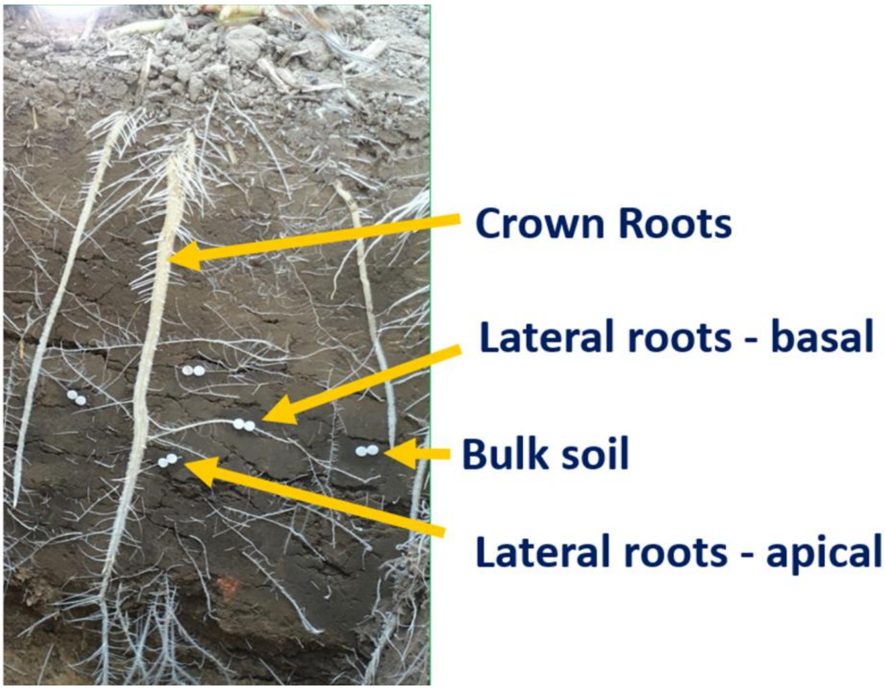

Figure 3 Sampling of rhizosphere soil solution

All the samples were extracted in 1 mL 80% (v/v) methanol for the analysis of organic acids and phytohormones. Carboxylates were analyzed for each treatment by pooling all the samples of rhizosphere soil solution collected from different zones of the root system in 2020, while they were analyzed separately for each sample in 2021. Additionally, plant phytohormones were also analyzed for all the samples in 2021. Carboxylates were determined by high-performance liquid chromatography (HPLC, Agilent 1290, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) as described by Behr et al. (2024) with additional identification of malate and citrate by a commercial enzymatic test (R-Biopharm, Darmstadt, Germany). Plant phytohormones were determined by HPLC method coupled with mass spectrometer (MS, QTrap 5500, AB Sciex) in the Core Facility, a central analytical laboratory department of University of Hohenheim (Behr et al. 2024; Flieger et al. 2018).

2.2.3. Statistical analysis

The trials were set up as split-plot designs, with the main plots arranged in a Latin square design in 2020 and an RCBD in 2021 (Ning et al. 2023). Both trials involved all the three treatment factors. A mixed-model approach was used to analyze the data in SAS v9.4 (SAS Institute, Cary, NC, USA). The model for 2020 is described as follows:

$$y_{hijkm} = \mu + r_i + c_j + m_{ij} + \alpha_k + \gamma_m + (\alpha\gamma)_{km} + e_{hijkm}, \tag{1}$$

where r_i, c_j, and m_{ij} stand for the design, whileα_k, β_l, and γ_m stand for the three treatment factors. Specifically, y_{hijkm} is the observation of plot h in the ith row and jth column treated with the kth level of maize cultivar and mth level of P fertilizer. Here, μ denotes the intercept, and r_i and c_j are the fixed effects of the ith complete row and jth column. The term m_{ij} is the random main plot effect associated with the area of two plots in a combination of row and column, α_k and γ_m are the fixed main effects of the kth maize cultivar and mth P fertilizer, respectively, $(\alpha\gamma)_{km}$ is the fixed two-way interaction effects of the corresponding factors involved, and e_{hijkm} is the plot error of y_{hijkm}. In 2021, the experiment underwent slight modifications. First, two control main plots were added per row. The main plot factor became either a combination of lime application level and P fertilizer or lime application level and maize cultivar. The adjustment allowed for testing the interaction between maize cultivar and lime application levels additionally under the no-fertilizer control treatment. Second, since the control plots were sown with four rows instead of eight rows, the data were weighted based on the number of rows per plot. Additionally, data from both lime treatments were used. Furthermore, data were measured for Ricardinio only. Therefore, the model is similar to Ning et al. (2023), but with the fixed-column effect removed, a weight statement included, and cultivar effects and interactions replaced by corresponding lime application effects. The model is given by

$$y_{hijlm} = \mu + r_i + c_j + m_{ij} + \beta_l + \gamma_m + (\beta\gamma)_{lm} + e_{hijlm}, \tag{4}$$

where β_l and $(\beta\gamma)_{lm}$ are the fixed effects for the lth lime application and its interaction effects with P fertilizer. All other effects are analogously defined as in (3).

Residuals were checked graphically for normal distribution and homogeneous variances in both statistical analyses. Global F tests were then conducted to assess differences between the levels of the main factors and their interactions. If significant differences were found, a Fisher's LSD test was performed at a significance level of $\alpha = 0.05$. The LSD test results were displayed using letter groupings (Piepho 2012).

3. Results

3.1. Carboxylates in the rhizosphere soil solution

In 2020, the organic acid anions found in the solution consisted of six different acids: malate, citrate, caffeate, p-coumarate, benzoate, and cinnamate. Based on the results of Jones (1998) and Gaume (2000), the roots of crops released organic acid anions, predominantly malate and citrate, to cope with P deficiency (Gaume 2000; Jones 1998). Malate and citrate were present in the rhizosphere soil solution in both years. However, at 93 DAS in 2020, there was no significant difference in organic acid anions affected by P fertilizer type and cultivar (Table S1), when the P-limitation effects continuously declined due to stimulation of lateral root growth in the P-deficient plants induced by RP (Grillet and Schmidt 2017; Müller et al. 2015).

Since P uptake primarily occurs in the apical root zones of young growing roots, it is important to examine the concentrations of di- and tricarboxylates that may be involved in P mobilization in different root zones (Neumann and Ludewig 2023; Smith 2002; van der Bom et al. 2023). The compounds of rhizosphere soil solution detected in 2021 varied with different root zones (Figure 4). Malate, citrate, and trans-aconitate were the quantitatively dominant di- and tricarboxylates detected in the maize rhizosphere as previously reported also in other studies, apart from trace amounts of succinic, fumaric, cis aconitic, and shikimic acids (Carvalhais et al. 2011; Gaume et al. 2001; Neumann and Römheld 2002). While trans-aconitate, an allelochemical, dominated in the basal zones of crown roots, citrate and malate were dominant in the apical zones of lateral roots, with malate dominating in the more basal root zones (Da Bortolo et al. 2018; Yan and Wang 2020). The finding that almost no di- and tricarboxylates were detected in the bulk soil solution, along with reports of malate, citrate, and trans-aconitate as dominant carboxylic root exudates in soil-free hydroponic culture, indicates that the detected carboxylates were released from maize roots (Carvalhais et al. 2011; Gaume et al. 2001; Neumann and Römheld 2002). Malate but particularly citrate, are potent chelators for Fe and Al at soil pH values below 7.0 (Jones 1998) and are also able to mobilize sparingly soluble P forms in soils. However, compared with Fe mobilization, much higher rhizosphere concentrations are required to mediate the solubilization of significant amounts of P relevant for plant nutrition. For citrate, as the most effective carboxylate in this context, the minimum concentrations of 5-10 µmol g^{-1} soil have been reported (Amann and Amberger 1988). Plants with a proven potential for citrate-mediated P mobilization, such as *Lupinus albus*, accumulated about 50 µmol g^{-1} rhizosphere soil (Dinkelaker et al. 1989). About 150 mg has been calculated for the amount of rhizosphere soil at a distance of 1 mm around a root segment of 1 cm (Bar-Yosef 1991). In the present study, a maximum citrate accumulation of about 20 nmol cm^{-1} root length has been detected in the apical root zones with the highest P uptake potential, where P mobilization should consequently be localized (Smith 2002). This amounts to a rhizosphere concentration of 0.133 µmol g^{-1} soil, which is almost two orders lower than the reported effective citrate concentration levels required for P mobilization. Due to a limited residence time (5 h) of growing root tips in a given soil volume, cumulative exudation over time would not be sufficient, and accordingly recent model calculations revealed citrate efflux rates of about 730 nmol h^{-1} cm^{-1} as critical values for P mobilization (McKay Fletcher et al. 2021; Neumann and Ludewig 2023), which is far from the maximum values detected in this study. These findings suggest that carboxylate-mediated P mobilization was probably not involved in P acquisition by the maize plants investigated, at least at the developmental stage studied (99 DAS). Carboxylate values with higher variability showed no significant differences between the treatments. Therefore, this could be attributed to the late sampling time.

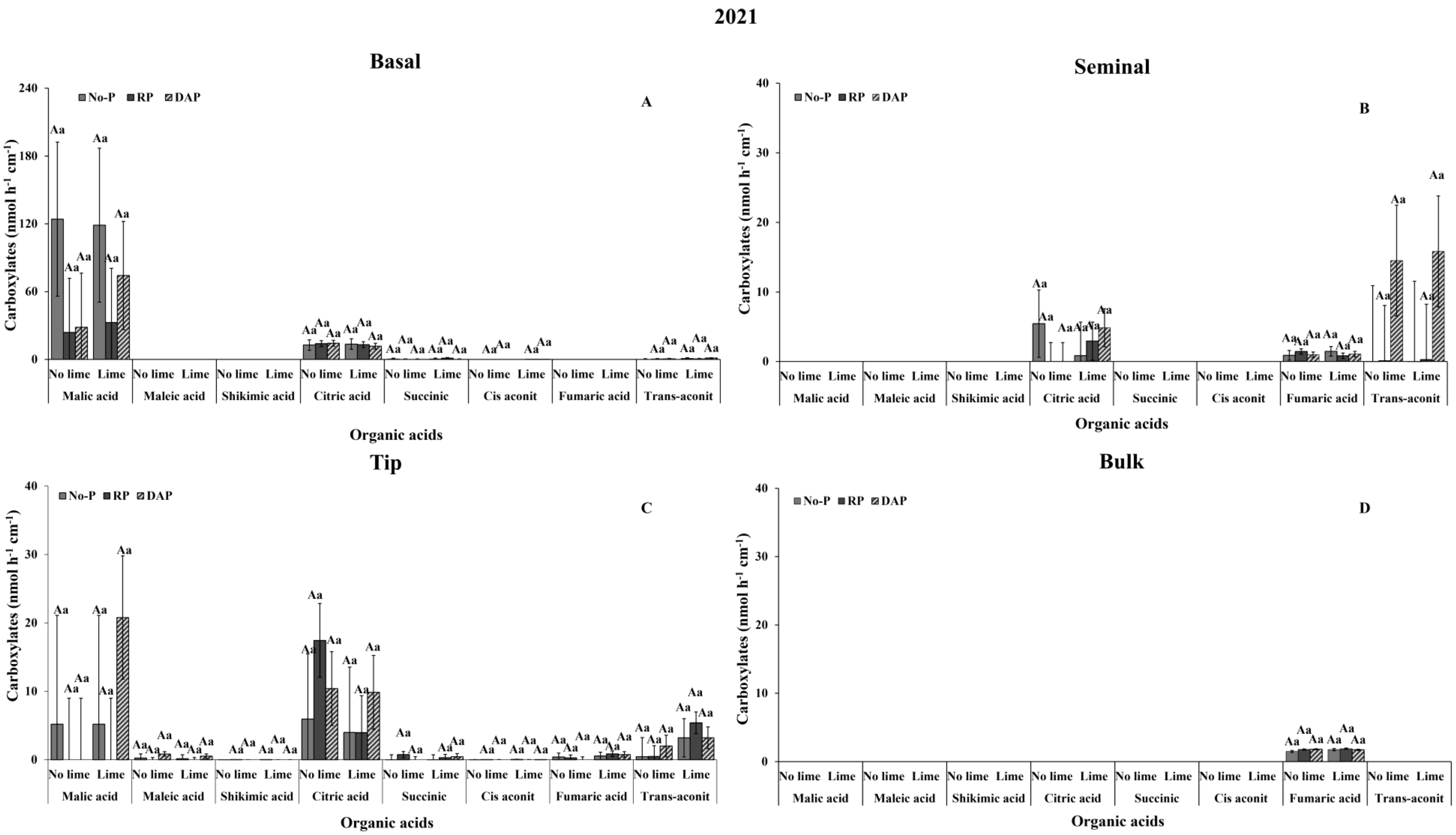

Figure 4 Effects of P-fertilizer type and lime application on the rate of organic acids (nmol cm^{-1}) in different zones of the root system at 99 DAS in 2021. Upper-case letters were used to show significance of cultivar within each P fertilizer type level, lower-case letters were used to show significance of P fertilizer type level within cultivar. Bars sharing at

least one identical letter were not significantly different based on Fisher´s LSD test, $p < 0.05$. Error bars represent the standard error of the least squares means. The detailed information of the data was shown in Table S2.

3.2. Root dry biomass

No difference in root growth due to P fertilizer type was observed in the maize seedling stage (Figure 5A), as the P status of the plants was still dominated by the seed P reserves. The seed P reserves cover the P demand of maize plants for approximately two weeks (Muhammad et al. 2015; Nadeem et al. 2011). Root dry biomass was only affected by cultivar at the early seedling stage (15 DAS, the first-leaf stage), as shown in Figure 5A. Ricardinio had 28% higher root dry biomass than Stabil at 15 DAS. Therefore, Ricardinio should indicate a higher biomass and/or nutrient base for plant growth, supporting a higher leaf area index at the early stage (Ning et al. 2023). After the seed P reserves are used up, P fertilizer or exogenous P begins to contribute to the growth of maize seedlings (Nadeem et al. 2013). Consequently, the significant interaction between P fertilizer and cultivar was observed at 34 DAS (the second-leaf stage). With the application of RP, Ricardinio had 76% higher root dry biomass than Stabil due to differences in the characteristics of the seeds.

At 48 DAS (the sixth-leaf stage), only P-fertilizer type affected root dry biomass in P1 ($P < 0.05$), and DAP led to 156% higher root dry biomass than RP (Figure 5B). The differences in the solubility of P fertilizer types resulted in the variation of P availability for plants, limiting plant growth (Gong et al. 2022; Ning et al. 2023). Severe P deficiency restricted the accumulation of root biomass (Liu et al. 2004). Zhan et al. (2019) presented that the root dry biomass of maize plants increased, up to 178%, with the increase of P fertilizer rate compared with P-deficient plants at the sixth-leaf stage. Cultivar showed no effects on root dry biomass at the grain-filling stage, only higher root dry biomass of Stabil in the position between neighboring plants. Stabil had 129% higher root dry biomass in P2 (Figure 5C), resulting in higher biomass and nutrient accumulation for shoot growth and the redistribution of biomass and nutrients (Azam et al. 2022). As shown, Stabil had higher shoot biomass at the sixth-leaf stage and harvest than Ricardinio (Ning et al. 2023). Stabil exhibited higher PUE. It is a relatively high-yielding cultivar compared with Ricardinio (Ning et al. 2023; Weiß et al. 2021). Therefore, we concluded that Stabil exhibited higher yielding and P acquisition characteristics, supported by the efficient root capture for soil resources. The preference of developing seedlings is to allocate P to the shoot, which is the active site for photosynthesis (Nadeem et al. 2013). P deficiency often influences the shoots greater than the roots, while seedlings with P deficiency showed reduced leaf expansion and biomass accumulation (Nadeem et al. 2013). Overall, cultivar differences in root system plasticity could be regarded as an appropriate target for marker aided selection to improve PUE of maize (Zhu et al. 2005). In addition, the efficiency of nutrient acquisition and biomass accumulation of the cultivar also can be an important criterion to improve PUE in cultivar selection.

