Journal of Plant Nutrition and Soil Science

WILEY-VCH

RESEARCH ARTICLE OPEN ACCESS

Interaction Between Nitrogen Forms, Phosphorus Forms, and Mineral Phases Drives Phosphorus Limitation and Uptake by Ryegrass in a Model System

Issifou Amadou[1,2] | Michel-Pierre Faucon[2] | Josep Peñuelas[3,4] | Jordi Sardans[3,4] | David Houben[2]

[1]Biogeochemistry of Forest Ecosystems, French National Research Institute for Agriculture, Food and Environment, Nancy, France | [2]Agroecology, Hydrogeochemistry, Environment and Resources, Research Unit 2018.C101, UniLaSalle, Beauvais, France | [3]Global Ecology Unit, Spanish National Research Council, Centre for Ecological Research and Forestry Applications—Blanes Centre for Advanced Studies—Autonomous University of Barcelona, Cerdanyola del Vallès, Catalonia, Spain | [4]Centre for Ecological Research and Forestry Applications, Cerdanyola del Vallès, Catalonia, Spain

Correspondence: Issifou Amadou (issifamadousana@gmail.com)

Received: 25 September 2025 | **Revised:** 1 May 2026 | **Accepted:** 6 May 2026

Academic Editor: Günter Neumann

Keywords: bioavailability | mineralization | nutrient limitation | phosphatase | rhizosphere

ABSTRACT

Background: Nitrogen (N) and phosphorus (P) cycles significantly influence ecosystem functioning, yet the interactions between their chemical forms and mineral phases in determining P limitation remain poorly understood.

Aims: We investigated the impact of interactions between P forms (organic vs. inorganic), N forms, and mineral types on plant P uptake and aimed to understand how these interactions affect rhizosphere processes governing P availability, including pH modifications and phosphatase activity.

Methods: Ryegrass (Lolium multiflorum) plants were grown in RHIZOtest-like devices in the presence of three P compounds (inositol hexakisphosphate—IHP, glycerophosphate—GLY, and inorganic P—IP) adsorbed onto two mineral phases (goethite and kaolinite), combined with two nitrogen forms (NO_3-N and NH_4-N).

Results: N addition shifted plant nutrition from N to P limitation, with the degree of this shift depending on P form and mineral type. Organic P sources induced stronger P limitation than IP. NO_3-N enhanced P acquisition more effectively than NH_4-N across all treatments. Mechanistically, N addition increased phosphatase activity but simultaneously depleted available P pools, with enzymatic P mobilization being the dominant process rather than pH-mediated effects. Clay minerals (kaolinite) showed lower P limitation than Fe oxyhydroxides (goethite) due to contrasting adsorption mechanisms.

Conclusions: In this model system, NO_3-N promoted greater P acquisition than NH_4-N through enhanced phosphatase activity rather than pH changes, and P limitation was more severe on Fe oxyhydroxides than on kaolinite. These findings suggest that selecting nitrate-based fertilization and adapting P management to soil mineralogy could help alleviate P limitation.

1 | Introduction

Phosphorus (P) limitation and its co-limitation with nitrogen (N) are significant ecological factors that influence ecosystem productivity and functioning worldwide (Hou et al. 2021; Vitousek et al. 2010). Understanding the interactions between N and P is crucial for managing plant P acquisition and optimizing agroecosystem yields. These interactions determine whether plants are limited by N or P alone or by both N and P together, which has important implications for ecosystem productivity (Dormaar and Ketcheson

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2026 The Author(s). *Journal of Plant Nutrition and Soil Science* published by Wiley-VCH GmbH.

1960; Grunes 1959; Thien and McFee 1972). In fact, a considerable portion of global cropland (32%) and pasture (43%) is P deficient, requiring increased P availability and fertilization to enhance yields and ensure food security (Lun et al. 2018). The disruption of the N cycle, caused by the increasing global demand for P and the widespread enrichment of N in biosphere, has significant consequences for ecosystem dynamics and agricultural production. Excessive N and P can lead to water and soil pollution, reduced soil fertility, and imbalanced nutrient availability, impacting both environmental and agricultural sustainability. The imbalance in soil N:P ratio is caused by the increasing global demand of P and N, but, at the same time, with a proportionally higher increase in the last 50 years of N than P emitted annually by human activities (Peñuelas et al. 2013, 2020). Although N fertilization rates have shown a greater increase compared to P fertilization rates, P tends to be more easily retained in soil (Fink et al. 2016; Singh and Schulze 2015). This retention has resulted in an accumulation of recalcitrant P forms in many long-term fertilized agricultural soils, which are not readily available for plant uptake (Zhu and Penuelas 2020). This has led to a widespread soil enrichment with N in some cropland areas, which has significant consequences on ecosystem dynamics and agricultural production (Wang et al. 2022; Xia et al. 2023; Zhu and Penuelas 2020). According to ecological stoichiometry theory, the N:P mass ratio in plants is strongly dependent on the protein:RNA ratio, and consequently the N:P mass ratios in plant photosynthetic organs govern plant growth rate (Elser et al. 2010). It is generally acknowledged that N:P mass ratios in plant biomass below 12 and above 16 indicate N and P (co-)limitation, respectively (He et al. 2014; Koerselman and Meuleman 1996; Zubillaga de Sanahuja et al. 2002). However, the mechanisms underlying N-induced P limitation are complex. Although N additions can directly influence P uptake by promoting plant growth, protein production (Sardans et al. 2017; Yu et al. 2022), and microbial phosphatase release to soil (Ercoli et al. 1996; Kome et al. 2019), the forms of N also interact with soil P pools, affecting P availability (Long et al. 2016; Schleuss et al. 2020). Notably, the inorganic N form (NH_4-N vs. NO_3-N) strongly influences P solubility and, consequently, P limitation (Lü et al. 2013; Peng et al. 2019). For instance, the nitrification process that converts NH_4-N to NO_3-N releases hydrogen ions (H^+). Similarly, NH_4-N uptake by roots stimulates the release of H^+ from the cytoplasm to the apoplasm to maintain electrical neutrality, and these H^+ are then released to the rhizosphere (Hinsinger et al. 2003). This lowers soil pH and increases the solubility of certain forms of phosphorus (Gómez-Suárez et al. 2020). Ammonium also stimulates microbes to release organic acids and enzymes that solubilize P from soil minerals (Fink et al. 2016; Singh and Schulze 2015). However, although NO_3-N can suppress some P-solubilizing microbes, both NH_4-N and NO_3-N can also compete with P for binding sites on soil particles. This competition can make P more soluble and more susceptible to desorption. Previous research on P cycling in ecosystems has predominantly been focused on inorganic phosphorus (IP) forms. However, organic phosphorus (OP) and its interactions with soil minerals have received less attention, despite the significant contribution of OP to plant nutrition depending on mineral phase (Amadou et al. 2021, 2022b). Advancing knowledge of the complex interactions among N form, P form, and soil minerals is crucial to comprehensively understand the processes regulating P cycling and acquisition under N enrichment. Targeted research across scales to elucidate these N–P-mineral interactions is vital for improving soil–plant biogeochemical models that integrate coupled nutrient cycles. Improved models could enhance predictions of shifting plant N–P co-limitation patterns and associated ecological impacts under global change (Deng et al. 2017; Goll et al. 2012). Overall, significant knowledge gaps exist regarding the influence of N–P-mineral interactions on P biogeochemistry and plant nutrition, especially for OP forms. Filling these research needs across diverse ecosystems will provide key insights for sustaining nutrient cycles and ecosystem productivity into the future. In this study, we examined the interactive impacts of N form, P form, and soil minerals on plant P limitation through a controlled greenhouse experiment with ryegrass. We used three P compounds (IP, inositol hexakisphosphate—IHP, and glycerophosphate—GLY) pre-adsorbed onto goethite and kaolinite to simulate natural soil conditions. We tested three specific hypotheses: (1) NO_3-N addition enhances P uptake more effectively than NH_4-N because nitrate-fed plants allocate more N resources to the synthesis of extracellular phosphatases under P-limiting conditions; (2) organic P forms (IHP, GLY) adsorbed onto mineral phases induce stronger P limitation than IP because they require both enzymatic cleavage and desorption before plant uptake, with the degree of limitation increasing with the strength of the mineral-P bond (goethite > kaolinite); and (3) N addition shifts plant nutrition from N limitation to P limitation, but the magnitude of this shift depends on the P source, with organic P forms producing higher N:P ratios (i.e., stronger P limitation) than IP across both mineral types.

By addressing these research questions through a mechanistic approach, we aim to enhance understanding of P cycling under N additions and improve soil–plant biogeochemical models that incorporate fully coupled N–P cycles.

2 | Materials and Methods

2.1 | Organic P Compounds, Mineral Phases, and N Forms

Two organic P compounds, myo-IHP, GLY, and IP form (KH_2PO_4), were chosen for the experiments. These OP compounds were selected on the basis of their prevalence in most soils and organic wastes. Each selected form differed from the others in P content and molecular size. The minerals were chosen as representative examples of two major classes of soil minerals commonly found in weathered soils: Fe oxyhydroxides and 1:1 phyllosilicate clay minerals. Two contrasting individual minerals were used. Goethite [Fe(OH)O] was chosen as representative of Fe oxyhydroxides; kaolinite was chosen as the clay mineral, specifically the 1:1 phyllosilicate. All minerals were obtained from Sigma-Aldrich Chemie S.a.r.l. and VWR (France) for the experiment. The two forms of N used were ammonium (NH_4-N) provided as $(NH_4)_2SO_4$ and nitrate (NO_3-N) provided as $Ca(NO_3)_2$.