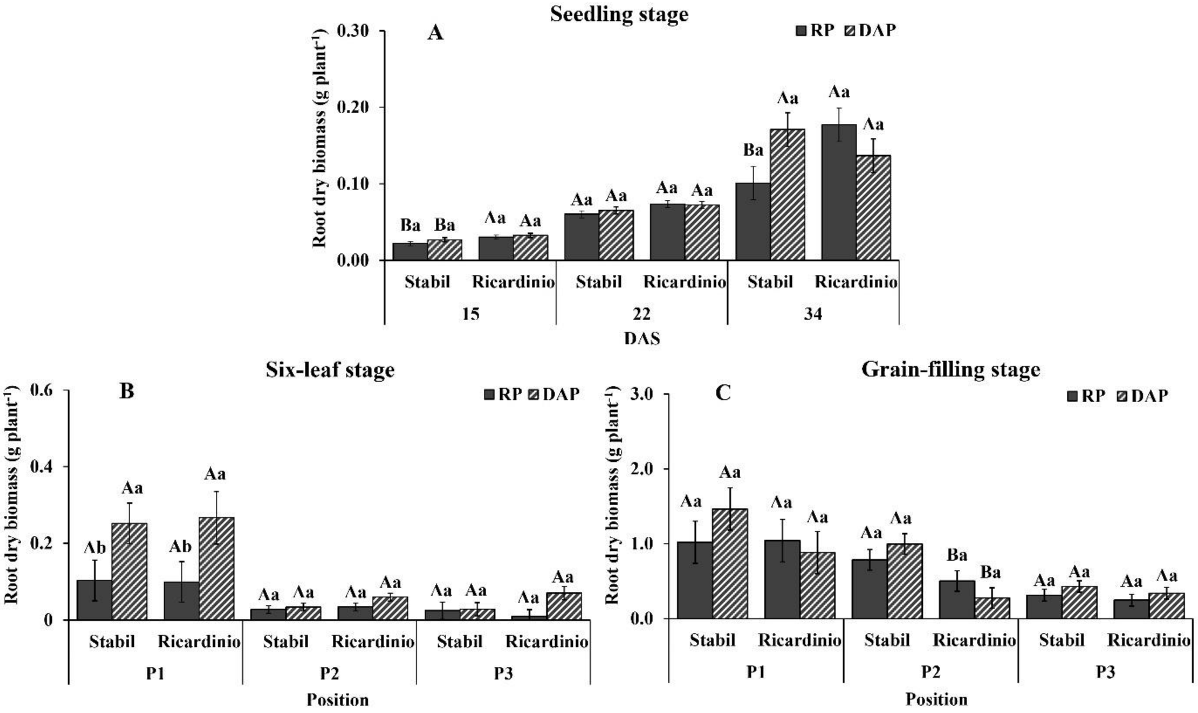

Figure 5 Effects of P fertilizer type and cultivar on root dry biomass at different DAS during seedling stage (A), on root dry biomass at different positions of the root system at sixth-leaf stage (48 DAS) (B) and grain-filling stage (122 DAS) (C) in 2020. Upper-case letters were used to show significance of cultivar within each P fertilizer type level, lower-case letters were used to show significance of P fertilizer type level within cultivar. Bars sharing at least one identical letter were not significantly different based on Fisher´s LSD test, $p < 0.05$. Error bars represent the standard error of the least squares means.

3.3. Root length

Under P limitation, increased malate exudation can mediate Fe mobilization at the root tips, and mobilized Fe^{3+} is reduced by apoplectic ascorbate (Grillet and Schmidt 2017). Subsequent precipitation of the extracellular Fe is triggered by P deficiency-induced ferroxidases (LPR1, LPR2) associated with ROS production (Müller et al. 2015). The oxidative stress induces the intracellular deposition of callose, which blocks the carbon supply to the root meristem. This inhibits root elongation and finally results in a higher density of root hairs and stimulated lateral root growth, enhancing spatial P acquisition (Grillet and Schmidt 2017; Müller et al. 2015). In this study, Fe mobilization via malate should be particularly expressed at the six-leaf stage under a certain soil pH condition (Liang et al. 2013; Mora-Macías et al. 2017). Consequently, the inhibition of root growth was reflected by reduced root biomass, root length, and RLD in P1 directly below the seeds in plants with RP supply, compared to those treated with DAP (Figure 5B and 6(A and B)). The effect of malate on adaptive modifications in root growth under P deficiency is highlighted by the dominance of malate in the rhizosphere soil solution. There could be a trend for higher values in P-limited plants with RP supply, as similarly reported in other studies with maize exposed to P-limitation (Carvalhais et al. 2011; Gaume et al. 2001; Neumann and Römheld 2002). P limitation on shoot growth and P uptake was been observed at 48 DAS in the six-leaf stage (Ning et al. 2023).

The differences in root dry biomass, root length, and RLD from the P fertilizer type observed in P1 disappeared at the grain-filling stage (Figure 5C and 6(D-F), 122 DAS). The effect of cultivar and P fertilizer type was only observed in the characteristics of roots in the position of P2 between neighboring plants. Stabil showed 54% higher root length and RLD, as well as fine root length than Ricardinio (Figure 6(D-F)), which was beneficial for P uptake (Ning et al. 2023; Zhang et al. 2022). There was no difference in root length, fine root length, and RLD of Stabil from P fertilizer observed. Therefore, Stabil with a relatively stable root system is characterized as a high-yielding cultivar and high P utilizer (Ning et al. 2023; Weiß et al. 2021). Stabil was able to take up more nutrients to support the growth of shoot. The root system of Ricardinio exhibited a stronger root morphological responses to P deficiency compared to Stabil. RP led to 89% higher root length and RLD and increased fine root length of Ricardinio than DAP in P2. This may reflect the observed stimulation of lateral root growth in P-deficient plants fertilized with RP, to improve spatial P acquisition (Müller et al. 2015). However, the inhibition of shoot growth induced by P limitation during vegetative growth was not compensated until the final harvest (Ning et al. 2023).

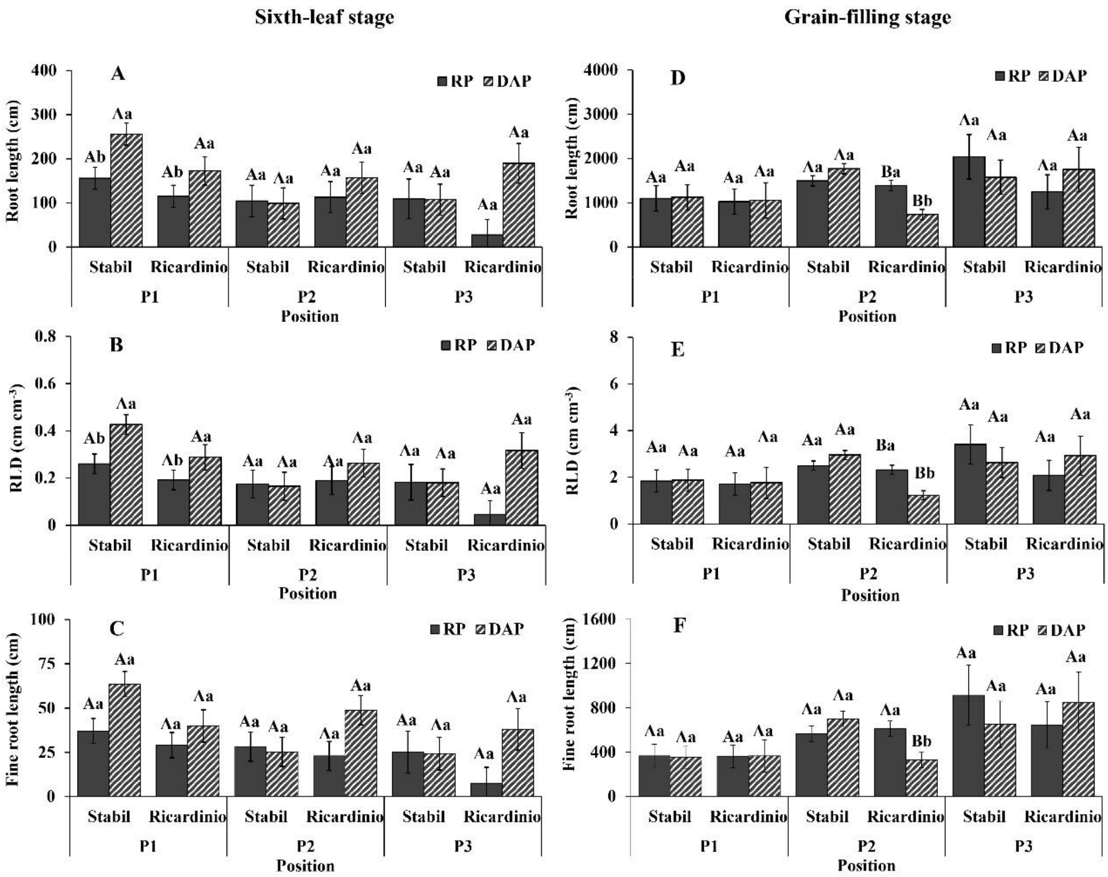

Figure 6 Effects of P fertilizer type and cultivar on root length, fine root length (0 < Diameter <= 0.2 (cm)), specific surface area, and root length density (RLD) at different positions (P1, P2, and P3) at the sixth-leaf stage (48 DAS, A-C) and the grain-filling stage (122 DAS, D-F). Upper-case letters were used to show significance of cultivar within each P fertilizer type level, lower-case letters were used to show significance of P fertilizer type level within cultivar. Bars sharing at least one identical letter were not significantly different based on Fisher´s LSD test, $p < 0.05$. Error bars represent the standard error of the least squares means.

3.4. Phytohormones in the rhizosphere soil solution

Table 2 Effects of P-fertilizer type and soil liming on the rate of phytohormones (nmol cm^{-1}) in different zones of the root system at 99 DAS in 2021 (ABA, Abscisic acid; MeIAA, Methyl-indole-3-acetic acid; MeJA, Methyl jasmonic acid). No significance of P fertilizer type and soil liming within cultivar (Ricardinio) is detected according to Fisher´s LSD test, *p* < 0.05.

Compounds	Name	No lime			Lime		
		No-P	RP	DAP	No-P	RP	DAP
ABA	Basal	12.41					
	Seminal	10.36 Aa	9.80 Aa	9.11 Aa	6.64 Aa	9.26 Aa	9.79 Aa
	Tip	8.86 Aa	8.47 Aa	8.56 Aa	8.86 Aa	8.73 Aa	8.32 Aa
	Bulk	7.79					
MeIAA	Basal	5.80					
	Seminal	7.13 Aa	6.27 Aa	5.28 Aa	6.54 Aa	6.68 Aa	6.38 Aa
	Tip	6.20 Aa	6.90 Aa	6.54 Aa	6.57 Aa	6.64 Aa	6.72 Aa
	Bulk	6.26					
MeJA	Basal	775.76					
	Seminal	876.52 Aa	795.08 Aa	782.30 Aa	824.80 Aa	834.02 Aa	815.59 Aa
	Tip	793.41 Aa	836.99 Aa	807.56 Aa	827.74 Aa	822.42 Aa	835.50 Aa
	Bulk	814.99					

P deficiency leads to increased ABA accumulation in root tips with a reduction in cytokinin in 4-day-old barley seedlings, inhibiting root elongation (Vysotskaya et al. 2020). Meanwhile, parallel to the increase in ABA, increased IAA interacts with ABA to stimulate root branching to adapt to P deficiency conditions. However, exogenous IAA increases root surface area, sugar release, and acid phosphatase activity, thereby enhancing organic P mineralization under P deficiency (Wittenmayer and Merbach 2005). The JA pathway is also activated in response to P deficiency, leading to an increase in JA content in the roots (Chacón-López et al. 2011; He et al. 2023; Khan et al. 2016). This, in turn, affects the architecture of the root system in Arabidopsis, allowing plants to thrive in low-nutrient conditions with increased root length. As shown in Table 2, the type of P fertilizer and lime application had no effect on the exudation rate of ABA in the different root zones (seminal and tip root). The results of the quantitative analysis of plant hormones were consistent with those of plant root morphology and rhizosphere organic acids during a similar stage (99 DAS, 2021). Soil liming, increased soil pH from 5.5 to

6.2 and enhanced plant ABA signaling to cope with P stress in legumes, as legumes showed a higher dependence on physiological responses to P deficiency compared to maize (Lyu et al. 2016; Rothwell et al. 2015). However, the difference in maize shoot growth from soil liming was much smaller compared with that from the P fertilizer type, even at the six-leaf stage (Ning et al. 2023). It is worth noting that plant hormones, such as IAA, ABA, and JA, can be produced by both plants and soil microorganisms, especially plant growth-promoting rhizobacteria in the rhizosphere (adjacent to the root surface) (Dodd et al. 2010; Patel et al. 2015). Rhizosphere bacteria mediate the plant hormone status, which aids in plant root growth and facilitates the transport of plant hormones, enabling plants to withstand stress (Dodd et al. 2010). In this stage, the root structure of maize remained unchanged, and the movement of P was not affected by different root zones, despite the secretion of various organic acids. Similar concentrations of the respective phytohormones were also detected in the bulk soil samples (Table 2). It is highly likely that the detected phytohormones were secreted by soil microorganisms. Therefore, quantitative phytohormone analysis of the early plant root systems is more meaningful but also presents better requirements for the timing of sampling and the restoration of root growth.

4. Conclusion

The root system plays a crucial role in P uptake. Cultivar with higher P uptake and yield potential exhibited a root system characterized by greater root biomass and root length, traits that enhance P acquisition under P-deficient soil conditions. While no differences were found in the concentrations of carboxylates and phytohormones in different root zones affected by P fertilizer type, cultivar, and lime application, variations in soil rhizosphere solution among different zones of the root system can be used in selecting P-efficient cultivars and P fertilizers to improve PUE. Future studies aiming to understand P mobilization in low P systems should consider the architecture of the root system at earlier growth stages, that may play a significant role in plant P acquisition.

Supplementary Material

Table S1 Effects of P-fertilizer type (Pf) and cultivar (Cul) on the rate of organic acids (nmol cm^{-1}) in different zones of root system at 93 DAS in 2020.

Organic acids	Malic acid		Citric acid		Caffeic acid		p-Coumaric acid		Benzoic acid		Cinnamic acid	
Cul / Pf	Stabil	Ricardinio	Stabil	Ricardinio	Stabil	Ricardinio	Stabil	Ricardinio	Stabil	Ricardinio	Stabil	Ricardinio
RP	6.09	5.56	0.43	0.42	0.11	0.12	0.10	0.08	0.28	0.30	0.13	0.10
DAP	4.56	4.88	0.47	0.47	0.10	0.10	0.08	0.08	0.25	0.23	0.12	0.11

Table S2 Effects of P-fertilizer type (Pf) and soil liming (Lime) on the rate of organic acids (nmol cm^{-1}) in different zones of root system at 99 DAS in 2021.

Organic acid		Malic acid		Maleic acid		Shikimic acid		Citric acid		Succinic		Cis aconit		Fumaric acid		Trans-aconit	
Name	Pf \ Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
Basal	No-P	124.16	118.73	-	-	-	-	12.70	13.59	0.28	0.28	0	0	-	-	0	0.81
	RP	23.83	32.71	-	-	-	-	13.99	13.11	0	1.11	0	0.01	-	-	0.31	0.44
	DAP	28.38	74.19	-	-	-	-	14.25	11.71	0	0	0	0.02	-	-	0.47	1.15
Seminal	No-P	-	-	-	-	-	-	5.45	0.85	-	-	-	-	0.90	1.46	0	0
	RP	-	-	-	-	-	-	0	2.94	-	-	-	-	1.44	0.84	0.11	0.28
	DAP	-	-	-	-	-	-	0	4.86	-	-	-	-	0.98	1.07	14.51	15.83
Tip	No-P	5.20	5.20	0.26	0.14	0	0.01	5.94	4.00	0	0	0	0.04	0.40	0.54	0.44	3.21
	RP	0	0	0	0	0.01	0	17.45	3.98	0.75	0.31	0.01	0	0.28	0.86	0.47	5.40
	DAP	0	20.79	0.83	0.52	0	0	10.40	9.86	0	0.45	0	0.02	0	0.76	2.01	3.22
Bulk	No-P	-	-	-	-	-	-	-	-	-	-	-	-	1.48	1.75	-	-
	RP	-	-	-	-	-	-	-	-	-	-	-	-	1.77	1.89	-	-
	DAP	-	-	-	-	-	-	-	-	-	-	-	-	1.81	1.73	-	-

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5 Publication III: Effect of P fertilizer placement and amount on early growth of maize (*Zea mays* L.) under drought stress

Publication III (submitted in Journal of Plant Nutrition and Soil Science):

Ning, F., Nkebiwe, P.M., Munz, S., Hartung, J., Zhang, P., Huang, S. and Graeff-Hönninger, S., 2024. Effect of P fertilizer placement and amount on early growth of maize (*Zea mays* L.) under drought stress.

Many studies investigated nutrient efficiency under different fertilizer placements. The placement of fertilizer has been advocated as an efficient approach to improve P uptake and increase maize yield. Furthermore, a meta-analysis has proven that deep placement of P fertilizer could enhance nutrient uptake and maize yield. In the context of frequent droughts and P depletion, more investigations are needed on maize production due to the increasing demand for maize under dual stresses. Therefore, in addition to how maize plants developed under P deficiency in publication I and publication II, publication III investigates the response of maize plants to different P fertilizer amounts and fertilizer placements under drought.

Effect of phosphate fertilizer placement and amount, and soil water content on early growth of maize (*Zea mays* L.)

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Keywords Deep P placement; P fertilizer amount; Drought; Maize growth; P uptake

Abstract

Background and aims

Drought stress (DS) reduces phosphorus (P) availability, and the scarcity of P resources exacerbates P stress. Deep placement of P fertilizer may alleviate P deficiency and DS.

Methods

A greenhouse trial was conducted involving three factors: P fertilizer amount (LP: 2 mg P 100 g^{-1} and HP: 12 mg P 100 g^{-1}), fertilizer placement (mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm)), and soil water content (drought stress (DS): 45% of soil water holding capacity (WHC) and well-watered (WW): 75% WHC). Root and shoot growth and P uptake were assessed at the fourth- and tenth-leaf stages.

Results

LP decreased shoot P content and root and shoot biomass compared with HP treatment. Under DS, root biomass increased in 0-18 and 10-18 cm depths at the fourth-leaf stage by 50% and 95%, respectively, compared to WW treatment. However, at the tenth-leaf stage, root biomass decreased by at least 41% across depths. Plants under DS showed lower shoot biomass and P uptake at both stages. Although L-DS did not improve shoot growth and P uptake by the tenth-leaf stage, over 55% higher root biomass and increased root length were observed in 10-18 cm depth compared to M-DS and L-DS treatments.

Conclusion

Deep fertilizer placement shows potential to alleviate combined stress of P deficiency and drought by increasing root growth in deeper soil. However, field experiments over a longer period are needed to determine if this positive response in root characteristics also promotes above-ground growth later.

Abbreviations

DAS	days after sowing
DM	dry matter
DS	drought stress
K	potassium
N	nitrogen
P	phosphorus
PUE	P use efficiency
RLD	root length density (cm cm^{-3})
SRL	specific root length (cm g^{-1})
WHC	water holding capacity

1. Introduction

Drought is a significant natural threat that results from different climate change-associated contexts, such as rising temperatures and lack of precipitation. The frequency and duration of drought are increasing in most parts of the world (Spinoni et al. 2014; Wang et al. 2021b). Drought develops gradually and is currently one of the most unpredictable weather hazards affecting crop production. Therefore, to cope with potential future drought stress (DS), research on field management practices such as irrigation and fertilization is crucial to improve drought tolerance in crop production.

Maize is one of the most important crops for humans, and currently, the demand for maize is still increasing (Tanumihardjo et al. 2020). DS-induced yield reduction in maize ranges from 33-46%, while the occurrences of DS during the vegetative period decreases yield by 19%-26% (Fawen et al. 2022; Mi et al. 2018; Daryanto et al. 2017). It restricts plant growth, due to slow root and shoot growth and development, reduced nutrient uptake, etc., ultimately limiting crop yields (Daryanto et al. 2017; Ge et al. 2012a). Maize root respond to DS by an increase in root length, and root length density (RLD: total length of roots per unit of soil volume, cm cm^{-3}), which are also the key traits considered in the selection for drought stress tolerance (Inès et al. 2022; Hussain et al. 2019; Kou et al. 2022). In addition to higher RLD, higher specific root length (SRL, the ratio of root length and root dry biomass, cm g^{-1}) is also a sign of morphological adaptation to the exploration of nutrients and water, indicating that the metabolic demand per unit of root length is reduced (Lyu et al. 2016; Lynch 2015).

Besides drought, maize is highly sensitive to the amount of plant-available P in soils (Colomb et al. 2000; Nadeem et al. 2011a). Especially, during the early growth stages, P deficiency has irreversible impacts, leading to reduced leaf area, decreased light interception, and finally limited biomass accumulation (Nadeem et al. 2011a; Ning et al. 2023). Higher SRL, root length, and RLD are specific root morphological traits that promote plant growth under P deficiency, enhancing P exploration in LP soils (Lyu et al. 2016; Liu 2021; Lynch 2015). P must reach the root surface by spatial root growth and diffusion; the latter being governed by the effective diffusion coefficient and the concentration gradient between soil and root surface (Suriyagoda et al. 2014). It is known that diffusion is restricted by drought (Suriyagoda et al. 2014). Thus, plant P uptake and utilization are commonly restricted under drought (He and Dijkstra 2014). The study of Klamer et al. (2019) indicated that supplying higher or optimal amounts of P under DS conditions during early maize development significantly increased both root and shoot biomass. Additionally, P uptake was twofold higher compared to a low P fertilizer treatment. This increase could be attributed to the development of denser root hairs. In general, longer and thinner roots, and thus less metabolic costs increase P uptake from soil (Lynch 2015; Lynch 2013). However, the reserves of phosphate rock - a common P fertilizer source - will be depleted in 300-400 years, predominantly mined in regions like Morocco, China, and the United States (Cooper et al. 2011). As the easiest and highest-quality deposits are exploited, the remaining reserves will be of lower grade, harder to extract, and more costly to process (Cordell et al. 2009). As mineral P fertilizer is produced mainly with rock phosphate, there is an urgent need for alternative approaches to improve PUE, especially under DS conditions (Gong et al. 2022; Klamer et al. 2019). Maize production is therefore vulnerable to the dual stress of P deficiency and drought during the early growth stage.

Generally, plant-available P decreases significantly with soil depth due to decreasing soil microbial activity, tillage, and plant residues (Oliveira et al. 2022). The application of fertilizers in appropriate soil depth might compensate for P deficiency in deeper soils. Appropriate deep-placed P fertilizer results in a higher proportion of roots in the deeper soil layers, root length and RLD, and improves plant photosynthetic performance by delaying the onset of leaf senescence (Chen et al. 2024). Nkebiwe et al. (2016) and Wu et al. (2022a) concluded that deep subsurface fertilizer placement (depth: > 10 cm) improves deep rooting, thereby resulting in 14%-27% higher yield and nutrient uptake, especially when P is applied together with nitrogen (N). Under seasonal drought, applying fertilizers at 10 cm soil depth resulted in 27% higher shoot biomass, 24% higher grain yield, and nutrient uptake in oilseed rape (Su et al. 2015).

Therefore, deep placement of fertilizers is proposed as a potential strategy to mitigate the effects of drought and P shortage to optimize maize production. Given the higher demand for P in maize plants from deeper soil layers, it is expected that a deep placement of P fertilizer will result in higher plant available P, benefiting root and shoot growth of maize. Hence, this study addressed the following hypotheses: Under drought conditions, 1.) maize plant growth and P uptake during the early stage can be restricted further by P deficiency, 2.) P fertilizer to lower soil layer leads to higher root length in deeper soil layers compared to upper soil layer fertilizer; 3.) P uptake can be improved by placed P fertilizer in lower soil layer, at different P fertilizer amounts. Here, the interaction of drought, fertilizer placement and amounts are illustrated by examining (i) root and shoot growth, (ii) P uptake, and (iii) PUE.

2. Materials and Methods

2.1. Greenhouse trial

A pot experiment was conducted in the Phytotechnikum research greenhouse of the University of Hohenheim (Stuttgart, Germany) from beginning of August until end of September 2022. Air temperature and humidity were monitored and recorded every ten minutes with a HOBO weather station (Onset Computer Corp Bourne, MA 02532, United States). Daily mean air temperature ranged from 21.6-28.1 °C and relative humidity ranged between 31.3-64.3% (Fig. S1). Additional lighting was provided by high-pressure sodium lamps (SOD AGRO 400, DH Licht GmbH, Wülfrath, Germany) from 5 a.m. to 9 a.m. and 6 p.m. to 9 p.m. Lamps were placed 2 m above the plant canopy in the middle row of each block.

The soil used in this pot trial was collected from 0-5 cm depth of a field low in P at the research station Oberer Lindenhof (48° 28′ 26″ N, 9° 18′ 12″ E) of the University of Hohenheim in November 2020. The collected soil was sieved through a 5-mm sieve and homogeneously mixed after air-drying for seven days. Selected physical and chemical soil properties are presented in Table 1.

Table 1. Physical and chemical properties of the experimental soil and the mixture of soil and sand used in the pot experiment.

Soil				Soil + Sand
Texture	**pH**	**Olsen-P** mg 100 g^{-1}	**K** mg 100 g^{-1}	**Olsen-P** mg 100 g^{-1}
Silty clay loam	5.3	1.4	2.0	1.0

The experiment comprised three factors, 1) two P fertilizer amounts applied as diammonium phosphate (DAP, 18% N–20% P) at the start of the trial: Low-P (LP): 2 mg P 100 g^{-1} soil dry matter (DM), and High-P (HP): 12 mg P 100 g^{-1} soil DM) with No-P fertilizer (NP) treatment as control, 2) three fertilizer placements: Mixed (M): homogenously applied and mixed to the entire soil depth (0-18 cm), Lower (L): homogenously applied and mixed only to the lower half of the soil depth (10-18 cm), Upper (U): homogenously applied and mixed only to the upper half of the soil depth (0-9 cm)), and 3) two soil water contents: drought stress (DS): 45% of soil water holding capacity (WHC) and well-watered (WW): 75% WHC. For each water content level, a control with no P-fertilizer application was added. In total, there were ten treatments as illustrated in Fig. 1.

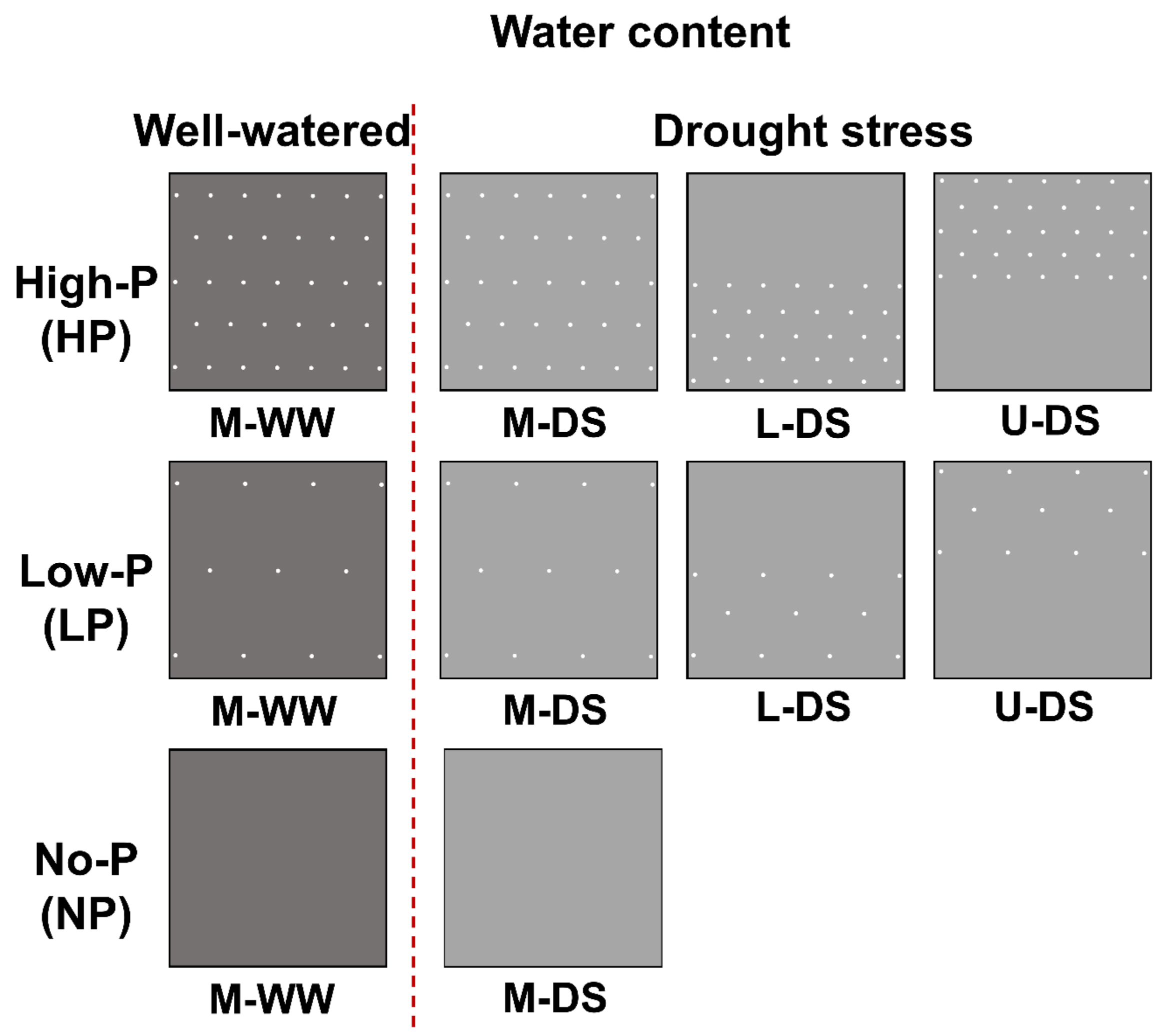

Fig. 1 Schematic diagram of the ten treatments in this trial. P fertilizer amount: No-P (NP), Low-P (LP, 2 mg 100 g^{-1} soil DM), and High-P (HP, 12 mg 100 g^{-1} soil DM), respectively; Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". The distribution and density of white dots illustrate fertilizer placement and the amount of P fertilizer added to the soil mixture.

The ten treatments differing in P fertilizer amount, placement, and water content were set up in Mitcherlich pots (20 cm diameter and 18 cm height). Each pot contained 6.4 kg soil mixture (70% soil and 30% quartz sand (DORSILIT® Nr.7, Gebrüder Dorfner GmbH Co, Germany)) with 15% water content.

After mixing soil and sand evenly, 0, 0.54 g, 3.26 g P were fertilized homogenously for all mixed (M) fertilizer treatments. For lower (L) placed fertilizer treatments, the lower half of the soil mixture was fertilized with 0.54 g or 3.26 g P accordingly, while the other half received no fertilizer. Upper (U) placed fertilizer treatments were the opposite. The difference in N fertilizer given by different amounts of DAP between treatments was balanced with stabilized ammonium sulfate fertilizer (21% N, NovaTec® Solub 21; Compo Expert, Münster, Germany) to achieve the same N quantity (150 mg N kg^{-1}). K was applied at a rate of 150 mg K kg^{-1}, and Mg was at a rate of 16 mg Mg kg^{-1} (Korn-Kali® + B, K+S Kali GmbH, Zielitz, Germany).

The mixed soil was filled into standard cylindrical Mitscherlich-pots (20 cm diameter and 18 cm height) according to the required depths of the experimental design. To protect the integrity of the soil mixture and even distribution and infiltration of irrigation water, 250 g of sand was evenly added to the top of the soil mixture in each pot.

Three seeds of the maize cultivar Ricardinio (S 230 / K 220, KWS SAAT SE & Co. KGaA, Einbeck, Germany) were sown at 3 cm depth in each pot on August 8th 2022. After sowing, water content of the substrate was adjusted to 60% WHC on the same day. At the one-leaf stage on August 15th 2022, plants were thinned to one plant per pot and drought stress treatments started. As plants treated with HP and M-WW (HP * M-WW) (Fig. 1) grew faster than others and consumed more water, water content of these pots was checked daily to meet the range of 60 to 75% to avoid plants in WW treatment suffering drought. If water content was below 60%, all pots were weighed and irrigated if needed to reach their target water content of 45% or 75% WHC for DS and WW treatments, respectively. The weight of plants was ignored for estimation of irrigation amounts.

Each treatment was destructively sampled on two harvest dates (the fourth-leaf stage (30 August, 22 days after sowing (DAS)) and the tenth-leaf stage (20 September, 43 DAS)) with four replicates (Fig. S2). The ten treatments and two harvest dates were laid out in a row-column design with three rows and seven columns, each replicated four times. The design had an incomplete factorial structure (Fig. S2). All pots were placed on four tables (2.5 m length × 1 m width × 1 m height) initially, corresponding to the four established blocks. The spacing between pots within each row was 0, and the distance of rows was 3 cm.

2.2. Data collection

Shoot biomass: Destructive samplings were conducted for the ten treatments with four replicates at each harvest after measuring *plant leaf area*. Shoot of each plant was cut at the soil surface and dried at 60 °C (FED 400, BINDER GmbH, Tuttlingen, Germany) to constant weight.

P analysis: All dried shoot samples were ground using a cutting mill equipped with a 0.5 mm sieve (Retsch cyclone mill twister, Retsch GmbH, 42781 Haan, Germany). After microwave digestion, shoot P concentration was measured by inductively coupled plasma-optical emission spectrometry (5110 ICP-OES; Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). Shoot P content was calculated by multiplying shoot P concentration with shoot dry biomass.

Root: After sampling of shoots, the entire soil from each pot was taken out and divided into the two horizontal soil depths: 0-9 cm and 10-18 cm. For each depth, roots were washed out carefully. Cleaned root samples were stored in 50% ethanol. At the second harvest, samples from the six treatments (M-DS, L-DS, and U-DS with HP and LP) were scanned using a root scanner (Epson Expression 10000Xl, Epson, Nagano, Japan). Root length was determined by analyzing the scanned images with WinRhizo (Regent Instruments Inc., Québec, Canada). Later, all root samples from the two harvests were dried at 60 ℃ for 3 d and dry matter was determined. Root length density (RLD, cm cm^{-3}) and specific root length (SRL, cm g^{-1}) were calculated for each pot and depth as follows (Kuchenbuch et al. 2009):

$$RLD = \frac{Root\ length}{\pi * r^2 * h}, \qquad (2)$$

while r refers to the radius of the pots and h refers to the height of the soil from which the root segment was harvested.

$$SRL = \frac{Root\ length}{Root\ dry\ biomass}, \qquad (3)$$

After the first harvest, all plants for the second harvest were placed directly on the ground (instead of the table) at the same point to prevent burning of leaves touching the lamps.

2.3. Statistical analysis

All data from the final harvest were analyzed with the following mixed model:

$$y_{ijklm} = \mu + b_i + r_{gi} + c_{hi} + \omega_j + \alpha_k + \tau_{kl} + (\alpha\omega)_{jk} + (\omega\tau)_{jkl} + \rho_{mj} + (\tau\rho)_{jklm} + e_{ijklm}, \quad (4)$$

where y_{ijklm} is the observation of water content j, P fertilizer application (with or without) k, P fertilizer amount l at fertilizer placement m tested in block i, μ is the intercept, b_i the fixed effect of block i, r_{gi} is the random effect of row g in block i, c_{hi} is the random effect of column h in block i, ω_j is the fixed effect of water content j, α_k is the fixed effect of P fertilizer application (with or without) k, τ_{kl} is the fixed effect of P fertilizer amount l nested in P fertilizer application (with or without) k, $(\alpha\omega)_{\omega jk}$ is the fixed interaction effect of P fertilizer application (with or without) k and water content j, $(\omega\tau)_{jk}$ is the fixed interaction effect of water content j and P fertilizer amount l nested within P fertilizer application (with or without) k, ρ_{mj} is the fixed effect of fertilizer placement m nested within water content j, $(\tau\rho)_{jkl}$ is the fixed interaction effect of fertilizer placement m in water content j and P fertilizer application (with or without) k at P fertilizer amount l, and e_{ijklm} is the error of y_{ijklm}. For data that were collected at both harvest dates, the model was extended by crossing all fixed effects with the factor harvest. This model can be described as follows:

$$y_{ijklmn} = \mu + b_i + r_{gi} + c_{hi} + \omega_j + \alpha_k + \tau_{kl} + (\alpha\omega)_{\omega jk} + (\omega\tau)_{jkl} + \rho_{mj} + (\tau\rho)_{jklm} + \beta_n + (b\beta)_{in} + (\omega\beta)_{jn} + (\alpha\beta)_{kn} + (\tau\beta)_{kln} + (\alpha\omega\beta)_{\omega jkn} + (\omega\tau\beta)_{jkln} + (\rho\beta)_{mjn} + (\tau\rho\beta)_{jklmn} + e_{ijklmn}, \quad (5)$$

where β_n is the fixed effect of harvest n, terms including β_n denote interaction effects with the n^{th} harvest and all other effects were analogously defined compared to model (4).