2.2 | Preparation of Goethite-P and Kaolinite-P Adsorption Complexes

The minerals (goethite and kaolinite) were loaded with the three compounds (IHP, GLY, and IP) to serve as a source of P for

ryegrass. Solutions containing 1 g L^{-1} P in 0.1 M KCl at pH 5.5 were prepared using the individual P compounds. The minerals were weighed in 100 g portions into 1-L bottles. Then, 200 mL of 0.1 M KCl was added, and the bottles were shaken for 24 h at 25°C to sufficiently hydrate the adsorption sites. The pH was maintained at 5.5 ± 0.05 by adding 0.1 M HCl or NaOH solution. To begin the formation of P–mineral complexes, each of the 1-L bottles containing the mineral phases was filled with 800 mL of each P solution. Then, a few drops of hexanol were added during the complexation step to prevent microbial degradation of the organic P compounds during the 24-h adsorption period. The bottles were shaken on a mechanical shaker for 24 h at 25°C and then centrifuged (3000 *g* for 15 min). The 1-L bottles were divided into several 250-mL bottles, centrifuged, and the supernatants were collected and removed. The P-laden minerals produced were shaken in deionized water and frozen at −20°C. To determine the amounts of adsorbed P, a certain volume of supernatants was immediately filtered through a 0.22-µm membrane syringe filter for P analysis. The concentration of P in the supernatants was determined by colorimetry (Ohno and Zibilske 1991) after hydrolysis of IHP and GLY to IP by the persulfate oxidation method (Peters and Van Slyke 1932). The amount of P adsorbed was calculated as the difference between the initial amount of P added and the amount of P remaining in the supernatant at the end of the experiment. The P loadings of the mineral–P complexes produced are shown in Table S1. All minerals were loaded to reach their adsorption maxima.

2.3 | Experimental Setup and Plant Growth

We used a RHIZOtest-like device (Bravin et al. 2010; Michaud et al. 2007; Figure S2) in which plant roots remain in contact with the substrate but are physically separated from the nutrient solution. It should be noted that this experimental system represents a simplified model of soil–plant interactions, using sterilized quartz mixed with pure mineral–P complexes as a growing substrate, rather than natural soil. This controlled approach allows isolation of specific mineral–N–P interactions, but the results should be interpreted in the context of this model system. Unlike natural soils, the substrate lacked organic matter, microbial communities (due to pre-sterilization), and the full complexity of mineral assemblages found in field conditions.

In a first step (preculture period), 0.20 g of ryegrass (*Lolium multiflorum*) seeds were grown for 14 days in hydroponics in a cylinder closed at the bottom by a screen allowing the development of the roots with a nutrient solution: $Ca(NO_3)_2$ (2 mmol L^{-1}), $MgSO_4$ (0.5 mmol L^{-1}), K_2SO_4 (0.7 mmol L^{-1}), KCl (0.1 mmol L^{-1}), KH_2PO_4 (0.1 mmol L^{-1}), $MnSO_4$ (0.5 µmol L^{-1}), $CuSO_4$ (0.5 µmol L^{-1}), $ZnSO_4$ (0.5 µmol L^{-1}), $(NH_4)_6Mo_7O_{24}$ (0.01 µmol L^{-1}), and Fe-EDTA (100 µmol L^{-1}). In a second phase (culture period), the 2-week-old seedlings were transplanted into polystyrene cylinder pots (125 mL volume) containing the growth media. The growing media consisted of a mixture of the previously prepared P–mineral complexes mixed with acid-washed quartz. Before the experiment, the quartz was sterilized by autoclaving (Gómez-Suárez et al. 2020) at 121°C for 2 h in order to eliminate potential microorganisms, thus allowing the results to be attributed to the effect of root activity alone. In addition, to avoid any P limitation for the plants during the experiment, we provided a total amount of P that was previously found to be sufficient to meet the plants' needs (Gómez-Suárez et al. 2020). Thus, the amount of mineral–P complex added to the growing medium was calculated to provide 10 mg of P in each treatment, whereas the amount of quartz was calculated to reach a final mass of 100 g in each pot. Then the pots containing the plants and growing medium were nested into other 250 mL cylinder pots containing a nutrient solution with a filter paper wick. The composition of this nutrient solution was the same as that used for the preculture period, except that P was not added and N was added as $Ca(NO_3)_2$ (4 mmol L^{-1}) or $(NH_4)_2SO_4$ (4 mmol L^{-1}). To make the amounts of Ca equal between the NO_3-N and NH_4-N treatments, $CaCl_2$ was added to the NH_4-N treatment. The devices were placed under controlled conditions (phytotron) under a 12 h photoperiod, constant temperature (25°C), and relative humidity (80%). No visible signs of ammonium toxicity (e.g., leaf chlorosis or necrosis) were observed in any treatment at harvest. Non-planted control treatments, in which the quartz-mineral–P complex was incubated without plants (hereafter called bulk substrate), were also set up to serve as references for assessing rhizosphere-induced changes in substrate properties (pH, available P). These controls were not included as a factor in the statistical analysis of plant response variables. The factorial design for plant-related variables comprised three factors: 3 P compounds (IHP, GLY, and IP) × 2 mineral phases (goethite and kaolinite) × 2 forms of nitrogen (NO_3-N and NH_4-N), with 4 replicates per treatment combination, totaling 72 planted devices plus 24 unplanted controls (96 devices in total).

2.4 | Plant Sampling and Analysis

The plants were harvested after 20 days of contact with mineral–P complexes. At harvest, shoots and roots were separated, and roots were gently rinsed with deionized water. Shoots and roots were then dried at 60°C for 72 h, weighed, and crushed prior to N and P analysis. The concentration of P in shoots and roots was determined colorimetrically (Ohno and Zibilske 1991) after mineralization by 65% HNO_3 digestion directly in a microwave Teflon vessel (Lange et al. 2014). In addition to P analysis, the N concentrations in shoot and root tissues were determined by dry combustion using an elemental analyzer (Dumas method, Flash EA 1112, Thermo Fisher Scientific). Dried and crushed samples were weighed into tin capsules prior to combustion analysis. Plant N refers to the N content of the entire plant, including roots and stems. Total plant P uptake was calculated by summing P uptake by roots (i.e., root P concentration multiplied by root dry mass) and P uptake by shoot (i.e., shoot P concentration multiplied by shoot dry mass). Total plant N uptake was calculated by summing N uptake by roots (i.e., root N concentration multiplied by root dry mass) and N uptake by shoots (i.e., shoot N concentration multiplied by shoot dry mass). The N:P mass ratio was then calculated by dividing total plant N concentration by total plant P concentration.

2.5 | Rhizosphere and Bulk-Mineral Layer Analysis

At the completion of the experiment, rhizosphere substrate (i.e., rhizosphere) and the bulk substrate were collected and dried at

ambient temperature. In accordance with the scheme proposed by DeLuca et al. (2015), each substrate sample was subjected to $CaCl_2$ extraction (0.01 mol L^{-1}), which extracts soluble and weakly adsorbed IP and emulates accessible P by interception and root diffusion. One gram of substrate was transferred to a polypropylene centrifuge tube to which 20 mL of 0.01 mol L^{-1} $CaCl_2$ was added and shaken for 3 h in a reciprocal shaker at 200 rpm at 20°C. After shaking, the pH (pH-$CaCl_2$) was measured in the suspension, and the extract was separated from the solid residue by centrifugation at 3000 × *g* for 15 min. In the extract, P concentrations were analyzed colorimetrically (Murphy et al. 2009) using a spectrophotometer (610 nm). ΔpH between rhizosheath and bulk substrate from unplanted pots postgrowth was then calculated.

After defrosting some samples, acid phosphomonoesterase activity in the rhizosheath was measured. Given that the substrate was pre-sterilized, the measured phosphatase activity is primarily attributed to root-derived (extracellular) acid phosphomonoesterases released by ryegrass roots into the rhizosphere. The phosphatase assay was conducted with a modified buffer at pH 6.5 (Tabatabai and Bremner 1969). Briefly, phosphatase activity was assessed via production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate during a 1 h incubation at 37°C with 0.5 g dry substrate, 0.2 mL toluene, 4 mL modified buffer, and 1 mL substrate. *p*-nitrophenol release was determined spectrophotometrically at 410 nm after stopping the reaction with 4 mL 0.5 M NaOH and 1 mL 0.5 M $CaCl_2$ and filtering.