To calculate PUE, the factor P fertilizer application (with or without) in model (4) was replaced by P fertilizer amount (g pot^{-1}), and a slope of P fertilizer amount on shoot P content (g plant^{-1} of the pot) was fitted. In this case, the slope is interpreted as PUE (Ning et al., 2023).

In all cases, model pre-requirements of normal distribution and homogeneous variances of residuals were checked graphically. In case of significant effects found via global *F* test, a Fishers LSD test was performed. Results of multiple comparison were presented via letter display (Piepho 2004). In case of PUE, estimated slopes and their pair-wise differences were estimated with their standard errors via contrasts. These contrasts were subsequently used to create a letter display. Since most parameters were affected by the interaction of P fertilizer amount and water content (Fig. S3), as well as P fertilizer amount and fertilizer placement (Fig. S4), the results will be presented in two separate sections.

3. Results

3.1. Shoot biomass accumulation

P fertilizer amount and soil water content had a significant impact on plant shoot dry biomass at the fourth- and tenth-leaf stages ($p < 0.001$). Fertilizer placement significantly affected shoot dry biomass at the tenth-leaf stage (Table S1). The interaction of P fertilizer amount and soil water content affected the accumulation of shoot dry biomass at the tenth-leaf stage.

Shoot dry biomass increased with the increase of P fertilizer amount and water content at the fourth-leaf stage (Fig. 2A). HP increased shoot dry biomass by 47% in comparison to LP. Shoot dry biomass of plants under WW conditions amounted to 1.83 g plant^{-1}, and thus was 87% higher than under DS conditions (0.98 g plant^{-1}). Under DS, plants in the U-DS treatment had 50% higher shoot dry biomass compared to L-DS, while fertilizer placement did not significantly affect shoot dry biomass (Fig. 2B).

At the tenth-leaf stage, plants in the HP-WW treatment resulted in the highest amount of biomass (25.98 g DM plant^{-1}), while LP decreased shoot dry biomass by 42% (Fig. 2C). In the HP-DS treatment, shoot dry biomass decreased by 55% compared to the WW treatment. Lowest shoot dry biomass was obtained with 7.28 g plant^{-1} in the LP treatment, indicating a 38% reduction in biomass when compared to HP plants under DS. L-DS reduced plant dry biomass by 20% compared to M-DS and U-DS (10.19 g plant^{-1}) on average across all P fertilizer amounts (Fig. 2D).

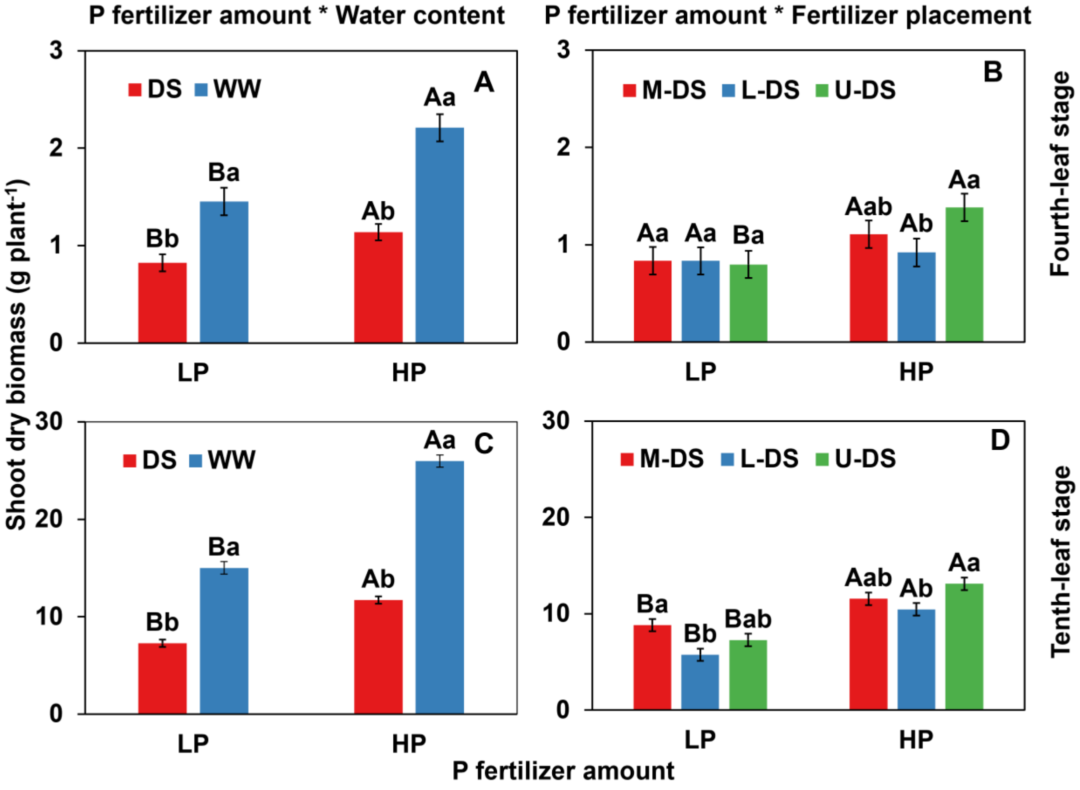

Fig. 2 Effects of P fertilizer amount, water content, and P fertilizer amount and fertilizer placement on plant shoot dry biomass at the fourth-leaf stage (A and B) and the tenth-leaf stage (C and D). P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

3.2. Shoot P uptake

3.2.1. Shoot P concentration

As shown in Table S1, P fertilizer amount significantly affected plant shoot P concentration at the fourth- and tenth-leaf stage ($p < 0.001$). Fertilizer placement significantly affected plant shoot P concentration at the fourth-leaf stage, while water content affected plant shoot P concentration at the tenth-leaf stage. The interaction of P fertilizer amount and fertilizer placement significantly influenced shoot P concentration at the tenth-leaf stage.

At the fourth-leaf stage, shoot P concentration of plants in the HP treatment was 3.12 g P kg^{-1}, indicating 56% higher P amounts in shoot dry biomass when compared with LP treatment (Fig. 3A). Under LP, L-DS did not decrease shoot P concentration among different fertilizer placements. Under HP, shoot P concentration (2.83 g P kg^{-1}) decreased by 16% in L-DS compared to M-DS, and did not differ from U-DS.

HP increased shoot P concentration by 44% (1.87 g P kg^{-1}) compared to LP (1.30 g P kg^{-1}) at the tenth-leaf stage (Fig. 3B). Under HP, L-DS showed 11% and 18% lower shoot P concentration than M-DS and U-DS treatments, respectively. With LP, a shoot concentration of 1.46 g P kg^{-1} was measured in the L-DS treatment, which was 25% higher than U-DS.

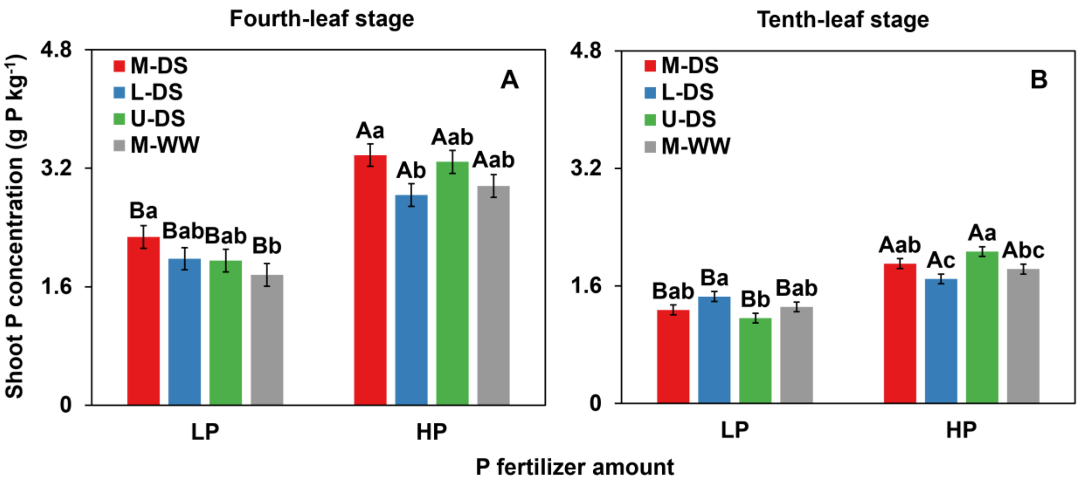

Fig. 3 Effects of P fertilizer amount, fertilizer placement, and water content on shoot P concentration at the fourth-leaf stage (A) and the tenth-leaf stage (B). P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75%

WHC). The two factors, fertilizer placement, and water content are combined with "-". Upper-case letters compare P fertilizer amounts within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount level. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

3.2.2. Shoot P content

P fertilizer amount, water content, and fertilizer placement significantly affected shoot P content at the fourth- and tenth-leaf stage. Shoot P content was also significantly affected by the interaction of P fertilizer amount and water content as well as P fertilizer amount and fertilizer placement at both harvest dates (Table S1).

At the fourth-leaf stage, shoot P content was 56% lower in LP compared to HP. In LP, plants under DS had 0.0017 g P plant^{-1}, while shoot P content of plants in the WW treatment increased by 58% (Fig. 4A). With HP, plants under DS and WW conditions had 119% and 274% higher shoot P contents compared with those with the dual stress of LP and DS, respectively. Plants under HP in the L-DS treatment exhibited a 32% decrease in shoot P content compared to those with M-DS and U-DS treatments. However, no significant difference was observed in shoot P content of plants with LP at different fertilizer placements (Fig. 4B).

LP decreased shoot P content by 58% compared to HP (0.035 g plant^{-1}) at the tenth-leaf stage, while in both P fertilizer amounts, shoot P content increased with increasing water content (Fig. 4C). HP under DS conditions increased shoot P content by 26% compared to LP with WW, in comparison to the treatment with the dual stress of LP and DS. With HP applied, L-DS exhibited a 20% and 35% decrease in shoot P content compared to M-DS and U-DS treatments, respectively (Fig. 4D). With LP, plants with L-DS and U-DS treatments had similar shoot P contents, which were 26% lower than those with M-DS.

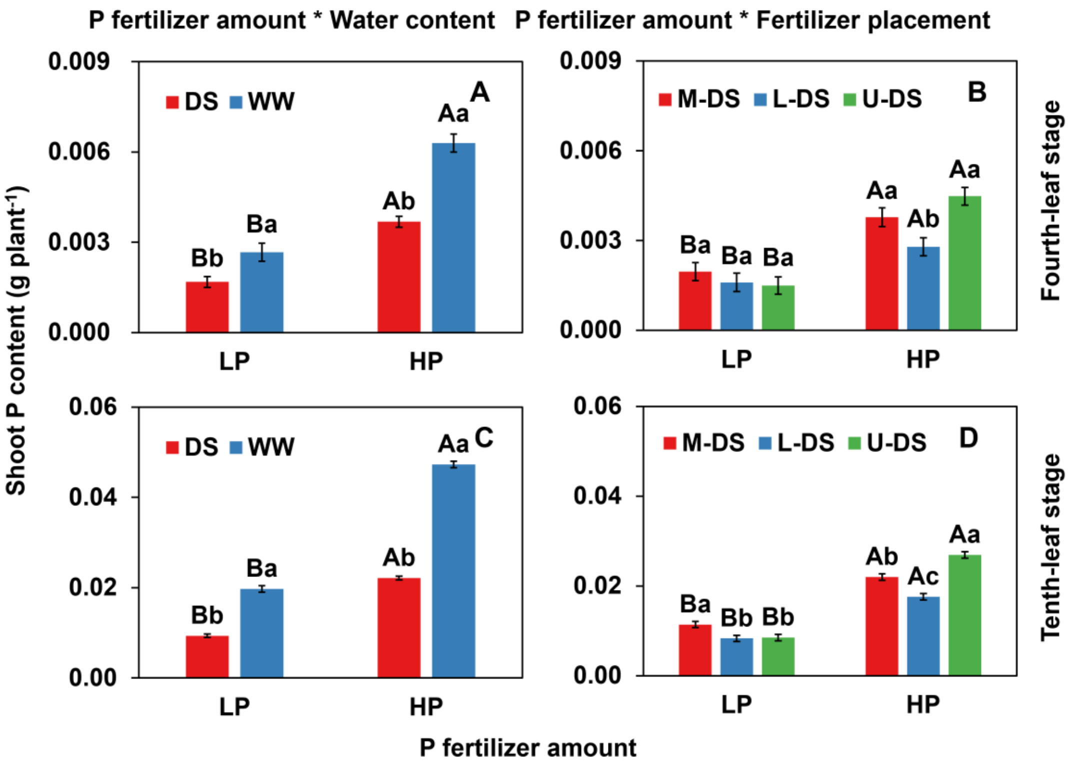

Fig. 4 Effects of P fertilizer amount, water content, and P fertilizer amount and fertilizer placement on shoot P content at the fourth-leaf stage (A and B) and the tenth-leaf stage (C and D). P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

3.2.3. P use efficiency

P use efficiency (PUE) was affected by the three factors: P fertilizer amount, water content, and fertilizer placement at the tenth-leaf stage ($p < 0.001$) (Table S1). The interaction between P fertilizer amount and water content, as well as between P fertilizer amount and fertilizer placement, also exhibited significance. Plants with LP showed a higher PUE, which was more than twice as high as those of the treatments with HP (Fig. 5). PUE of drought stressed plants was 42% lower than PUE of plants that were under WW condition. PUEs of plants with L-DS and U-DS were similar in the LP treatments, which was around 30% lower than those with M-DS. In HP, plants with M-DS and U-DS exhibited 27% and 57% higher PUE than those with L-DS, respectively.

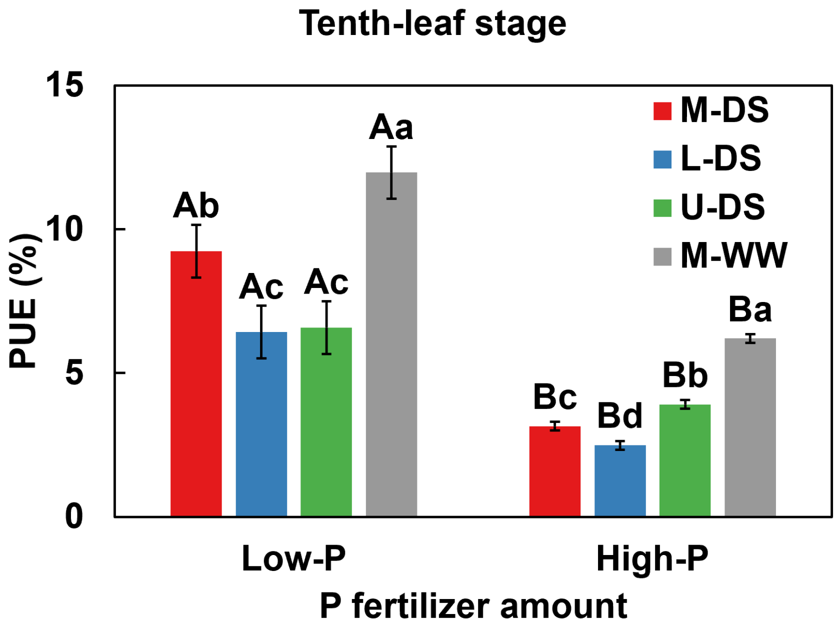

Fig. 5 Effects of P fertilizer amount, fertilizer placement, and water content on P use efficiency (PUE) at the tenth-leaf stage. P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content, are combined with "-". Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, *p* < 0.05. Error bars indicate the standard error of the least squares means.

3.3. Root growth and development

3.3.1. Root biomass

At the fourth-leaf stage, water content significantly affected root dry biomass in 10-18 cm depth and total root dry biomass in 0-18 cm depth. Fertilizer placement affected root dry biomass in both depths of 0-9 cm and 10-18 cm, as well as the total root dry biomass (Table S2). Root dry biomass at the tenth-leaf stage was significantly affected by P fertilizer amount, water content, and their interaction. Fertilizer placement showed a significant impact on root dry biomass in the depth of 0-9 cm and total root dry biomass at the tenth-leaf stage, while no difference was observed in 10-18 cm.

At the fourth-leaf stage, root dry biomass in 10-18 cm and the total root dry biomass in 0-18 cm depth increased by 95% and 75% under DS compared to WW conditions, (Fig. 6 B and C). Plants in the LP treatment indicated 12% lower total root dry biomass (0-18 cm depth) compared to plants in HP (Fig. 6C). On average across P fertilizer amounts (LP and HP), U-DS increased root dry biomass in 0-9 cm and 0-18 cm depths, while plants in L-DS showed 26% and 50% higher root dry biomass in 10-18 cm depth compared to plants in M-DS and U-DS, respectively (Fig. 6D).

At the tenth-leaf stage, DS with LP and HP reduced root dry biomass in 0-9 cm depth by 34% and 46% (Fig. 6E), in 10-18 cm depth by 50% and 69% (Fig. 6F), and total root dry biomass by 57% and 40% (Fig. 6G), respectively, compared to WW condition. On average across P fertilizer amounts (LP and HP), L-DS decreased root dry biomass in 0-9 cm depth by 44% compared to M-DS and U-DS, and total root dry biomass by 23% (Fig. 6H). No effect of fertilizer placement on root dry biomass in 10-18 cm depth was found (Fig. 6H).

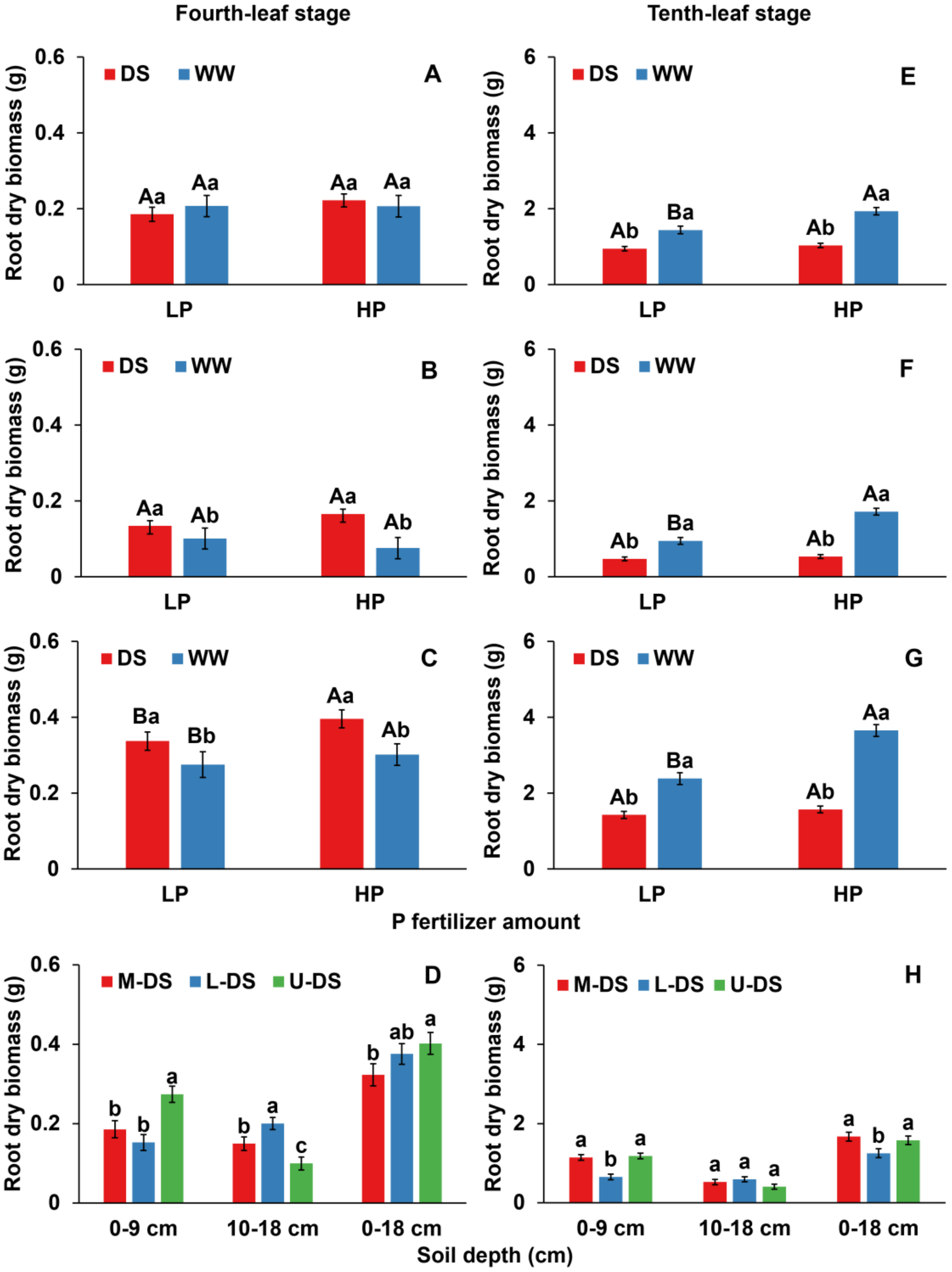

Fig. 6 Effects of P fertilizer amount and water content (A-C, E-G), and effects of fertilizer placement (D, H) on maize root dry biomass (g) in different soil depths (0-9 cm, 10-18 am, and 0-18 cm) at the fourth-leaf stage (A-D) and the tenth-leaf stage (E-H). P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed

fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

3.3.2. Root length

Significant differences in root length and root length density (RLD) under DS occurred only for fertilizer placement (Table S3). Under DS conditions, fertilizer placement affected root length and RLD in 0-9 cm and 10-18 cm depths at the tenth-leaf stage, but no significant effects on root length and RLD were observed for the total root. On average across P fertilizer amount, root length and RLD in 0-9 cm depth with M-DS and U-DS were 132% higher than those with L-DS, while M-DS and L-DS increased root length and RLD by 114% in 10-18 cm depth than U-DS (Table 2).

As shown in Table S3, specific root length (SRL) under DS was affected by fertilizer placement and the interaction between P fertilizer amount and fertilizer placement at the tenth-leaf stage. L-DS increased SRL by 67% and 175% compared to M-DS and U-DS treatments (Table 2). For M-DS and U-DS, no significance in SRL was found between LP and HP, while with L-DS, roots with LP exhibited an increase in SRL of 22% compared to those with HP.

Table 2 Effects of P fertilizer amount and fertilizer placement on maize root length, root length density (RLD), and specific root length (SRL) in different soil depths under drought stress (DS) at the tenth-leaf stage. P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC). Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Numbers with at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

P fertilizer amount	**Fertilizer placement**	**Water content**	**Root length (cm)**			**RLD (cm cm^{-3})**			**SRL (cm g^{-1})**
			0-9 cm	**10-18 cm**	**0-18 cm**	**0-9 cm**	**10-18 cm**	**0-18 cm**	
LP	M	DS	7071 Aa	4351 Aa	11421 Aa	13246 Aa	8150 Aa	10698 Aa	2775 Ab
	L	DS	3066 Ab	6476 Aa	9542 Aa	5744 Ab	12132 Aa	8938 Aa	5768 Aa
	U	DS	9345 Aa	2608 Ab	11952 Aa	17506 Aa	4885 Ab	11195 Aa	1696 Ab
HP	M	DS	6492 Aa	5952 Aa	12444 Aa	12162 Aa	11150 Aa	11656 Aa	3385 Ab
	L	DS	3335 Ab	6267 Aa	9603 Aa	6248 Ab	11741 Aa	8995 Aa	4517 Ba
	U	DS	6733 Aa	3326 Ab	10059 Aa	12613 Aa	6231 Ab	9422 Aa	2042 Ac

4. Discussion

4.1. Effects of P fertilizer amount and drought on root growth, P uptake, and shoot growth in early maize

P strongly affects maize shoot and root growth and development, as well as nutrient uptake (Ning et al. 2023; Ho et al. 2005). In our study, effects of DS were detected on root growth earlier compared to P deficiency. At the fourth-leaf stage, DS resulted in higher total root dry biomass, particularly at 10-18 cm depth. Increased root biomass in deeper layers enhanced water exploration under DS conditions (Lynch 2013; Hermans et al. 2006). At the tenth-leaf stage, root dry biomass decreased with the reduction of P fertilizer amount and water content. At this point, the plants were probably not adequately supplied with nutrients (Li et al. 2021a), caused by LP and DS. Under LP, WW resulted in 39%, 77%, and 52% higher root biomass in 0-9 cm depth, 10-18 cm depth, and the entire soil mixture, respectively, compared to DS. Besides higher root dry biomass, P exploration in LP soils can be enhanced by longer roots, thinner root hairs, and higher specific root length for nutrient acquisition (Postma et al. 2017; Lynch 2015). However, in our study, no difference was observed in dry root biomass, root length, and RLD between LP and HP in different soil depths under drought stress (Fig. 6 and Table 2). In the report by Klamer et al. (2019), root dry biomass in HP under DS was more than doubled compared to the LP treatments under WW conditions six weeks after sowing, where water control started at 19 DAS and plants were gradually exposed to DS. In our study, the drought stress might have been too severe to show this positive effect of HP.

Shoot growth, however, was affected by P deficiency and DS at the fourth-leaf stage. In our study, LP resulted in a smaller leaf area, reduced (-31%) shoot dry biomass and shoot P concentration, and lower (-54%) shoot P content compared to HP at both growth stages, which is consistent with the study of Zhan et al. (2019). The shoot P concentration of the plants with HP amounted to 3.1 g P kg^{-1} at the fourth-leaf stage, reaching the minimum shoot P concentration threshold (Li et al. 2021a; Zhan et al. 2019). Therefore, maize plants under WW and HP conditions should be free from DS and P deficiency. DS probably restricted plant growth due to a low nutrient diffusion rate in the soil (Suriyagoda et al. 2014). It resulted in 50% lower shoot dry biomass at the fourth- and tenth-leaf stages, 21% lower P concentration at the tenth-leaf stage, and 40% and 53% lower shoot P content at the fourth- and tenth-leaf stages, respectively, compared to WW conditions, with the impact of DS diminishing as P amounts increased (Zhan et al. 2019). A meta-analysis showed that the difference of plants under dual stress highly depends on various processes, such as plant nutrient uptake capacity, root system plasticity, P rate, and the severity and duration of drought cycles (Suriyagoda et al. 2014). The dual stress of LP and DS resulted in the lowest shoot biomass and P uptake (Fig. 2 and Fig. 4), as DS not only restricted P diffusion to plants but also exacerbated the impact of P deficiency on shoot growth, consistent with Kaya et al. (2020). The results showed that plants with HP under DS accumulated less shoot dry biomass, had higher shoot P concentration and higher P content than those with LP under WW conditions. Differently, the magnitude of the increase in shoot dry biomass and P uptake in response to increased soil water content was greater than that of increased P fertilizer amounts, which might be due to the relatively lower initial soil water content (Klamer et al. 2019).

4.2. Effects of P fertilizer amount and fertilizer placement on root growth, P uptake, and shoot growth in early maize under drought

Different P fertilizer placements could affect the root structure vertically in the soil profile, and influence plant growth and nutrient uptake (Nkebiwe et al. 2016; Wu et al. 2022). Higher root length, RLD, and SRL are specific root morphological traits that promote plant growth under P deficiency, enhancing P exploration in LP soils (Lyu et al. 2016; Liu 2021; Lynch 2015). At the fourth-leaf stage, plant root and shoot growth have already been under nutrient stress due to drought, while root growth in 10-18 cm was enhanced with L-DS treatments compared to M-DS and U-DS for nutrient and water uptake. Until the tenth-leaf stage, U-DS led to higher root dry biomass and higher root length and RLD in 0-9 cm depth compared to L-DS. Root systems with enhanced topsoil foraging acquire P more efficiently than others of equivalent size, allowing plants to have superior P acquisition and growth in LP soils (Lynch and Brown 2001). HP applied to 0-9 cm depth resulted in higher shoot dry biomass and P uptake than in 10-18 cm depth, when compared to LP. This benefit persisted until the tenth-leaf stage, even though P was placed in 10-18 cm depth. L-DS contributed to higher root dry biomass, root length, and RLD in 10-18 cm depth compared with U-DS (Fig. S5 (B and D) and Fig. S6). Wu et al (2022a) presented that plants with deep-placed fertilizer (depth: 15 cm or 25 cm) exhibited higher root length and RLD at the silking stage. Fertilizer placement at 15 cm induced the center of gravity of the maize root system to shift downward from the depth of 5 cm to 15 cm depth compared to a fertilizer placement at 5 cm depth, and the roots were more evenly distributed vertically (Chassot et al. 2001). L-DS fertilizer at both P fertilizer amounts contributed to higher SRL compared to M-DS and U-DS (Table 2). Therefore, L-DS optimized root exploitation for P and water at low metabolic costs under DS (Lynch 2015). As the induced fine root proliferation due to P fertilizer placement triggers an organic acid ion response in the root system in response to P deficiency, which could benefit plant P uptake (Zhang et al. 2023).

In our study, there was no advantage of L-DS in terms of shoot dry biomass, and P uptake, especially in HP up to the tenth-leaf stage (Fig. 2 and Fig. 4). Wu et al. (2022a) presented the advantage of fertilizer placement at 15 cm and 25 cm on shoot biomass starting from jointing to maturity stage compared with fertilizer placed at 5 cm under field conditions. 25% higher shoot biomass was observed in the treatment where fertilizers were applied at 5 cm depth compared to treatments where fertilizer was applied at 15 cm, 25 cm at around 30 DAS. The gap in shoot dry biomass, P content, and PUE between L-DS and U-DS under LP was smaller compared with HP. In LP, shoot dry biomass and P uptake were lower with L-DS and U-DS compared to M-DS. However, in the case of HP, U-DS resulted in higher shoot dry biomass and P uptake. The smaller variation in shoot biomass accumulation and P uptake at the early stage between L-DS and U-DS under LP conditions, compared to HP conditions, allowed plants in the L-DS treatment to catch up in growth and P uptake when compared to those in U-DS. Plants in L-DS could obtain sufficient nutrients from deeper soil layers at later stages, rather than plants in U-DS, which received sufficient P supply at the very early stage (Zhan et al. 2019; Wu et al. 2022a). It is worth noting that sulfur, an essential nutrient, has become a limiting factor for plant growth in many areas, potentially affecting nutrient uptake and overall plant performance. The vertical distribution of sulfur in soil typically decreases with depth (Sankhyan et al. 2024). In our study, the introduction of sulfur through ammonium sulfate to balance N in DAP in LP and NP treatments may have influenced the effects of P fertilizer placement, particularly by enriching the upper soil layer with additional nutrients. This raises the possibility that differences in sulfur availability, rather than P alone, could have contributed to the observed effects on shoot biomass and P uptake. To fully understand the effects of P fertilizer placement, it is necessary to assess soil sulfur status and account for its potential influence. Despite these considerations, L-DS promoted an optimized root system architecture which is adapted to the dual stress of drought and P deficiency, thereby increasing the potential for sustainable maize production.

5. Conclusions

Soil water content and P amount impacted growth and development of maize plants in the juvenile phase. DS and LP diminished biomass accumulation and reduced P acquisition, compromising their ability to accumulate photosynthates and nutrients to support plant growth. The addition of drought further limited maize plant growth and development on top of P deficiency. While L-DS did not show an advantage in shoot biomass or P uptake up to the tenth-leaf stage compared to M-DS and U-DS, it promoted key root traits, such as greater root length, RLD, and SRL. These traits are critical for deep rooting, enabling plants to explore larger soil volumes for P and water in deeper layers, which is particularly beneficial under drought conditions. Our results provide detailed information on how growth and development of maize shoots and roots were affected by the amount of P fertilizer and soil water content. In the context of drought, to compensate for P deficiency, excessive P application risks higher P losses and may not effectively enhance maize production. This study highlights the potential of optimizing fertilizer placement depth to improve maize performance under P depletion and climate stress. However, field experiments over an entire growth period are necessary to determine whether the observed positive response in root characteristics also enhances above-ground growth in later stages.