2.6 | Data Processing and Analysis

All statistical analyses were performed using R software version 3.5.0. The impact of factors (substrate minerals, P compounds, N forms, and their interactions) on response variables was modeled by generalized linear models (GLMs) using the glm() function from the stats package (R Core Team 2023). The Rcmdr package (Fox 2005) was used as a graphical interface for statistical analyses. The GLM model was used to avoid mathematical transformation of the variables and to get rid of the strict assumption that "the residuals will follow a conditionally normal distribution" (McCullagh and Nelder 2019; Warton et al. 2016) and allow the use of a variety of other distributions of the exponential family for the residuals (Cohen et al. 2014). GLMs were fitted using a Gamma distribution with a log link function, which is appropriate for continuous, strictly positive response variables that may exhibit heteroscedasticity (McCullagh and Nelder 2019). All factors were tested individually, and the models were compared on the basis of second-order Akaike information (AIC), with the lowest relative value being considered the best fit. Specifically, seven nested GLM models were compared: three single-factor models (N form, P compound, mineral type), three two-way interaction models (N form × P compound, N form × mineral type, and mineral type × P compound), and one full three-way interaction model (N form × mineral type × P compound). For each response variable, the model with the lowest AIC*c* value was selected as the best fit (Table 1). For all response variables, the three-way interaction model provided the best fit, as indicated by the lowest AIC*c* values. The ANOVA of the best fit GLM model with a chi-square test generates the deviance analysis table that gives the significance level of all factors. As the three-way interaction was significant for most response variables, post hoc pairwise comparisons among all treatment combinations (mineral type × P compound × N form) were performed using Tukey's honest significant difference (HSD) test at $p < 0.05$. For this purpose, each unique treatment combination was treated as a single factor level in a one-way ANOVA framework. As recommended by Schabenberger and Pierce (2001), the results of ANOVA were presented by showing the degree of freedom (Df), *F*-ratio (*F*; i.e., the between-group variance divided by the within-group variance), and *p* value (i.e., the lowest probability level at which the means are considered significant).

TABLE 1 | Generalized linear model (GLM) model results testing the impact of factors: N form (NO_3-N or NH_4-N), minerals (goethite and kaolinite), and P forms (inorganic phosphorus [IP], inositol hexakisphosphate [IHP], and glycerophosphate [GLY]) on N:P mass ratio and total P uptake.

Explanatory factor	N:P mass ratio			P uptake		
	AIC*c*	*F*	*p*	AIC*c*	*F*	*p*
N forms	463	4.1	Ns	84	65.03	**
P forms	480	11.5	**	62	131.38	**
Minerals	482	5.3	*	98	18.08	***
N forms × P forms	464	19.5	**	31	11.09	***
N forms × Minerals	465	5.3	*	87	3.24	*
Minerals × P forms	300	21.1	***	53	26.98	**
N forms × Minerals × P forms	255	30.9	***	−35	142	***

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

3 | Results

3.1 | Phosphorus Uptake and Plant Nutrient Limitation

The interaction between N forms, P forms, and mineral phases was the predominant factor affecting N:P mass ratio (Figure 1), P uptake (Figure 2), and plant biomass (Figure S1), as evidenced by the best model fit (Table 1; interaction main effect on N:P mass ratio including total substrate P concentrations, $F_{55} = 30.9$, $p < 0.001$, and on P uptake, $F_{40} = 142$, $p < 0.001$). As a result of this interaction, N:P mass ratios varied markedly across treatments, ranging from 2.5 ± 0.42 to 32.31 ± 2.00 (Figure 1). Treatments without N addition consistently showed N:P mass ratios below 12 (from 2.5 ± 0.42 to 10.5 ± 2.00). Whole-plant N and P concentrations are presented in Table S3 and compared with published sufficiency values for *L. multiflorum* (Bergmann 1992). Without N addition, plant N concentrations ranged from 3.6 mg g^{-1} (Go-GLY, indicating severe N deficiency) to 60.8 mg g^{-1} (Go-IP), whereas P concentrations ranged from 1.3 mg g^{-1} (Go-GLY, below the critical deficiency threshold of 2.1 mg g^{-1}) to 9.2 mg g^{-1} (K-IP, above the sufficiency range). Nitrogen addition substantially increased plant N concentrations across all treatments (81.8–187.3 mg g^{-1}), well above the sufficiency range of 28–42 mg g^{-1} (Bergmann 1992),

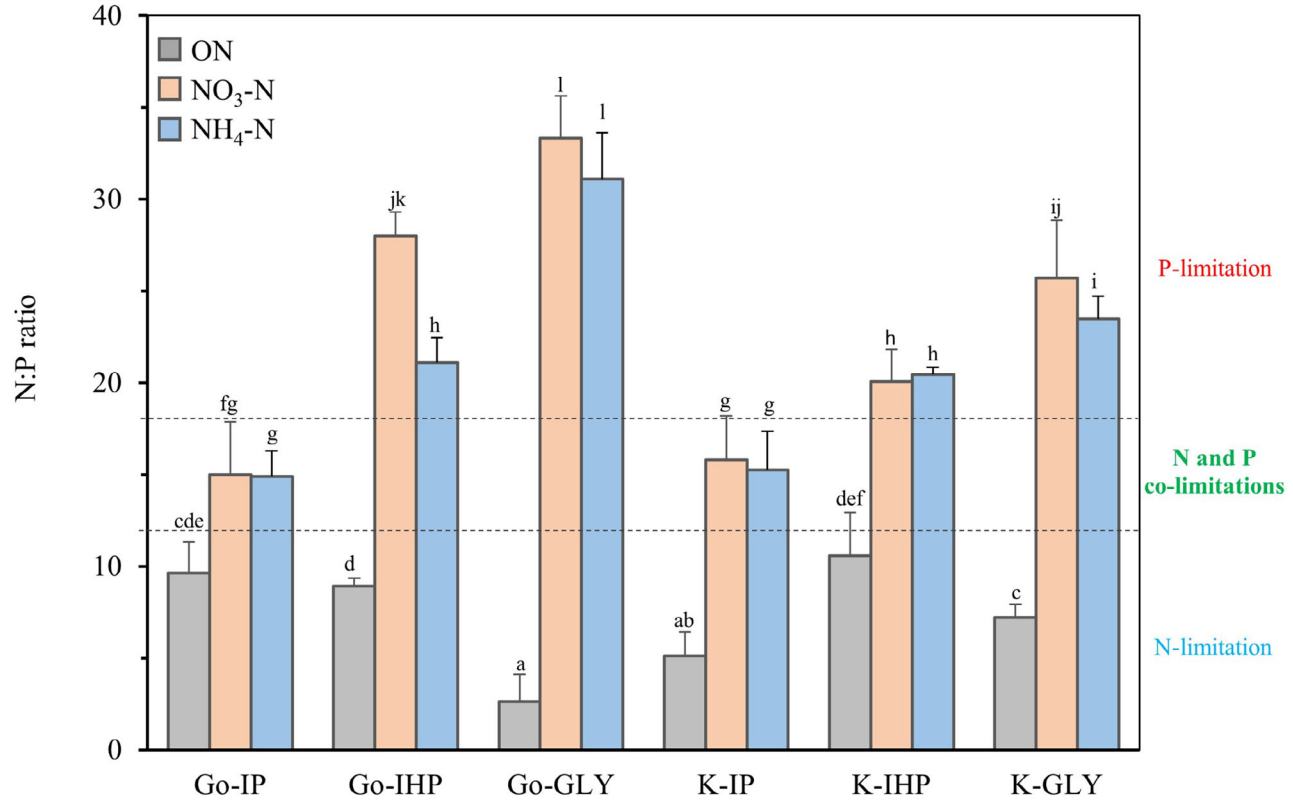


FIGURE 1 | Effect of N forms and their interactions with minerals-P complexes on N:P ratios. The interaction effect of N, P compounds, and mineral phases on N:P ratios was significant ($p < 0.001$) according to GLM model results testing the impact of factors (N forms, P compounds, mineral phases, and their combined effect). Means with different letters were significantly different according to Tukey's HSD test at $p < 0.05$ (with Sidak adjustment for multiple comparisons). Error bars indicate standard error. N forms (NO_3-N: nitrate and NH_4-N: Ammonium); mineral phases (Go: goethite and K: kaolinite); Phosphorus compounds (IHP: myo-inositol hexakisphosphate, GLY: glycerophosphate and IP: KH_2PO_4); The dashes (-) identify the mineral-phosphorus complexes produced by the adsorption of P forms onto mineral phases. Example: Go-IHP refers to goethite and myo-inositol hexakisphosphate complexes. The N:P ratio has been used to indicate plant N or P limitation conditions, that is, N limitation when N:P < 14, P limitation when N:P > 16, and putative NP co-limitation between 14 and 16 (Koerselman and Meuleman 1996; Zheng et al. 2017).

indicating luxury N consumption. In contrast, P concentrations showed more modest responses to N addition, remaining within or below the sufficiency range (3.5–5.0 mg g^{-1}) for most organic P treatments. This pattern of N excess combined with marginal-to-sufficient P supply in OP treatments confirms that the elevated N:P ratios reflect genuine P limitation rather than an artifact of the ratio calculation.

However, N addition increased the N:P mass ratio across all treatments (mineral-P complexes). Importantly, with N addition, N:P mass ratio differed strongly depending on the P source (Figure 1). Mineral-IP complexes elicited lower N:P mass ratios in plants (14.8–15.8) than mineral-OP complexes (19.12–32.2) (Figure 1).