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Supplementary Information

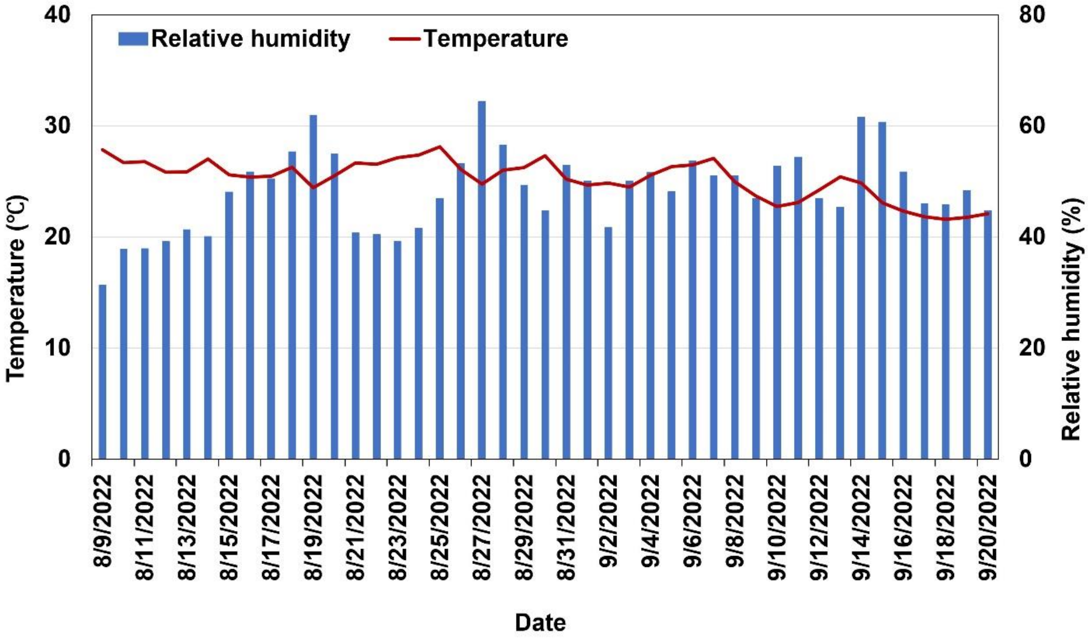
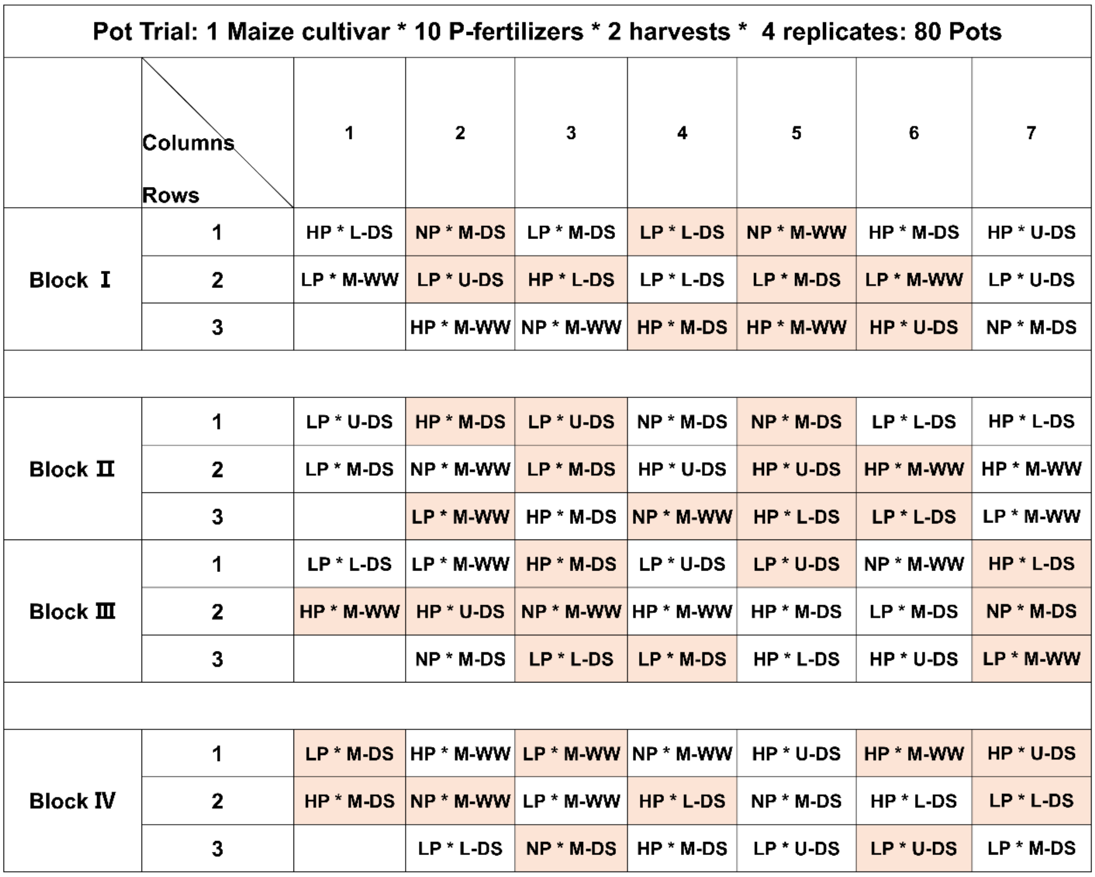


Fig. S1 Daily values of mean temperature and relative humidity during the experimental period.



Pot Trial: 1 Maize cultivar * 10 P-fertilizers * 2 harvests * 4 replicates: 80 Pots

	Columns Rows	1	2	3	4	5	6	7
Block Ⅰ	1	HP * L-DS	NP * M-DS	LP * M-DS	LP * L-DS	NP * M-WW	HP * M-DS	HP * U-DS
	2	LP * M-WW	LP * U-DS	HP * L-DS	LP * L-DS	LP * M-DS	LP * M-WW	LP * U-DS
	3		HP * M-WW	NP * M-WW	HP * M-DS	HP * M-WW	HP * U-DS	NP * M-DS
Block Ⅱ	1	LP * U-DS	HP * M-DS	LP * U-DS	NP * M-DS	NP * M-DS	LP * L-DS	HP * L-DS
	2	LP * M-DS	NP * M-WW	LP * M-DS	HP * U-DS	HP * U-DS	HP * M-WW	HP * M-WW
	3		LP * M-WW	HP * M-DS	NP * M-WW	HP * L-DS	LP * L-DS	LP * M-WW
Block Ⅲ	1	LP * L-DS	LP * M-WW	HP * M-DS	LP * U-DS	LP * U-DS	NP * M-WW	HP * L-DS
	2	HP * M-WW	HP * U-DS	NP * M-WW	HP * M-WW	HP * M-DS	LP * M-DS	NP * M-DS
	3		NP * M-DS	LP * L-DS	LP * M-DS	HP * L-DS	HP * U-DS	LP * M-WW
Block Ⅳ	1	LP * M-DS	HP * M-WW	LP * M-WW	NP * M-WW	HP * U-DS	HP * M-WW	HP * U-DS
	2	HP * M-DS	NP * M-WW	LP * M-WW	HP * L-DS	NP * M-DS	HP * L-DS	LP * L-DS
	3		LP * L-DS	NP * M-DS	HP * M-DS	LP * U-DS	LP * U-DS	LP * M-DS

Fig. S2 Experimental design. All the pink colored pots were harvested at the second harvest date. P fertilizer amount: No-P (NP), Low-P (LP, 2 mg 100 g^{-1} soil DM), and High-P (HP, 12 mg 100 g^{-1} soil DM), respectively; Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-".

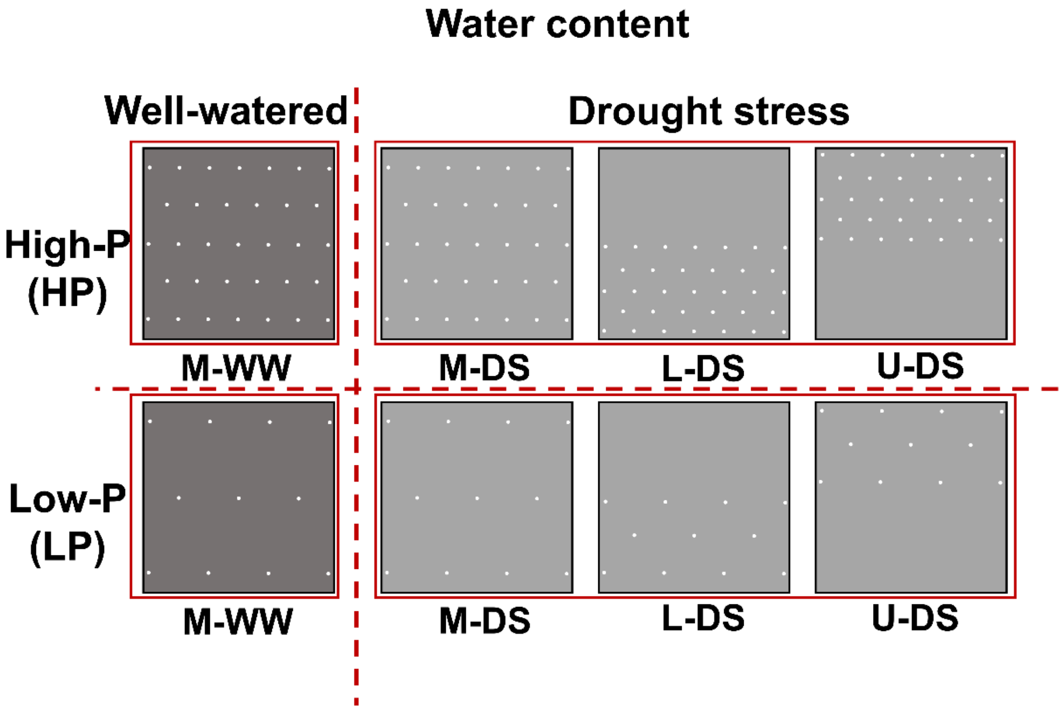


Fig. S3 Graphical representation of the interaction of P fertilizer amount and water content, bounded by red dashed lines. P fertilizer amount: No-P (NP), Low-P (LP, 2 mg 100 g^{-1} soil DM), and High-P (HP, 12 mg 100 g^{-1} soil DM), respectively; Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". The distribution and density of white dots illustrate fertilizer placement and the amount of P fertilizer added to the soil mixture.

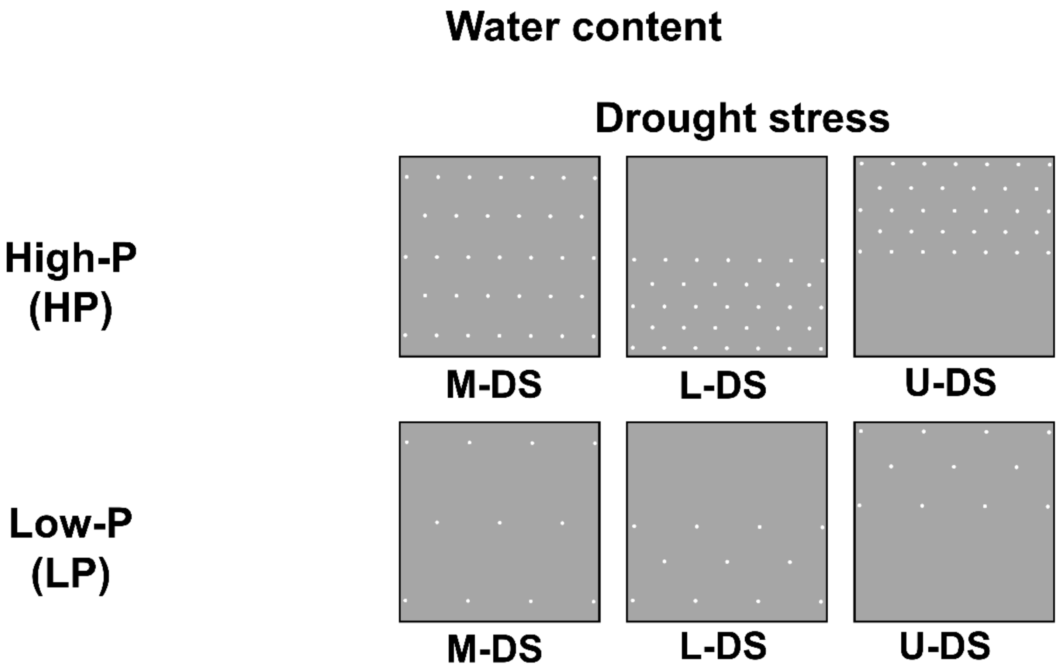


Fig. S4 Graphical representation of the interaction of P fertilizer amount and fertilizer placement. P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM), and High-P (HP, 12 mg 100 g^{-1} soil DM), respectively; Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC). The two factors, fertilizer placement, and water content are combined with "-". The distribution and density of white dots illustrate fertilizer placement and the amount of P fertilizer added to the soil mixture.

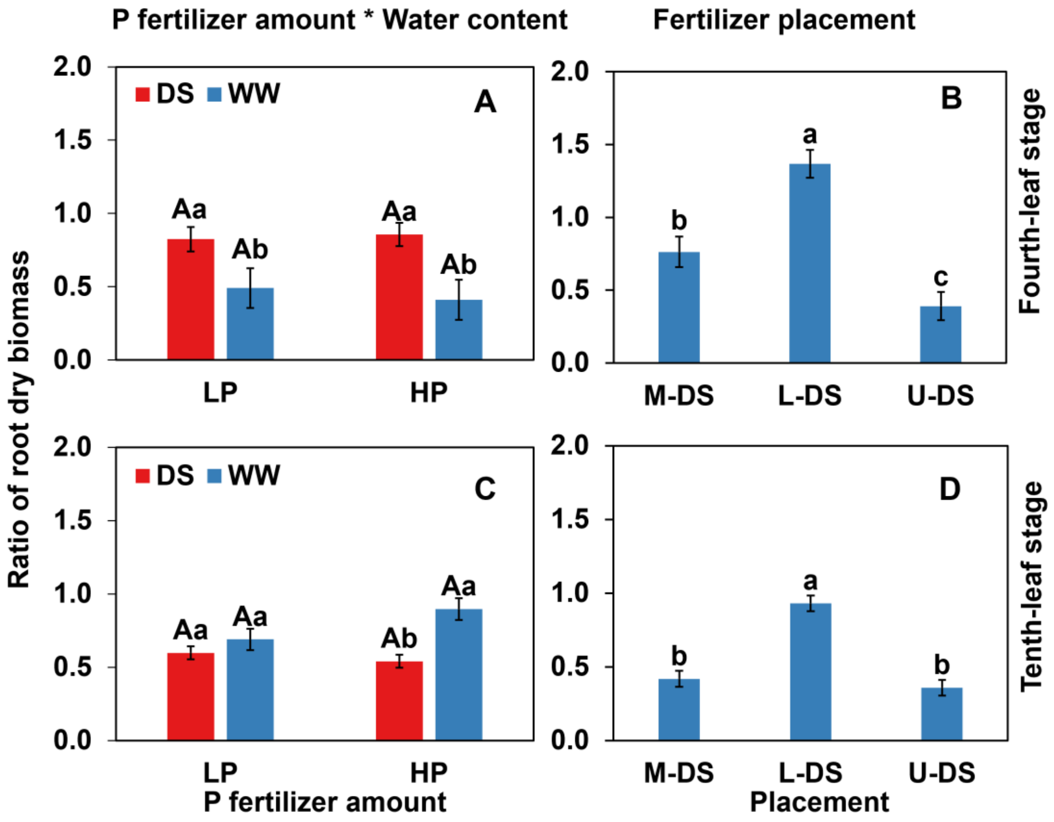


Fig. S5 Interaction of P fertilizer amount and water content, and effects of fertilizer placement on the ratio of root dry biomass distributed in 10-18 cm and 0-9 cm depths at the fourth-leaf stage (A and B) and the tenth-leaf stage (C and D). P fertilizer amount: Low-P (LP, 2 mg 100 g^{-1} soil DM) and High-P (HP, 12 mg 100 g^{-1} soil DM); Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC) and Well-watered (WW, 75% WHC). The two factors, fertilizer placement, and water content are combined with "-". Upper-case letters compare P fertilizer amount within each water content or fertilizer placement level, while lower-case letters compare water content or fertilizer placement levels within each P fertilizer amount. Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

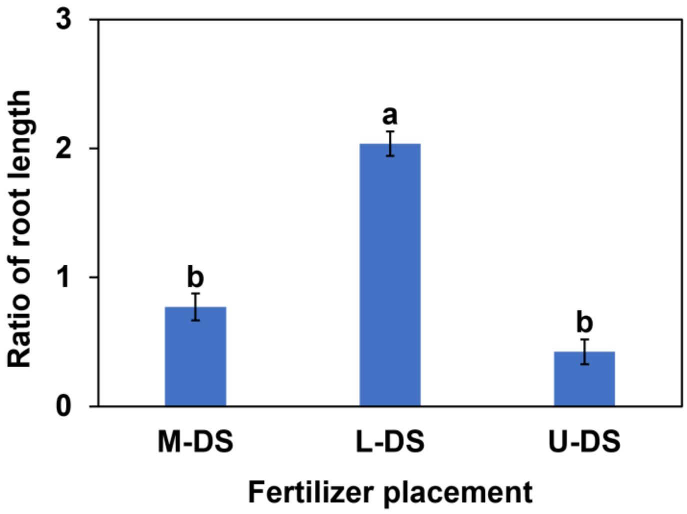


Fig. S6 Effects of fertilizer placement on the ratio of root length distributed in 10-18 cm and 0-9 cm depths at the tenth-leaf stage. Fertilizer placement: mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm) placed fertilizer, respectively; Water content: Drought stress (DS, 45% WHC). The two factors, fertilizer placement, and water content are combined with "-". Bars headed by at least one identical letter did not differ significantly according to Fisher´s LSD test, $p < 0.05$. Error bars indicate the standard error of the least squares means.

Table S1 ANOVA for the effects of P fertilizer amount, fertilizer placement, and water content on plant shoot dry biomass, P concentration, P content, and PUE. Py, P fertilizer application (with or without); Wcon, soil water content; Pa, P fertilizer amount; P, fertilizer placement. ns: not significant at $\alpha = 0.05$.

Factor	**Shoot dry biomass**		**P concentration**		**P content**		**PUE**
	g plant^{-1}		**g P kg^{-1}**		**g plant^{-1}**		**%**
	Fourth-leaf stage	**Tenth-leaf stage**	**Fourth-leaf stage**	**Tenth-leaf stage**	**Fourth-leaf stage**	**Tenth-leaf stage**	**Tenth-leaf stage**
Py	<.001	<.001	<.001	<.001	<.001	<.001	.
Wcon	<.001	<.001	ns	<.001	<.001	<.001	<.001
Py*Wcon	0.015	<.001	ns	<.001	<.001	<.001	<.001
Pa(Py)	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Pa*Wcon(Py)	ns	<.001	ns	ns	0.002	<.001	<.001
P(Py*Wcon)	ns	0.004	0.035	ns	0.036	<.001	<.001
P*Pa(Py*Wcon)	ns	ns	ns	<.001	0.011	<.001	<.001

Table S2 ANOVA for the effects of P fertilizer amount, fertilizer placement, and water content on root dry biomass, the ratio of root dry biomass distributed in 10-18 cm and 0-9 cm depths, and root:shoot ratio of plant dry biomass at the fourth- and tenth-leaf stage. Py, P fertilizer application (with or without); Wcon, soil water content; Pa, P fertilizer amount; P, fertilizer placement. ns: not significant at $\alpha = 0.05$.