This differential response in nutrient limitation was closely reflected in P uptake patterns. Nitrogen addition amplified P uptake, especially when N was applied as NO_3-N (Figure 2; Table S2). Total P uptake correlated positively with both available-P and phosphatase activity (Table 2). Nevertheless, P uptake from mineral-OP complexes remained lower despite N addition (Figure 2). Moreover, NO_3-N increased P uptake and availability more than NH_4-N across all mineral–P complexes (Figures 2 and 3b). Overall, N addition shifted plant nutrition from N limitation to P limitation, with the degree of P limitation varying according to the P source type.

3.2 | Rhizosphere Properties and Biochemical Responses

Nitrogen addition induced significant changes in rhizosphere biochemical properties that were closely linked to P dynamics. N addition consistently increased phosphatase activity across all treatments (Figure 3a), and this enhanced enzyme activity correlated positively with P uptake (Table 2). However, available P concentrations simultaneously decreased with N addition (Figure 3b). The decline in available P followed a specific gradient: mineral-IP > mineral-IHP > mineral-GLY (Figure 3b), which paralleled the increasing N:P mass ratios observed in plant tissues (Figure 1).

The relationship between P availability and plant nutrition status was further confirmed across all treatments, as available P and N:P mass ratio correlated negatively (Table 2). Plant P limitation intensified as available P declined (Figures 1 and 3b, Table 2). Elevated phosphatase activity (Figure 3a) and enhanced availability of P in the rhizosphere (Figure 3b) were observed

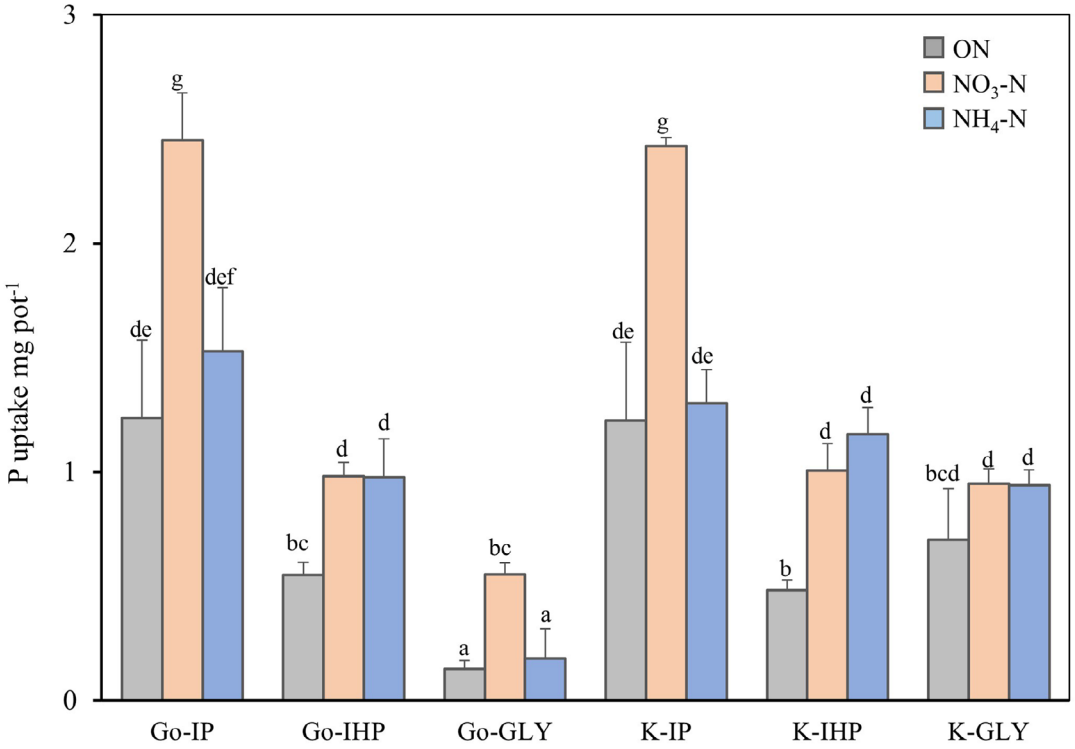


FIGURE 2 | Effect of N, P, and mineral phase interactions on total P uptake by ryegrass plants with different mineral-P complexes. The interaction effect of N, P compounds, and mineral phases on total P uptake by ryegrass was significant ($p < 0.001$) according to GLM model results testing the impact of factors (N forms, P compounds, mineral phases, and their combined effect). Means with different letters were significantly different according to Tukey's HSD test at $p < 0.05$ (with Sidak adjustment for multiple comparisons). Error bars indicate standard error. N forms (NO_3-N: nitrate and NH_4-N: ammonium); mineral phases (Go: goethite and K: kaolinite); phosphorus compounds (IHP: myo-inositol hexakisphosphate, GLY: glycerophosphate and IP: KH_2PO_4); the dashes (-) identify the mineral-phosphorus complexes produced by the adsorption of P forms onto mineral phases. Example: Go-IHP refers to goethite and myo-inositol hexakisphosphate complexes.

TABLE 2 | Spearman product-moment correlation (r values) between total P uptake, foliar N and P, and rhizosphere properties.

	N:P	Total P uptake
Rhizosphere pH	−0.036	0.159
Available P	−0.209*	0.537***
Phosphatase activity	0.381***	0.463***

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

under N addition. Notably, no significant pH effects were detected following NO_3-N or NH_4-N addition (Figure 3c). In sum, N addition enhanced phosphatase activity but simultaneously depleted available P pools, with NO_3-N form being more effective than NH_4-N in mobilizing P from mineral complexes.

3.3 | Relationships Between Mineral Types, P Forms, and Plant Responses

The extent of P limitation and plant responses varied substantially between mineral types. Phosphorus limitation was significantly higher with goethite-P than kaolinite-P complexes across all P forms (Figure 1). The two OP forms also showed contrasting behavior depending on N availability. Without N addition, the measured level of P limitation (N:P mass ratio) was lower in mineral-IHP complexes compared to mineral-GLY. However, with N addition, the measured level of P limitation in mineral-IHP became higher than that observed in mineral-GLY (Figure 1). This reversal pattern highlighted the differential response of OP forms to N inputs depending on their chemical structure.

The complex interactions observed in this multifactorial system were reflected in several significant correlations across treatments. Both phosphatase activity and available P concentration correlated positively with P uptake (Table 2). Available P and N:P mass ratio showed a significant negative correlation (Table 2). These relationships were consistent across treatments regardless of mineral type or P form. Overall, mineral type significantly influenced P bioavailability, with OP forms showing contrasting responses to N addition depending on their chemical structure.

4 | Discussion

4.1 | Interactive Effects of N and P Forms on Plant Nutrient Limitation

Our findings demonstrate that the interaction between N forms, P forms, and mineral phases was the predominant factor affecting

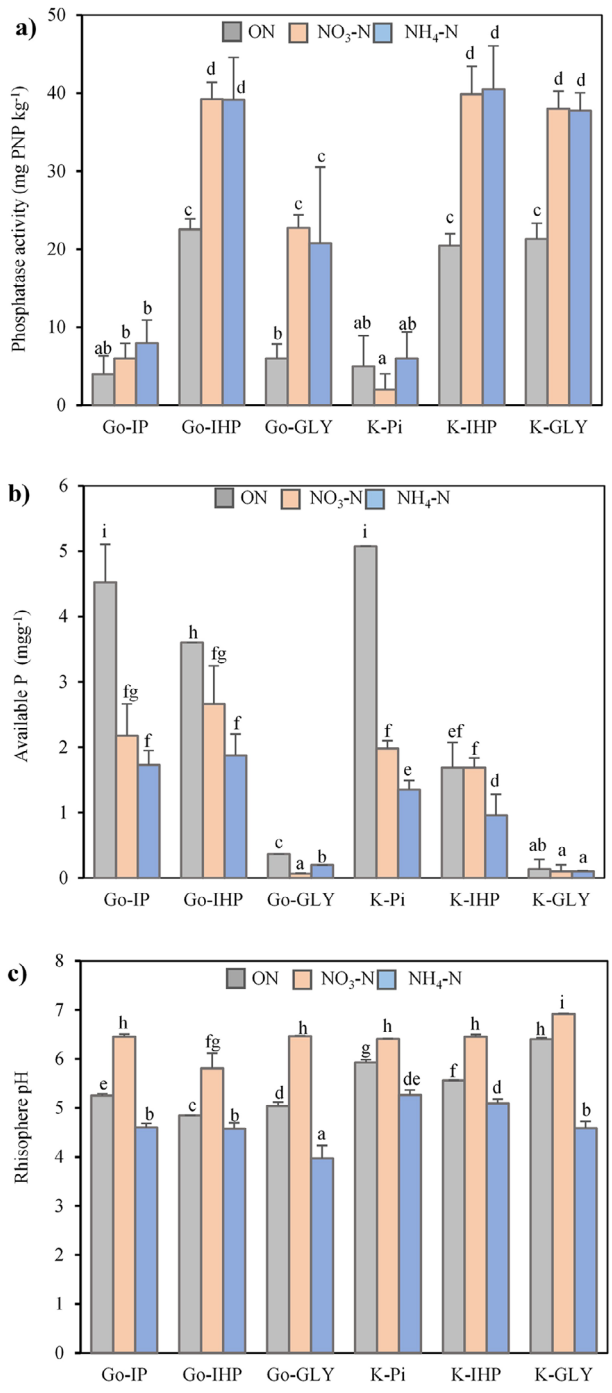


FIGURE 3 | Effect of N, P, and mineral phase interactions on (a) phosphatase activity, (b) available P, and (c) rhizosphere pH. Means with different letters were significantly different according to Tukey's HSD test at $p < 0.05$ (with Sidak adjustment for multiple comparisons). Error bars indicate standard error. N forms (NO_3-N: nitrate and NH_4-N: Ammonium); mineral phases (Go: goethite and K: kaolinite); phosphorus compounds (IHP: myo-inositol hexakisphosphate, GLY: glycerophosphate and IP: KH_2PO_4); the dashes (-) identify the mineral-phosphorus complexes produced by the adsorption of P forms onto mineral phases. Example: Go-IHP refers to goethite and myo-inositol hexakisphosphate complexes.