Factor	**Fourth-leaf stage**				**Tenth-leaf stage**			
	Root dry biomass (g)			**10-18: 0-9**	**Root dry biomass (g)**			**10-18: 0-9**
	0-9 cm	**10-18 cm**	**0-18 cm**		**0-9 cm**	**10-18 cm**	**0-18 cm**	
Py	ns	ns	ns	ns	<.001	<.001	<.001	0.004
Wcon	ns	<.001	<.001	0.003	<.001	<.001	<.001	0.031
Py*Wcon	ns	ns	ns	ns	0.005	<.001	<.001	0.033
Pa(Py)	ns	ns	0.009	ns	0.004	<.001	<.001	ns
Pa*Wcon(Py)	ns	ns	ns	ns	0.018	<.001	<.001	ns
P(Py*Wcon)	<.001	<.001	0.020	<.001	<.001	ns	0.031	<.001
P*Pa(Py*Wcon)	ns	ns	ns	ns	ns	ns	ns	ns

Table S3 ANOVA for the effects of P fertilizer amount and fertilizer placement with 45% WHC on root length and root length density (RLD, cm cm^{-3}) in different depths, the ratio of root length distributed in 10-18 cm and 0-9 cm depths, and specific root length (SRL, cm g^{-1}) at the tenth-leaf stage. Py, P fertilizer application (with or without); Wcon, soil water content; Pa, P fertilizer amount; P, fertilizer placement. ns: not significant at $\alpha = 0.05$.

Factor	**Tenth-leaf stage**							
	Root length cm				**RLD m m^{-3}**			**SRL**
	0-9 cm	**10-18 cm**	**0-18 cm**	**10-18: 0-9**	**0-9 cm**	**10-18 cm**	**0-18 cm**	**cm g^{-1}**
Pa(Py)	ns	ns	ns	ns	ns	ns	ns	ns
P(Py*Wcon)	<.001	<.001	ns	<.001	<.001	<.001	ns	<.001
P*Pa(Py*Wcon)	ns	ns	ns	ns	ns	ns	ns	0.042

6 General Discussion

The main objective of this Doctoral Thesis was to evaluate various approaches to crop management to improve P utilization in maize production systems under P deficiency and drought stress. In **Paper I**, the response of different maize plants to different types of P fertilizer under soil liming was presented. The effects of P deficiency on root morphology and physiology were presented in **Paper II**. In addition, the effects of P fertilizer placement and the amount of P fertilizer on the early growth and development of maize under drought stress were shown in **Paper III**. Furthermore, the potential for improving PUE and maize production through deep fertilizer placement was elucidated by analyzing P uptake, root growth, and distribution.

The general discussion will integrate the various factors discussed in **Papers I-III** to evaluate the potential of crop management in improving PUE in the context of predicted P depletion and frequent droughts. In addition, it will provide a deeper insight into the management of P in maize production systems and the potential reduction of P losses.

6.1 Potential of improving PUE

6.1.1 Cultivar selection

Recent research indicates that the best-performing maize cultivars in terms of P use can be identified through multi-location analysis, regardless of the starter fertilizer applied (Weiß et al. 2021). P fertilizer has no influence on grain yield across locations, even though it significantly affects the growth and development of early maize plants (Roller et al. 2022). Therefore, choosing maize cultivars with better performance in P use is a fundamental guarantee for resilient crop production in variable environments (Weiß et al. 2021; Bailey-Serres et al. 2019). In paper I, the cultivar “Stabil” showed higher plant productivity and P uptake than “Ricardinio” under low-P soil conditions. These results were consistent with those observed in Einbeck, which has a similar environment in terms of temperature and precipitation but with higher P content in the soil (Weiß et al. 2021). Therefore, even though the same hybrid of maize exhibits varying performances under different conditions (Weiß et al. 2021), selecting cultivars with high P utilization and yield characteristics based on the specific environments is essential to achieve high yield and PUE under low-P conditions and in the context of P depletion.

The characteristics of maize roots, which are vital organs for plant production, differ morphologically and physiologically among cultivars. In publication II, only morphological responses of roots to different types of P fertilizer were found; no physiological responses were observed. Compared to Stabil, the root morphology, root growth, and distribution of Ricardinio are more sensitive to P availability related to P fertilizer type. Cultivars with high root biomass and RLD indicate higher water and P uptake, higher leaf area index and photosynthesis, and therefore higher yields (Qi et al. 2012). The colonization of arbuscular mycorrhizal fungi (AMF) or changes in maize root exudates showed weak trends in modern cultivars (Li et al. 2021b). However, different cultivars perform differently under various environmental conditions, especially stress conditions (Weiß et al. 2021; Roller et al. 2022).

The selection for specific root traits is important and likely to be very productive during the processes of breeding crops for low fertility (Lynch 2007; Lynch et al. 2005). Different cultivars vary in nutrient uptake and fertilizer management based on root characteristics. Cultivars with higher root exudates could enhance plant P uptake and P availability. Increased plant P availability could also suppress root hair development. Maize breeders looking to develop cultivars tolerant to P deficiency should focus on several key traits. Tolerant genotypes can maintain normal growth, produce higher biomass, and exhibit increased root-to-shoot ratios compared to sensitive lines (Sun and Zheng 2022; Gong et al. 2011). When screening for tolerant lines, it is crucial to consider traits such as relative dry weight, P-deficiency symptoms, chlorophyll content, shoot and root length, leaf area, and root volume (Azam et al. 2022). Tolerant lines exhibit higher P uptake efficiency, P utilization efficiency, and expression of P-responsive genes (Sun and Zheng 2022). Therefore, cultivars with high-yielding and P-efficient characteristics in low-P soils should be selected based on the targeted environment.

6.1.2 Potential of crop management (liming, rotation, and intercropping)

The availability of P for plants varies based on different soil pH (Barrow et al. 2020). Soil pH affects nutrient availability by influencing the absorption and dissolution processes of soil and affecting the rate of P uptake by plants. Barrow and Hartemink (2023) pointed out that the plant effect is stronger than the soil effect on P availability, and uptake decreases with increasing pH. Paper I observed that a higher soil pH after lime application decreased shoot biomass by 15% and reduced P uptake at the sixth-leaf stage. However, no significant effects from lime application were observed at the harvest stage. Additionally, paper II revealed no changes in root morphology and physiology from lime application (soil pH modified from 4.9 to 6). Lime primarily functions in the rhizosphere, affecting biotic processes such as root exudation, rhizosphere microbial activity, and the turnover of soil organic P, and abiotic processes like solubilization, adsorption, fixation, and even occlusion of P in the soil. In maize, the rhizosphere affected phosphatase activity and phosphorus-mineralizing-related bacteria more strongly than P fertilization (Guo et al. 2022). Thus, rhizosphere-based P management provides an efficient perspective to improve P utilization and crop production through effective P application practices, e.g., fertilizer placement. In addition to root organic acid anions in the root exudates, phytohormones are also essential for plants to regulate growth (Tariq et al. 2022). Many studies have reported that phytohormones play an essential role in the response to P deficiency.

Crop rotation and intercropping are also mainly attributed to rhizosphere interactions between different plant species regarding changes in root morphology and physiology in plants. The mechanisms behind these practices involve changes in the physical and chemical properties of the soil, as well as the transfer of various forms of P in soil. The root system acts as a mediator in the process of P acquisition from soil to support the growth of shoots. The roots and microbes in the rhizosphere interact between soil and the root system, influencing and being influenced by soil chemical properties, soil pH, and root exudate acids. The grain yield and P uptake were observed to be twice as much in the legume-maize intercropping system compared to the sole maize cropping system (Pypers et al. 2007). As presented, the soil pH with residues of the 1st crop from velvet bean varied from 4.8 to 5.7 over time, while the soil pH with/without residues of the 1st crop from maize and without residues of the 1st crop from velvet bean remained relatively stable (Pypers et al. 2007). It might be owing to the root-induced chemical changes, especially by arbuscular mycorrhizal fungi (AMF) infection (Pypers et al. 2007; Vanlauwe et al. 2000). Consequently, the intensity and quantity of P availability in the topsoil also increased generally (Pypers et al. 2007). The solubilization of RP was enhanced in the subsequent season (maize) in soils previously grown with legumes (Pypers et al. 2007). Zhang et al. (2016) noted that in the intercropping system of faba bean and maize, the root morphological plasticity of maize is stimulated by the presence of faba bean, resulting in increased shoot growth. Faba bean showed strong physiological but poor morphological root plasticity, while maize was the opposite (Gao et al. 2019). The colonization of AMF by faba bean was much higher than that of maize under either low or high P conditions, benefiting mobilization (Tang et al. 2020; Cozzolino et al. 2013). AMF symbiosis improves root vigor and promotes nutrient accumulation at various sites, affecting the content of organic acids in root exudates (Ma et al. 2022). Additionally, enhanced root exudates, such as citrate and acid phosphatase, by faba bean facilitate soil P availability, thereby increasing crop productivity and nutrient use efficiency of maize plants (Zhang et al. 2016). Under low plant-available P soils, maize plants grow and acquire nutrients more efficiently when intercropped with faba bean than under high plant-available P soils. This is owing to marked decreases in AMF species colonization for crops and acid phosphatase activity, especially in faba bean plants (Wen et al. 2019a). The growth and productivity of plants might be reduced or suppressed by high P in the soils.

The intercropping system of legumes, such as faba bean, soy, and lupin, with maize not only enhances soil rhizosphere acidification and activates soil P but also improves the metabolism of maize under drought conditions (Latati et al. 2016; Schwerdtner and Spohn 2022; Subramanian and Charest 1995). This facilitation is particularly effective in P-deficient conditions and can improve P fertilizer recovery efficiency (Sugihara et al. 2021). The intensity of plant-plant facilitation increases under P-deficient and drought conditions, with maize consistently benefiting from intercropping (Zhu et al. 2023). Root isolation experiments confirm that these benefits are driven by interspecific rhizosphere interactions (Zhu et al. 2023). Overall, intercropping legumes with maize is a sustainable agricultural strategy that can improve soil fertility, enhance plant P uptake, and increase crop yields in various environmental conditions (Schwerdtner and Spohn 2022; Latati et al. 2016). It is also meaningful for poor soils in the context of resource depletion and improving the use efficiency of RP, especially under more frequent drought scenarios.

6.1.3 Potential of P fertilizer management for improving PUE

More soluble P fertilizers, such as SSP, MAP, and DAP, are needed to achieve higher yields and meet human demand. However, the selection of P fertilizer could vary with cropping system soil conditions, such as soil pH (Zhao et al. 2022). In paper I, RP led to a 33% lower silage yield and 29% lower P content at harvest, with a 37% lower PUE than DAP. The lime application showed no effect on PUE, even when different fertilizers were applied. The relative effectiveness of RP was less than a tenth compared with that of superphosphate (Bolland and Barrow 1988). High application rates of slowly soluble P-fertilizer had even worse effects (Barrow and Bolland 1990). CMP, an alkaline fertilizer, improves soil pH and soil physical and chemical properties in acid soil. In contrast, DAP, a weak alkali-soluble P fertilizer, is transformed into plant available forms ($CaHPO_4$ and $Ca_8H_2(PO_4)_5 \cdot 5H_2O$) in neutral soil (Zhao et al. 2022). Therefore, applying CMP is recommended as an approach to improve maize production and soil quality. The use of slow-release P fertilizers to enhance PUE is controversial, as the absorption capacity of soil affects P diffusion more than fertilizer protection (Volf and Rosolem 2021). The soil characteristics mainly drive the increases in P availability or diffusion. Especially in maize production, plant roots acquire P mainly through larger exploration volumes due to root elongation rather than through dissolving P in the soil, making it available for plant uptake by legumes.

Maintaining an appropriate level of P supply at the root zone can maximize the efficiency of plant roots in mobilizing and acquiring P from the rhizosphere (Shen et al. 2011). This is achieved through an integration of root morphological and physiological adaptive strategies. Additionally, roots exude more efficient organic acids and phytohormones, which aid in the release of P from the rhizosphere (Li et al. 2021b). However, in paper II, no significant differences were observed in the rhizosphere soil solution regarding P availability based on the types of P fertilizers. Similarly, maize root systems respond to P deficiency mainly through morphological changes rather than physiological responses, as reported in many studies. However, root exudates vary depending on the position and parts of the root system. The results establish a connection between root system architecture and the presence of root organic acids and phytohormones.

Localized P fertilizer has been shown to enhance plant root development, including increased RLD and a lower rhizosphere pH, compared to broadcast application in the fertilizer depot (Zhang et al. 2023). Quinn et al. (2020) further supported this, demonstrating that sub-surface fertilizer application improved maize yield. These morphological and physiological responses enhance the release and capture of P. Generally, having a shallow root system enriched at the top is very beneficial for the absorption of P from the top layer due to the higher distribution of P (Zhu et al. 2005). However, crop growth is subject to many limitations, and inadequate P at the later stage is one of them, especially under drought stress (Lynch and Wojciechowski 2015). Deep fertilizer placement provides a potential solution to address this limitation. Strategically placing P fertilizers at greater depths in the soil ensures that crops have access to this vital nutrient throughout their growth cycle. P fertilizers need to be placed deep enough to ensure they remain in the moist soil longer than the plant growth period (Eck and Fanning 1961). The results in paper III showed that the advantages in maize growth led by a high P rate (12 mg 100 g^{-1}) were greatly limited under drought compared to those under sufficient water, especially in terms of root traits. At the R3 growth stage of soybean, which is the beginning of pod development, strip-tillage with deep banded P fertilizer enhanced more profound root growth, improved resilience to induced drought, and reduced yield loss due to drought stress (Hansel et al. 2017). Slow-release P fertilizer needs to be applied in cooperation with fertilizer placement due to the enriched root promotion. It provides approaches to optimize PUE under the dual stress of drought and P deficiency. Wang et al. (2024) present an optimized P fertilizer type and placement strategy.

Luo et al. (2024) emphasized the importance of optimizing P fertilizer management to enhance PUE and reduce environmental costs. Optimized P fertilizer management, which includes selecting the appropriate P fertilizer type, application rate, and application methods, could enhance maize productivity by 23.3% and PUE by 39.2% (Luo et al. 2024). Additionally, it reduced the P application rate by 12.4%-34.1% and the environmental cost by 24.3% (Luo et al. 2024). Optimized management of P fertilizer can enhance maize growth in P-deficient conditions or low P input maize production systems.

6.2 Outlook

The response of different maize cultivars to P deficiency varies. Cultivars with higher yielding and P utilization characteristics significantly improve PUE, accompanied by a pronounced increase in root growth. Given the late sampling stage and the field conditions, no differences in rhizosphere metabolites were observed due to the type of P fertilizer, maize cultivar, and soil liming. However, P deficiency has been observed to inhibit the growth of the root system in the early stages of maize development while promoting root system growth in the later stages, as observed with P deficiency.

Fertilizer placement significantly promotes the growth and elongation of the maize root system under very low P conditions. However, until the ten-leaf stage, the benefits of applying fertilizer in deeper soil zones are needed to compensate for the effects of P deficiency during the earlier growth stage. The benefits of deep fertilizer placement for plant roots might also extend to shoot growth and nutrient uptake during the later stage, thus improving maize production.

Overall, the thesis indicates that there are still unsolved research questions that should be considered in future studies. To come up with improved recommendations for farmers on P fertilization strategies, the mechanisms behind root self-regulation in terms of root morphology and rhizosphere metabolites in the early growth of maize with different PUE after perceiving P deficiency under specific soil conditions seem to be of major importance. In addition, clarification is needed on the promotion of root growth through the placement of P fertilizer. It remains to be seen how root growth might interact with different metabolites from different root zones under drought conditions. Lastly, future studies need to develop an optimal strategy for choosing P fertilizer type, amount, and placement under combined P deficiency and drought stress based on the principles of 4Rs nutrient stewardship.

7 Summary

Phosphorus (P) is a nonrenewable and finite resource for all living things. It plays a crucial role as an essential nutrient in crop production. However, plants have low efficiency in utilizing P due to its immobility and low bioavailability. P deficiency can cause irreversible effects, particularly during the early stages of maize growth. Drought further exacerbates nutrient uptake challenges, especially for P, by limiting its diffusion in the soil. Therefore, the dual stress of drought and P deficiency restricts plants' shoot and root growth. It is necessary to investigate the interaction between P deficiency and drought and better understand the response mechanisms, as the effect of P deficiency on plant growth precedes the plant's own drought regulatory mechanisms.

In **Paper I**, the effects of placed diammonium phosphate (DAP) and rock phosphate (RP) on the growth and development of two maize cultivars (Stabil and Ricardinio) were investigated combined with soil liming. Maize cultivars differed in their P utilization characteristics under low-P field conditions. The results showed that RP resulted in a lower leaf area index and light interception than DAP. This led to a 33% lower silage yield and a 29% lower P content at harvest. The PUE of RP was found to be 18%, which is 37% lower than that of DAP. Furthermore, soil liming reduced shoot biomass and caused a 35% decrease in shoot P content at the six-leaf stage. Maize cultivar ‘Stabil’ showed higher yield and P uptake. This paper demonstrated that placed RP could not be used as a substitute for DAP in silage maize production regardless of the application of lime to the soil.