P uptake and limitation. The range of N:P mass ratios, from 2.5 ± 0.42 to 10.5 ± 2.00 (Figure 1), indicated N limitation (N:P mass ratio < 12) in treatments without N addition. This range was consistent with those reported in other studies (Güsewell 2004; von Oheimb et al. 2010; Yang et al. 2019). The N:P thresholds used here to diagnose nutrient limitation (N:P < 14 for N limitation, N:P > 16 for P limitation, and putative N–P co-limitation between 14 and 16; Koerselman and Meuleman (1996) have been widely validated across diverse soil, climate, and vegetation contexts (Aerts and Chapin III 2000; Tessier and Raynal 2003). Although these thresholds involve some degree of uncertainty, the key finding of this study resides in the significant variation of N:P ratios across treatments, revealing how mineral type, P form, and N form interactively control the shift between N and P limitation. Individual N and P concentrations (Table S3), compared to published sufficiency values for *L. multiflorum* (Bergmann 1992), further confirmed that the observed shifts reflected genuine changes in nutrient limitation status.

However, N addition increased the N:P mass ratio across treatments (mineral-P complexes), denoting a shift from N to P limitation (Sardans et al. 2017; Yang et al. 2019). Importantly, with N addition, N:P mass ratio differed between mineral-IP and mineral-OP treatment sources (Figure 1). Mineral-IP elicited lower N:P mass ratios in plants (14.8–15.8) than mineral-OP (19.12–32.2) (Figure 1), implying co-limitation by N and P for IP but switch to P limitation with OP, irrespective of minerals. This corroborates that P source nature is a variable that strongly influences nutrient limitation in plants (Amadou et al. 2022b; Long et al. 2016). The P limitation with OP likely stems from lower OP availability and uptake versus IP forms (Figures 2 and 3b), as widely demonstrated (Bollyn et al. 2017). These results are consistent with the high affinity of OPs for mineral phases, as well as their recalcitrance and stability in soil, and especially the need for prior mineralization (Amadou et al. 2022b; Ganta et al. 2020). Here, decreasing available P after N addition (mineral-IP > mineral-IHP > mineral-GLY; Figure 3b) paralleled increasing N:P mass ratios (Figure 1). Moreover, available P and N:P mass ratio correlated negatively (Table 2), with plant P limitation intensifying as available P declined (Long et al. 2016; Zheng et al. 2017) (Figures 1 and 3b, Table 2). Hence, higher P availability from IP mitigates P limitation from IP versus OP (Figures 1 and 3b). In summary, the interaction between N and P, depending on their chemical form, determines plant nutrient limitation. Nitrogen additions shift limitation from N to P, but the degree of P limitation reached depends on the P source. These findings have implications for fertilizer management. They showed that N and P limitations are interdependent and influenced by mineral interactions. To prevent imbalanced fertilization, inputs should match the specific soil P forms. With dominant IP forms, N and P likely co-limit, requiring balanced application. However, with prevalent OP forms, P limitation will be stronger in the soil–plant system. These findings suggest that in systems where P is predominantly in organic form (such as soils amended with manure or compost), P limitation may intensify upon N enrichment. In contrast, when IP dominates, N–P co-limitation is more likely. It should be noted, however, that OP sources may offer the advantage of a slower, more sustained P release, which could be beneficial for long-term soil fertility (Frossard et al. 2000). These observations, derived from a controlled model system, warrant further validation under field conditions with

natural soils containing complex mineral assemblages and active microbial communities.

4.2 | Nitrogen-Driven Shifts in P Bioavailability and Uptake

Nitrogen addition also amplified P uptake, especially when N is applied as NO_3-N (Figure 1; Table S2), highlighting combined N–P–mineral effects on P dynamics. Elevated phosphatase activity (Figure 3a) and the availability of P in the rhizosphere (Figure 3b) under N addition likely enabled enhanced P uptake, as both increase the possibility that a phosphatase enzyme meets an OP compound and catalyzes its hydrolysis after desorption from the minerals (Craine and Jackson 2010; Long et al. 2016). Consistently, total P uptake correlated positively with both available-P and phosphatase activity (Table 2). Most likely, the addition of N allowed plants to synthesize N-costly phosphatases (Schleuss et al. 2020), which then made OP available for plant uptake (Helfenstein et al. 2018). Thus, N addition apparently intensified P recycling from IP and OP pools across minerals (Widdig et al. 2019). However, lower P uptake from mineral-OP complexes despite N addition (Figure 2) could arise from great cost/difficulty to desorb OP (Amadou et al. 2022a; Bollyn et al. 2017) and because once desorbed, OPs require enzymatic cleavage before uptake (Klotzbücher et al. 2020). Thus, in mineral-OP systems, N addition can rapidly exhaust available-P (Figure 3b), inducing high P limitation (Figure 1). In mineral-IP systems, N addition does not systematically constrain P, leading more probably to potential N–P co-limitation.

The differential effectiveness of NO_3-N and NH_4-N in enhancing P acquisition can be partly attributed to their contrasting effects on rhizosphere chemistry. As expected, NH_4-N addition acidified the rhizosphere, whereas NO_3-N addition induced alkalinization. Surprisingly, these pH shifts did not influence N:P ratios or P uptake, which contrasts with patterns commonly reported in the literature. This result indicates that phosphatase activity played a more decisive role than pH changes, at least for the OP forms examined in this study. A potential explanation could be that NH_4-induced acidification increases the number of positive charges on soil constituents, such as oxides and kaolinite, thereby enhancing P sorption and reducing its availability. Yet, the absence of significant pH effects on P dynamics in our experiment suggests that this mechanism did not operate under the tested conditions. However, it should be noted that the low buffering capacity of the quartz-mineral substrate may have amplified pH changes relative to natural soils, while simultaneously limiting the ecological relevance of these changes. In well-buffered natural soils, the pH effects of N form on P solubility may be more or less pronounced depending on the soil's acid-base buffering capacity. Instead, our findings reinforce the view that direct biological processes, particularly enzymatic mobilization, predominated over abiotic, pH-mediated mechanisms in controlling P availability. Overall, N additions increase P uptake via enhanced phosphatase activity and P availability. However, the greater stability of OP forms on mineral phases and their lower desorption capacity limit their availability for plant uptake. In fact, if the OP is adsorbed by the minerals, the phosphatase activities would have a limited or no effect on its mineralization. This illustrates challenges in managing OP in soils: Although N can accelerate P cycling enzymatically, higher affinity of OP for minerals still limits plant P availability. It highlights the need for integrated N and P management in organic systems to prevent P drawdown. Targeted crops and amendments may help manage tightly bound OP.

4.3 | Mineral-Specific Effects on Phosphorus Dynamics

Phosphorus limitation varies substantially between mineral types due to their contrasting physicochemical properties, which affect P retention (Bai et al. 2021; Yan et al. 2014). Limitation of P was significantly higher with goethite-P than kaolinite-P complexes (Figure 1). The P limitation was therefore less marked on clay minerals than on Fe oxyhydroxides. This may be due to the fact that the anion/cation uptake process on clays is different from that on oxides. Phosphorus adsorption on clay is based on electrostatic interaction, and as an anion, NO_3-N, can easily replace orthophosphates (Ganta et al. 2019; Hori et al. 1985). In contrast, with their net positive charge at typical soil pH values, Fe oxyhydroxides attract and hold negatively charged ions (anions), such as phosphate (PO_4^{3-}). This process, known as ligand exchange, involves the phosphate anion forming a covalent bond with the Fe atom on the oxide surface. This bond is significantly stronger than the electrostatic interaction between anions and clay minerals (Chitrakar et al. 2006). The two OP forms also showed large differences in limitation levels. Without N addition, the measured level of P limitation (N:P mass ratio) was lower in mineral-IHP complexes compared to mineral-GLY. However, with N addition, the measured level of P limitation in mineral-IHP becomes higher than that observed in mineral-GLY (Figure 1). This suggests that adding N helps mobilize IHP, making P less limiting than other forms of OP, such as GLY. As a result, we found that the uptake of P from the IHP form was higher than that from the GLY form (Figure 2). This highlights the key role of N–P-mineral interactions in OP dynamics, especially for the major and most limiting forms (Craine and Jackson 2010; Hou et al. 2021). These results also suggest a new mechanism by which N inputs could accelerate IHP cycling (the main OP form in soils), delaying its onset of limitation. Hence, P limitation depends on mineral phase, with goethite inducing stronger P limitation than kaolinite. IP is more available than OP across minerals, but N addition can increase P availability from IHP. This suggests that P fertilizer and availability must be tailored to specific mineral compositions. Characterizing mineralogy is critical to develop P management plans tailored to OP forms and dynamics. Additionally, it is of utmost importance in optimizing agroecosystem models to enhance the accuracy of predicting P availability. Moreover, N addition increased phosphatase activity (Figure 3a) but decreased available P concentration (Figure 3b). Both phosphatase activity and available P concentration correlated positively with P uptake (Table 2), suggesting that in the presence of OP sources, available P and phosphatase activity influence P uptake and limitation for all forms. Lower available P concentration with N addition (Figure 3b) may stem from greater P uptake, and the slower replenishment from mineral complexes cannot be sufficient to avoid P limitation (Lund et al. 2009; Sardans and Peñuelas 2012). Studies also show N influences rhizosphere properties (Marklein and Houlton 2012; Zheng et al. 2017), and in low P systems,

available P decreases, increasing limitation (Lü et al. 2013). Thus, N supply can increase P uptake from mineral IP and OP, altering plant P limitation.

4.4 | Implications for Nutrient Management and Research Perspectives

Our results provide several insights for nutrient management, though the controlled conditions of this model system require caution when extrapolating to field settings. The use of a pre-sterilized mineral substrate, in the absence of organic matter and microbial communities, represents a simplified system compared to natural soils. In particular, the measured phosphatase activity is exclusively root-derived, whereas microbial phosphatases typically dominate P mobilization in natural soils (Nannipieri et al. 2011). Additionally, the dense root mat formed in the RHIZOtest-like device may have concentrated root exudates relative to field conditions, and the experiment was conducted with a single plant species. These aspects should be considered when interpreting the magnitude of the effects observed here. Despite these simplifications, the controlled approach allowed us to isolate fundamental mineral-N–P interactions that would be difficult to disentangle in complex natural soils. A key finding is the strong dependence of P limitation on mineral type, which has direct practical relevance. In soils dominated by Fe oxyhydroxides, P retention through ligand exchange is strong and P limitation is more severe, whereas in soils dominated by clay minerals, such as kaolinite, electrostatic P retention is weaker and more easily disrupted by competing anions (e.g., NO^{3-}). This implies that P fertilizer rates and forms should be adapted to the dominant mineralogy of the target soil. Beyond the mineral effect, the form of N fertilizer also emerged as an important lever for P management. Our finding that NO_3-N enhanced P uptake more effectively than NH_4-N across all mineral-P complexes suggests that nitrate-based fertilizers may be preferable in P-limited systems where mineral-P interactions constrain P availability. Regarding organic P management, our results showed that although OP forms are less immediately available than IP, N addition can accelerate their mobilization, especially for IHP. This has implications for soils receiving organic amendments, such as manure, compost, or crop residues. Although the OP contained in these amendments may initially be retained by soil minerals, appropriate N inputs may enhance its cycling through increased phosphatase activity. In addition, organic acids released during organic matter decomposition can compete with phosphate for adsorption sites on mineral surfaces, thereby displacing previously sorbed P and increasing its availability (Guppy et al. 2005). The integration of organic amendments with adequate N fertilization could thus provide a synergistic approach to enhance P availability in soils with high P-fixing capacity.

These findings open several research perspectives. Future studies should validate the observed mechanisms in natural soils with contrasting mineral assemblages, including calcareous soils where Ca-P precipitation adds further complexity to P dynamics. Incorporating active microbial communities and soil organic matter would allow a more comprehensive assessment of the relative contributions of biotic and abiotic processes to P mobilization. Extending this work to multiple plant species with contrasting P acquisition strategies (e.g., legumes, mycorrhiza-dependent species) would help assess the generality of the patterns identified here (Lambers et al. 2006; Richardson et al. 2009). Ultimately, integrating these mineral-specific N–P interactions into soil–plant biogeochemical models could improve predictions of nutrient limitation under varying fertilization regimes.

5 | Conclusions

This study confirms that the complex interplay between N forms, P sources, and mineral phase fundamentally shapes P bioavailability and plant nutrient dynamics. N addition shifted plant nutrition from N to P limitation, but the degree of this shift depended critically on P chemical form and mineral type. Organic P forms showed stronger limitation than IP, with mineral-specific differences reflecting contrasting adsorption mechanisms between clay minerals and Fe oxyhydroxides. Enhanced phosphatase activity rather than pH changes was the dominant mechanism controlling P mobilization under N enrichment. These findings, obtained in a simplified model system, provide mechanistic insights into how coupled N–P-mineral interactions govern P bioavailability. Although extrapolation to field conditions requires caution, the identified mechanisms, particularly the dominant role of enzymatic P mobilization over pH effects, have implications for nutrient management and ecosystem modeling. Future work should validate these interactions under field conditions, incorporating microbial communities, organic matter, and diverse plant species to assess the generality of the observed patterns.

Author Contributions

Issifou Amadou prepared and wrote the manuscript; Michel-Pierre Faucon and David Houben defined the outline, main highlights, revisions, and suggestions of the manuscript, tables, and figures. All authors discussed the design and the results and read and agreed to submission of the manuscript.

Acknowledgments

The authors thank the Campus UniLaSalle Beauvais and Programme Opérationnel FEDER/FSE Picardie 2014–2020 for their support for the start of the global research project in agriculture focusing on sustainable management of soil fertility and eco-efficiency of phosphorus management in farming systems. Special thanks to Stephane Firmin for his significant contribution to the analysis and measurement of carboxylates. Thanks to the technical staff (Adeline Adam, Céline Roisin, Aurore Coutelier, and Philippe Jacolot) for their kind assistance. Thanks to Nicolas Honvault for his technical and scientific advice. Special thanks to the members of the thesis jury (Sylvain Pellerin, Cornelia Rumpel, Claude Plassard and Frédéric Gerard) and thesis committee (Frederic Gerard, Alain Mollier, Gilles Colinet and Olivier Pourret) for their suggestions and comments.

Open access publication funding provided by COUPERIN CY26.

Funding

This work was supported by Campus UniLaSalle Beauvais and the Programme Opérationnel FEDER/FSE Picardie 2014–2020, within the framework of a global research project in agriculture focusing on sustainable management of soil fertility and ecoefficiency of phosphorus management in farming systems.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aerts, R., and F. S. Chapin III. 2000. "The Mineral Nutrition of Wild Plants Revisited : A Re-Evaluation of Processes and Patterns." *Advances in Ecological Research* 30: 1–67. https://doi.org/10.1016/S0065-2504(08)60016-1.

Amadou, I., M.-P. Faucon, and D. Houben. 2022a. "New Insights Into Sorption and Desorption of Organic Phosphorus on Goethite, Gibbsite, Kaolinite and Montmorillonite." *Applied Geochemistry* 143: 105378. https://doi.org/10.1016/j.apgeochem.2022.105378.

Amadou, I., M.-P. Faucon, and D. Houben. 2022b. "Role of Soil Minerals on Organic Phosphorus Availability and Phosphorus Uptake by Plants." *Geoderma* 428: 116125. https://doi.org/10.1016/j.geoderma.2022.116125.

Amadou, I., D. Houben, and M.-P. Faucon. 2021. "Unravelling the Role of Rhizosphere Microbiome and Root Traits in Organic Phosphorus Mobilization for Sustainable Phosphorus Fertilization. A Review." *Agronomy* 11, no. 11: 2267. https://doi.org/10.3390/agronomy11112267.

Bai, S., W. Lü, S. Chen, J. Han, Z. Liu, and A. S. Giwa. 2021. "Different Adsorption Behavior of Inorganic and Organic Phosphorus on Synthetic Schwertmannite: Assessment and Mechanism of Coexistence." *Journal of Environmental Chemical Engineering* 9, no. 5: 106056. https://doi.org/10.1016/j.jece.2021.106056.

Bergmann, W. 1992. *Nutritional Disorders of Plants : Development, Visual and Analytical Diagnosis*. Gustav Fischer.

Bollyn, J., J. Faes, A. Fritzsche, and E. Smolders. 2017. "Colloidal-Bound Polyphosphates and Organic Phosphates Are Bioavailable: A Nutrient Solution Study." *Journal of Agricultural and Food Chemistry* 65, no. 32: 6762–6770. https://doi.org/10.1021/acs.jafc.7b01483.

Bravin, M. N., A. M. Michaud, B. Larabi, and P. Hinsinger. 2010. "RHIZOtest: A Plant-Based Biotest to Account for Rhizosphere Processes When Assessing Copper Bioavailability." *Environmental Pollution* 158, no. 10: 3330–3337. https://doi.org/10.1016/j.envpol.2010.07.029.