Paper II explored the impact of different types of P fertilizer (DAP and RP) on the root systems of maize. The results showed that P deficiency in the early stages of growth hindered root growth. However, in later stages, the roots exhibited enhanced lateral root growth in response to P deficiency. Although the differences in organic acids and phytohormones across different zones of the maize root system were not significant due to the delayed sampling, it is still feasible and necessary to conduct further investigations on organic acids and phytohormones in various root locations.

Paper III tested deep P fertilizer placement as a strategy to alleviate combined drought and P deficiency stress in maize. It was tested under greenhouse conditions involving three factors: P fertilizer amount (low-P: LP, and high-P: HP), fertilizer placement (mixed (M, 0-18 cm), lower (L, 10-18 cm), and upper (U, 0-9 cm)), and soil water content (DS, 45% of soil water holding capacity (WHC)) and well-watered: WW, 75% WHC) and well-watered: WW). LP decreased shoot P content and both root and shoot biomass compared to HP. Under DS, root biomass increased by 50% and 95% in 0-18 and 10-18 cm depth at the fourth-leaf stage compared to WW treatment. However, root biomass decreased by at least 41% in different depths at the tenth leaf stage. Plants under DS consistently exhibited lower shoot biomass and P uptake at both stages. Although L-DS did not improve shoot growth and P uptake until the tenth-leaf stage, more than 55% higher root biomass and increased root length could be found in 10-18 cm depth compared to M-DS and U-DS treatments. This could potentially enhance P exploration in a larger soil volume and enable water absorption from deeper soil layers. However, no advantage in P uptake was observed with LP and HP until the ten-leaf stage.

In conclusion, this thesis highlights the importance of optimizing P utilization strategies in maize production systems facing the dual challenges of P deficiency and drought stress. While soil liming and cultivar selection can help, high-solubility P fertilizers like DAP remain irreplaceable by RP due to their superior ability to support root development. It also discussed the possibilities and mechanisms for mitigating P and water- deficiency by promoting root growth in deeper soil layers through applying P fertilizers. This study provides a comprehensive evaluation of the feasibility of various maize cultivation and management practices under combined P deficiency and drought conditions, offering valuable references and practical guidelines for sustainable maize production in resource-limited environments.

8 Zusammenfassung

Phosphor (P) ist eine nicht erneuerbare und begrenzte Ressource, die für alle Lebewesen von entscheidender Bedeutung ist. Es spielt eine zentrale Rolle als essenzieller Nährstoff in der Pflanzenproduktion. Pflanzen weisen jedoch eine geringe Effizienz bei der Nutzung von P auf, da es im Boden unbeweglich ist und nur eine geringe Bioverfügbarkeit hat. Ein P-Mangel kann irreversible Schäden verursachen, insbesondere in den frühen Wachstumsstadien von Mais. Trockenheit verschärft die Nährstoffaufnahmeprobleme, insbesondere für P, indem sie dessen Diffusion im Boden einschränkt. Die doppelte Belastung durch Trockenheit und P-Mangel begrenzt daher das Wachstum von Spross und Wurzeln. Es ist notwendig, die Wechselwirkung zwischen P-Mangel und Trockenheit zu untersuchen und die Reaktionsmechanismen besser zu verstehen, da die Auswirkungen von P-Mangel auf das Pflanzenwachstum den eigenen regulatorischen Mechanismen der Pflanze bei Trockenstress vorausgehen.

In **Paper I** wurden die Auswirkungen von platziertem Diammoniumphosphat (DAP) und Rohphosphat (RP) auf das Wachstum und die Entwicklung von zwei Maissorten (Stabil und Ricardinio) in Kombination mit Bodenkalkung unter Feldbedingungen mit niedrigem P-Gehalt in den Jahren 2020 und 2021 untersucht. Die Ergebnisse zeigten, dass RP im Vergleich zu DAP zu einem niedrigeren Blattflächenindex und einer geringeren Lichtinterzeption führte. Dies führte zu einem um 33 % niedrigeren Biomasseertrag und einem um 29 % niedrigeren P-Gehalt in der Trockenmasse bei der Ernte. Der PUE-Wert von RP lag bei 18 % und damit um 37 % niedriger als der von DAP. Darüber hinaus verringerte die Bodenkalkung die Sprossbiomasse und verursachte einen Rückgang des P-Gehalts im Spross im Sechs-Blatt-Stadium um 35 %. Die Maissorte „Stabil“ wies höhere Erträge und eine höhere P-Aufnahme auf. Aus der Studie kann geschlussfolgert werden, dass RP nicht als Ersatz für DAP im Anbau von Silomais fungieren kann, unabhängig ob eine Kalkung des Bodens und damit eine pH-Wert Änderung erfolgt.

In **Paper II** wurden die Auswirkungen verschiedener P-Düngerarten (DAP und RP) auf das Wurzelsystem von Mais untersucht. Die Ergebnisse zeigten, dass ein P-Mangel in den frühen Wachstumsstadien das Wurzelwachstum limitiert. In späteren Stadien zeigten die Wurzeln jedoch als Reaktion auf den P-Mangel ein verstärktes Seitenwurzelwachstum. Die Untersuchung verschiedener in der Wurzel gebildeter Hormone zeigten keine signifikanten Unterschiede bei organischen Säuren und Phytohormonen in den verschiedenen Zonen des

Maiswurzelsystems. Dies sollte jedoch in weiteren Studien mit einer höher aufgelösten Probenahme weiter untersucht werden.

In **Paper III** wurde das Potenzial einer P-Düngung in unterschiedlichen Bodentiefen in Kombination mit Trockenstress und damit unterschiedlichen Bodenwassergehalten untersucht. Der Versuch fand unter Gewächshausbedingungen statt und umfasste die drei Faktoren: P-Düngermenge (low-P: LP, und high-P: HP), Düngerplatzierung (gemischt (M, 0-18 cm), unten (L, 10-18 cm), und oben (U, 0-9 cm)), und Bodenwassergehalt (DS, 45% der Wasserhaltekapazität (WHC)) und bewässert: WW, 75% WHC). LP verringerte den P-Gehalt in den Sprossen sowie die Biomasse von Wurzeln und Sprossen im Vergleich zu HP. Unter DS nahm die Wurzelbiomasse in 0-18 und 10-18 cm Tiefe im Vier-Blatt-Stadium im Vergleich zur WW-Behandlung um 50% und 95% zu. Allerdings nahm die Wurzelbiomasse im Zehnblattstadium in verschiedenen Tiefen um ~ 41 % ab. Pflanzen unter DS wiesen in beiden Stadien durchweg eine geringere Sprossbiomasse und P-Aufnahme auf. Obwohl L-DS das Triebwachstum und die P-Aufnahme bis zum zehnten Blattstadium nicht erhöhte, konnte in 10-18 cm Tiefe eine um mehr als 55% höhere Wurzelbiomasse und eine größere Wurzellänge im Vergleich zu den Behandlungen M-DS und U-DS festgestellt werden. Dies könnte möglicherweise die P-Exploration in einem größeren Bodenvolumen verbessern und die Wasseraufnahme aus tieferen Bodenschichten ermöglichen, auch wenn bei LP und HP bis zum Zehnblattstadium kein Vorteil bei der P-Aufnahme beobachtet wurde.

Abschließend hebt diese Dissertation die Bedeutung der Optimierung von Phosphor (P)-Nutzungsstrategien in Maisproduktionssystemen hervor, die mit den doppelten Herausforderungen von P-Mangel und Trockenstress konfrontiert sind. Während Bodenkalkung und die Auswahl geeigneter Sorten hilfreich sein können, bleiben hochlösliche P-Dünger wie Diammoniumphosphat (DAP) aufgrund ihrer überlegenen Fähigkeit, das Wurzelwachstum zu fördern, durch Rohphosphat (RP) unersetzlich. Die Arbeit diskutiert außerdem die Möglichkeiten und Mechanismen zur Minderung von P- und Wasserdefiziten, insbesondere durch die Förderung des Wurzelwachstums in tieferen Bodenschichten mittels gezielter P-Düngerapplikation. Diese Studie bietet eine umfassende Bewertung der Machbarkeit verschiedener Anbau- und Managementpraktiken für Mais unter kombinierten P-Mangel- und Trockenheitsbedingungen und liefert wertvolle Referenzen sowie praktische Leitlinien für eine nachhaltige Maisproduktion in ressourcenbegrenzten Umgebungen.

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11 Supplementary Materials[2]

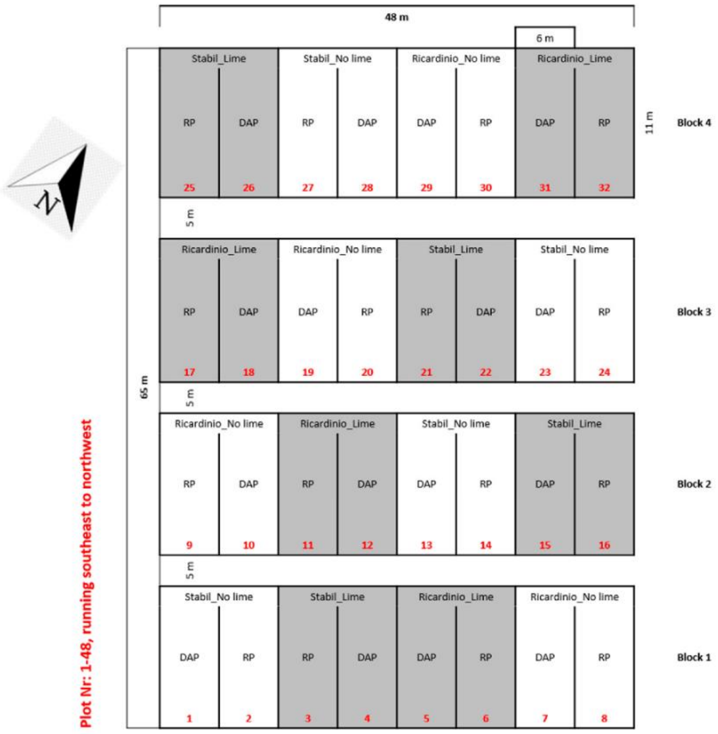

Figure S1. Experimental design in 2020. RP, rock phosphate; DAP, diammonium phosphate; with (Lime) and without (No lime) lime application. Main-plot factor is given on the top and sub-plot factor is presented below.

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[2] V refers to Publication I.

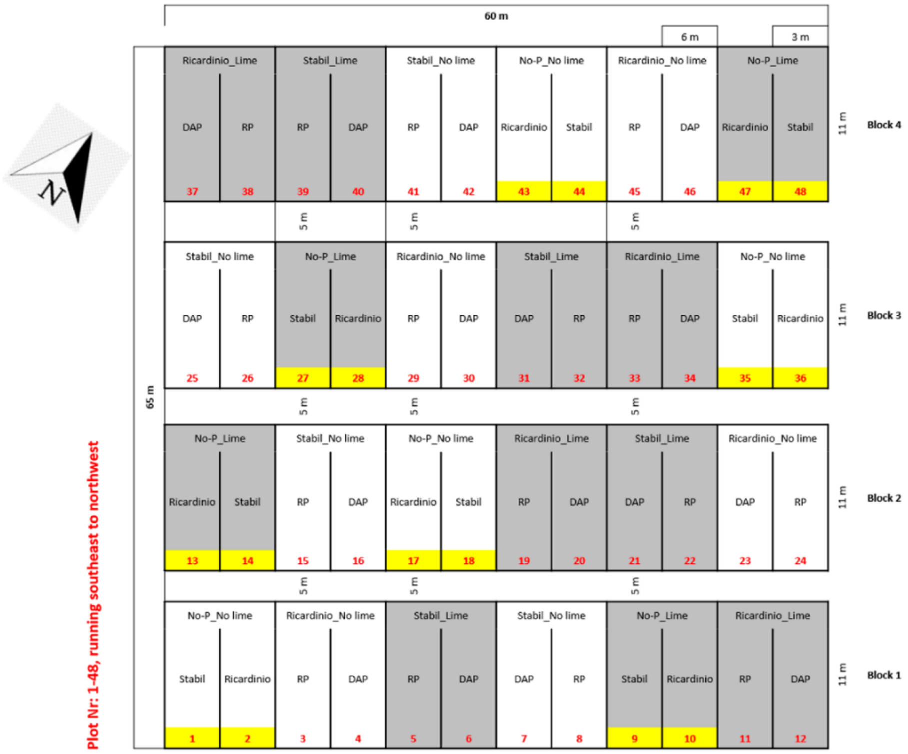

Figure S2. Experiment design in 2021.

Figure S2. Experimental design in 2021. No-P, no P-fertilizer applied; RP, rock phosphate; DAP, diammonium phosphate; with (Lime) and without (No lime) lime application. Main-plot factor is given on the top and sub-plot factor is presented below.

Table S1. Simple means for interactions of P-fertilizer (Pf), cultivar (Cul), lime application (Lime) on different traits in 2020 and at different stages.

Treatment			**LAI**									**PAR**			**Biomass**		**P concentration**
												%			**kg DM ha^{-1}**		**g P kg^{-1}**
Lime	**Cul**	**Pf**	**15**	**20**	**34**	**48**	**62**	**76**	**90**	**104**	**118**	**65**	**93**	**118**	**Six-leaf stage**	**Harvest**	**Six-leaf stage**
No lime	Stabil	RP	0.01	0.03	0.16	0.33	1.07	3.56	4.26	3.74	4.05	22.6	75.9	88.1	151	10728	2.80
		DAP	0.02	0.03	0.07	0.79	2.68	5.16	4.51	4.22	4.22	49.5	91.2	94.0	460	16631	3.38
	Ricardinio	RP	0.02	0.04	0.14	0.26	0.77	2.77	3.28	3.30	3.37	27.3	78.8	89.1	111	11352	2.45
		DAP	0.02	0.04	0.10	0.72	2.15	4.21	3.81	3.52	4.13	45.0	91.1	93.2	412	15478	3.10
Lime	Stabil	RP	0.02	0.04	0.11	0.22	1.07	3.45	3.72	3.41	3.94	33.9	76.8	89.4	99	12578	2.41
		DAP	0.02	0.03	0.08	0.75	2.14	3.85	3.47	3.54	3.59	38.9	89.2	92.1	431	15926	3.75
	Ricardinio	RP	0.02	0.03	0.12	0.27	1.12	3.18	3.12	2.78	3.74	28.2	75.5	89.1	122	10641	2.44
		DAP	0.02	0.03	0.06	0.60	2.03	3.68	4.04	3.50	3.42	45.2	86.3	91.6	364	14272	3.25

Table S2. Simple means for interactions of P-fertilizer (Pf), cultivar (Cul), lime application (Lime) on different traits in 2021 and at different stages.

Treatment			**PAR**	**Biomass**		**P concentration**	**P content**		**PUE**	
			%	**kg DM ha^{-1}**		**g P kg^{-1}**	**mg P plant^{-1}**		**%**	
Lime	**Cul**	**Pf**	**99**	**Six-leaf stage**	**Harvest**	**Six-leaf stage**	**Six-leaf stage**	**Harvest**	**Six-leaf stage**	**Harvest**
No lime	Stabil	No-P	85.4	882	12796	2.39	20.21	176.89	-	-
		RP	85.5	182	8996	2.04	3.21	126.65	0.50	18.52
		DAP	94.4	1357	16634	4.16	46.42	215.30	7.16	32.30
	Ricardinio	No-P	81.1	136	7559	1.89	2.35	106.56	-	-
		RP	82.1	180	7863	1.98	3.20	122.12	0.48	18.43
		DAP	96.1	1344	15053	3.85	48.43	214.22	6.89	29.25
Lime	Stabil	No-P	82.0	193	8663	2.26	3.85	125.18	-	-
		RP	87.6	151	8986	2.16	3.09	138.80	0.43	18.89
		DAP	92.1	1028	13681	3.79	35.55	204.48	5.26	28.57
	Ricardinio	No-P	81.9	167	7685	2.08	3.25	99.09	-	-
		RP	83.4	150	8011	2.00	2.66	116.14	0.40	17.64
		DAP	89.2	945	11323	4.65	31.89	180.33	5.22	27.34

Table S3. ANOVA for the effects of P-fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on LAI at different DAS in 2020. ns: not significant at $\alpha = 0.05$.

DAS	**15**	**20**	**34**	**48**	**62**	**76**	**90**	**104**	**118**
Pf	ns	ns	<0.001	<0.001	<0.001	<0.001	ns	0.044	ns
Cul	ns	ns	ns	ns	ns	0.035	ns	ns	ns
Lime	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pf × Cul	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pf × Lime	ns	ns	ns	ns	ns	0.039	ns	ns	ns
Cul × Lime	ns	0.006	ns	ns	ns	ns	ns	ns	ns
Pf × Cul × Lime	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table S4. ANOVA for the effects of P-fertilizer (Pf), cultivar (Cul), lime application (Lime), and their interactions on the percentage of canopy absorbed PAR (%) at 65, 93, and 118 DAS in 2020, and at 99 DAS in 2021. ns: not significant at $\alpha = 0.05$.

Year	**2020**			**2021**
DAS	**65**	**93**	**118**	**99**
Pf	<0.001	<0.001	0.005	<0.001
Cul	ns	ns	ns	ns
Lime	ns	ns	ns	ns
Pf × Cul	ns	ns	ns	ns
Pf × Lime	ns	ns	ns	ns
Cul × Lime	ns	ns	ns	ns
Pf × Cul × Lime	ns	ns	ns	ns