Chitrakar, R., S. Tezuka, A. Sonoda, K. Sakane, K. Ooi, and T. Hirotsu. 2006. "Phosphate Adsorption on Synthetic Goethite and Akaganeite." *Journal of Colloid and Interface Science* 298, no. 2: 602–608. https://doi.org/10.1016/j.jcis.2005.12.054.

Cohen, P., S. G. West, and L. S. Aiken. 2014. *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. Psychology Press.

RCore Team. 2023. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Craine, J. M., and R. D. Jackson. 2010. "Plant Nitrogen and Phosphorus Limitation in 98 North American Grassland Soils." *Plant and Soil* 334, no. 1–2: 73–84. https://doi.org/10.1007/s11104-009-0237-1.

DeLuca, T. H., H. C. Glanville, M. Harris, et al. 2015. "A Novel Biologically-Based Approach to Evaluating Soil Phosphorus Availability Across Complex Landscapes." *Soil Biology and Biochemistry* 88: 110–119. https://doi.org/10.1016/j.soilbio.2015.05.016.

Deng, Q., D. Hui, S. Dennis, and K. C. Reddy. 2017. "Responses of Terrestrial Ecosystem Phosphorus Cycling to Nitrogen Addition: A Meta-Analysis." *Global Ecology and Biogeography* 26, no. 6: 713–728.

Dormaar, J. F., and J. W. Ketcheson. 1960. "The Effect of Nitrogen Form and Soil Temperature on the Growth and Phosphorus Uptake of Corn Plants Grown in the Greenhouse." *Canadian Journal of Soil Science* 40, no. 2: 177–184. https://doi.org/10.4141/cjss60-023.

Elser, J. J., W. F. Fagan, A. J. Kerkhoff, N. G. Swenson, and B. J. Enquist. 2010. "Biological Stoichiometry of Plant Production: Metabolism, Scaling and Ecological Response to Global Change." *New Phytologist* 186, no. 3: 593–608. https://doi.org/10.1111/j.1469-8137.2010.03214.x.

Ercoli, L., M. Mariotti, A. Masoni, and F. Massantini. 1996. "Effect of Temperature and Phosphorus Fertilization on Phosphorus and Nitrogen Uptake by Sorhgum." *Crop Science* 36, no. 2: 348–354. https://doi.org/10.2135/cropsci1996.0011183X003600020023x.

Fink, J. R., A. V. Inda, T. Tiecher, and V. Barrón. 2016. "Iron Oxides and Organic Matter on Soil Phosphorus Availability." *Ciência e Agrotecnologia* 40, no. 4: 369–379. https://doi.org/10.1590/1413-70542016404023016.

Fox, J. 2005. "The R Commander: A Basic-Statistics Graphical User Interface to R." *Journal of Statistical Software* 14: 1–42. https://doi.org/10.18637/jss.v014.i09.

Frossard, E., L. M. Condron, A. Oberson, S. Sinaj, and J. C. Fardeau. 2000. "Processes Governing Phosphorus Availability in Temperate Soils." *Journal of Environmental Quality* 29, no. 1: 15–23. https://doi.org/10.2134/jeq2000.00472425002900010003x.

Ganta, P. B., O. Kühn, and A. A. Ahmed. 2019. "QM/MM Simulations of Organic Phosphorus Adsorption at the Diaspore–Water Interface." *Physical Chemistry Chemical Physics* 21, no. 44: 24316–24325. https://doi.org/10.1039/C9CP04032C.

Ganta, P. B., O. Kühn, and A. A. Ahmed. 2020. "QM/MM Molecular Dynamics Investigation of the Binding of Organic Phosphates to the 100 Diaspore Surface." *Frontiers in Forests and Global Change* 3: 71. https://doi.org/10.3389/ffgc.2020.00071.

Goll, D. S., V. Brovkin, B. R. Parida, et al. 2012. "Nutrient Limitation Reduces Land Carbon Uptake in Simulations With a Model of Combined Carbon, Nitrogen and Phosphorus Cycling." *Biogeosciences* 9, no. 9: 3547–3569.

Gómez-Suárez, A. D., C. Nobile, M.-P. Faucon, O. Pourret, and D. Houben. 2020. "Fertilizer Potential of Struvite as Affected by Nitrogen Form in the Rhizosphere." *Sustainability* 12, no. 6: 2212. https://doi.org/10.3390/su12062212.

Grunes, D. L. 1959. "Effect of Nitrogen on the Availability of Soil and Fertilizer Phosphorus to Plants." *Advances in Agronomy* 11: 369–396.

Guppy, C., N. Menzies, P. Moody, and F. Blamey. 2005. "Competitive Sorption Reactions Between Phosphorus and Organic Matter in Soil: A Review." *Australian Journal of Soil Research* 43: 189–202. https://doi.org/10.1071/SR04049.

Güsewell, S. 2004. "N:P Ratios in Terrestrial Plants: Variation and Functional Significance." *New Phytologist* 164, no. 2: 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x.

He, M., F. A. Dijkstra, K. Zhang, et al. 2014. "Leaf Nitrogen and Phosphorus of Temperate Desert Plants in Response to Climate and Soil Nutrient Availability." *Scientific Reports* 4, no. 1: 6932. https://doi.org/10.1038/srep06932.

Helfenstein, J., F. Tamburini, C. von Sperber, et al. 2018. "Combining Spectroscopic and Isotopic Techniques Gives a Dynamic View of Phosphorus Cycling in Soil." *Nature Communications* 9, no. 1: 1–9.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003. "Origins of Root-Mediated pH Changes in the Rhizosphere and Their Responses to Environmental Constraints: A Review." *Plant and Soil* 248, no. 1: 43–59. https://doi.org/10.1023/A:1022371130939.

Hori, T., M. Moriguchi, M. Sasaki, S. Kitagawa, and M. Munakata. 1985. "Preconcentration of Some Phosphorus-Containing Anions by Adsorption on Hydrated Iron(III) Oxide." *Analytica Chimica Acta* 173: 299–303. https://doi.org/10.1016/S0003-2670(00)84967-3.

Hou, E., D. Wen, L. Jiang, et al. 2021. "Latitudinal Patterns of Terrestrial Phosphorus Limitation Over the Globe." *Ecology Letters* 24, no. 7: 1420–1431. https://doi.org/10.1111/ele.13761.

Klotzbücher, A., F. Schunck, T. Klotzbücher, et al. 2020. "Goethite-Bound Phosphorus in an Acidic Subsoil Is Not Available to Beech (*Fagus sylvatica*

L.).” *Frontiers in Forests and Global Change* 3: 94. https://doi.org/10.3389/ffgc.2020.00094.

Koerselman, W., and A. F. M. Meuleman. 1996. “The Vegetation N:P Ratio: A New Tool to Detect the Nature of Nutrient Limitation.” *Journal of Applied Ecology* 33, no. 6: 1441–1450. https://doi.org/10.2307/2404783.

Kome, G. K., R. K. Enang, F. O. Tabi, and B. P. K. Yerima. 2019. “Influence of Clay Minerals on Some Soil Fertility Attributes: A Review.” *Open Journal of Soil Science* 9, no. 9: 155–188. https://doi.org/10.4236/ojss.2026.163003.

Lambers, H., M. W. Shane, M. D. Cramer, S. J. Pearse, and E. J. Veneklaas. 2006. “Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits.” *Annals of Botany* 98, no. 4: 693–713.

Lange, B., M.-P. Faucon, P. Meerts, M. Shutcha, G. Mahy, and O. Pourret. 2014. “Prediction of the Edaphic Factors Influence Upon the Copper and Cobalt Accumulation in Two Metallophytes Using Copper and Cobalt Speciation in Soils.” *Plant and Soil* 379, no. 1: 275–287. https://doi.org/10.1007/s11104-014-2068-y.

Long, M., H.-H. Wu, M. D. Smith, et al. 2016. “Nitrogen Deposition Promotes Phosphorus Uptake of Plants in a Semi-Arid Temperate Grassland.” *Plant and Soil* 408, no. 1–2: 475–484. https://doi.org/10.1007/s11104-016-3022-y.

Lü, X.-T., S. Reed, Q. Yu, N.-P. He, Z.-W. Wang, and X.-G. Han. 2013. “Convergent Responses of Nitrogen and Phosphorus Resorption to Nitrogen Inputs in a Semiarid Grassland.” *Global Change Biology* 19, no. 9: 2775–2784. https://doi.org/10.1111/gcb.12235.

Lun, F., J. Liu, P. Ciais, et al. 2018. “Global and Regional Phosphorus Budgets in Agricultural Systems and Their Implications for Phosphorus-Use Efficiency.” *Earth System Science Data* 10, no. 1: 1–18. https://doi.org/10.5194/essd-10-1-2018.

Lund, M., T. R. Christensen, M. Mastepanov, A. Lindroth, and L. Ström. 2009. “Effects of N and P Fertilization on the Greenhouse Gas Exchange in Two Northern Peatlands With Contrasting N Deposition Rates.” *Biogeosciences* 6, no. 10: 2135–2144. https://doi.org/10.5194/bg-6-2135-2009.

Marklein, A. R., and B. Z. Houlton. 2012. “Nitrogen Inputs Accelerate Phosphorus Cycling Rates Across a Wide Variety of Terrestrial Ecosystems.” *New Phytologist* 193, no. 3: 696–704.

McCullagh, P., and J. A. Nelder. 2019. *Generalized Linear Models*. Routledge.

Michaud, A. M., M. N. Bravin, M. Galleguillos, and P. Hinsinger. 2007. “Copper Uptake and Phytotoxicity as Assessed in Situ for Durum Wheat (*Triticum turgidum durum* L.) Cultivated in Cu-Contaminated, Former Vineyard Soils.” *Plant and Soil* 298, no. 1: 99–111. https://doi.org/10.1007/s11104-007-9343-0.

Murphy, P. N. C., A. Bell, and B. L. Turner. 2009. “Phosphorus Speciation in Temperate Basaltic Grassland Soils by Solution ^{31}P NMR Spectroscopy.” *European Journal of Soil Science* 60, no. 4: 638–651. https://doi.org/10.1111/j.1365-2389.2009.01148.x.

Nannipieri, P., L. Giagnoni, L. Landi, and G. Renella. 2011. “Role of Phosphatase Enzymes in Soil.” In *Phosphorus in Action*, edited by E. Bünemann, A. Oberson, and E. Frossard, 215–243. Springer. https://doi.org/10.1007/978-3-642-15271-9_9.

Ohno, T., and L. M. Zibilske. 1991. “Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green.” *Soil Science Society of America Journal* 55, no. 3: 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x.

Peng, Y., Z. Peng, X. Zeng, and J. H. Houx. 2019. “Effects of Nitrogen-Phosphorus Imbalance on Plant Biomass Production: A Global Perspective.” *Plant and Soil* 436, no. 1–2: 245–252. https://doi.org/10.1007/s11104-018-03927-5.

Penuelas, J., I. A. Janssens, P. Ciais, M. Obersteiner, and J. Sardans. 2020. “Anthropogenic Global Shifts in Biospheric N and P Concentrations and Ratios and Their Impacts on Biodiversity, Ecosystem Productivity, Food Security, and Human Health.” *Global Change Biology* 26, no. 4: 1962–1985. https://doi.org/10.1111/gcb.14981.

Peñuelas, J., B. Poulter, J. Sardans, et al. 2013. “Human-Induced Nitrogen–Phosphorus Imbalances Alter Natural and Managed Ecosystems Across the Globe.” *Nature Communications* 4, no. 1: 2934. https://doi.org/10.1038/ncomms3934.

Peters, J. P., and D. D. Van Slyke. 1932. *Quantitative Clinical Chemistry*. Williams & Wilkins.

Richardson, A. E., P. J. Hocking, R. J. Simpson, and T. S. George. 2009. “Plant Mechanisms to Optimise Access to Soil Phosphorus.” *Crop and Pasture Science* 60, no. 2: 124–143. https://doi.org/10.1071/CP07125.

Sardans, J., O. Grau, H. Y. Chen, et al. 2017. “Changes in Nutrient Concentrations of Leaves and Roots in Response to Global Change Factors.” *Global Change Biology* 23, no. 9: 3849–3856. https://doi.org/10.1111/gcb.13721.

Sardans, J., and J. Peñuelas. 2012. “The Role of Plants in the Effects of Global Change on Nutrient Availability and Stoichiometry in the Plant-Soil System.” *Plant Physiology* 160, no. 4: 1741–1761. https://doi.org/10.1104/pp.112.208785.

Schabenberger, O., and F. J. Pierce. 2001. *Contemporary Statistical Models for the Plant and Soil Sciences*. CRC Press.

Schleuss, P. M., M. Widdig, A. Heintz-Buschart, K. Kirkman, and M. Spohn. 2020. “Interactions of Nitrogen and Phosphorus Cycling Promote P Acquisition and Explain Synergistic Plant-Growth Responses.” *Ecology* 101, no. 5: e03003. https://doi.org/10.1002/ecy.3003.

Singh, B., and D. G. Schulze. 2015. “Soil Minerals and Plant Nutrition.” *Nature Education Knowledge* 6, no. 1: 1.

Tabatabai, M. A., and J. M. Bremner. 1969. “Use of *p*-Nitrophenyl Phosphate for Assay of Soil Phosphatase Activity.” *Soil Biology and Biochemistry* 1, no. 4: 301–307. https://doi.org/10.1016/0038-0717(69)90012-1.

Tessier, J. T., and D. J. Raynal. 2003. “Use of Nitrogen to Phosphorus Ratios in Plant Tissue as an Indicator of Nutrient Limitation and Nitrogen Saturation.” *Journal of Applied Ecology* 40, no. 3: 523–534. https://doi.org/10.1046/j.1365-2664.2003.00820.x.

Thien, S. J., and W. W. McFee. 1972. “Effect of Nitrogen on Phosphorus Transport Systems in *Zea mays* L.” *Soil Science Society of America Journal* 36, no. 4: 617–620. https://doi.org/10.2136/sssaj1972.03615995003600040033x.

Vitousek, P. M., S. Porder, B. Z. Houlton, and O. A. Chadwick. 2010. “Terrestrial Phosphorus Limitation: Mechanisms, Implications, and Nitrogen–Phosphorus Interactions.” *Ecological Applications* 20, no. 1: 5–15. https://doi.org/10.1890/08-0127.1.

von Oheimb, G., S. A. Power, K. Falk, et al. 2010. “N:P Ratio and the Nature of Nutrient Limitation in Calluna-Dominated Heathlands.” *Ecosystems* 13, no. 2: 317–327. https://doi.org/10.1007/s10021-010-9320-y.

Wang, R., B. Bicharanloo, E. Hou, Y. Jiang, and F. A. Dijkstra. 2022. “Phosphorus Supply Increases Nitrogen Transformation Rates and Retention in Soil: A Global Meta-Analysis.” *Earth’s Future* 10, no. 3: e2021EF002479. https://doi.org/10.1029/2021EF002479.

Warton, D. I., M. Lyons, J. Stoklosa, and A. R. Ives. 2016. “Three Points to Consider When Choosing a LM or GLM Test for Count Data.” *Methods in Ecology and Evolution* 7, no. 8: 882–890. https://doi.org/10.1111/2041-210X.12552.

Widdig, M., P.-M. Schleuss, A. R. Weig, et al. 2019. “Nitrogen and Phosphorus Additions Alter the Abundance of Phosphorus-Solubilizing Bacteria and Phosphatase Activity in Grassland Soils.” *Frontiers in Environmental Science* 7: 185. https://doi.org/10.3389/fenvs.2019.00185.

Xia, S., J. Jiang, F. Liu, et al. 2023. “Phosphorus Addition Promotes Plant Nitrogen Uptake Mainly via Enhancing Microbial Activities: A Global Meta-Analysis.” *Applied Soil Ecology* 188: 104927. https://doi.org/10.1016/j.apsoil.2023.104927.

Yan, Y. P., F. Liu, W. Li, F. Liu, X. H. Feng, and D. L. Sparks. 2014. "Sorption and Desorption Characteristics of Organic Phosphates of Different Structures on Aluminium (oxyhydr)Oxides: Sorption-Desorption of Organic P on Al Oxyhydroxides." *European Journal of Soil Science* 65, no. 2: 308–317. https://doi.org/10.1111/ejss.12119.

Yang, D., H. Mao, and G. Jin. 2019. "Divergent Responses of Foliar N:P Stoichiometry During Different Seasons to Nitrogen Deposition in an Old-Growth Temperate Forest, Northeast China." *Forests* 10, no. 3: 257. https://doi.org/10.3390/f10030257.

Yu, H., X. Lu, T. Miki, K. Matsubae, Y. Sasaki, and T. Nagasaka. 2022. "Sustainable Phosphorus Supply by Phosphorus Recovery From Steelmaking Slag: A Critical Review." *Resources, Conservation and Recycling* 180: 106203. https://doi.org/10.1016/j.resconrec.2022.106203.

Zheng, L.-L., Q. Zhao, Z.-Y. Yu, S.-Y. Zhao, and D.-H. Zeng. 2017. "Altered Leaf Functional Traits by Nitrogen Addition in a Nutrient-Poor Pine Plantation: A Consequence of Decreased Phosphorus Availability." *Scientific Reports* 7, no. 1: 7415. https://doi.org/10.1038/s41598-017-07170-3.

Zhu, Y.-G., and J. Penuelas. 2020. "Changes in the Environmental Microbiome in the Anthropocene." *Global Change Biology* 26, no. 6: 3175–3177. https://doi.org/10.1111/gcb.15086.

Zubillaga, M. M., J. P. Aristi, and R. S. Lavado. 2002. "Effect of Phosphorus and Nitrogen Fertilization on Sunflower (*Helianthus annuus* L.) Nitrogen Uptake and Yield." *Journal of Agronomy and Crop Science* 188, no. 4: 267–274. https://doi.org/10.1046/j.1439-037X.2002.00570.x.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: jpln70086-sup-0001-SuppMat.pdf.

Supporting File 2: jpln70086-sup-0002-SuppMat.docx.