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Phosphorus Dynamics in Japanese Blueberry Field: Long-Term Accumulation and Fractionation across Soil Types and Depths

Chun Lu [1], Soh Sugihara [2], Haruo Tanaka [2], Ryosuke Tajima [3], Shingo Matsumoto [4] and Takuya Ban [1,*]

[1] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan; chunlu2008@gmail.com
[2] Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan; sohs@cc.tuat.ac.jp (S.S.); haruo@cc.tuat.ac.jp (H.T.)
[3] Graduate School of Agricultural Science, Tohoku University, Sendai 980-8572, Japan; ryosuke.tajima.a4@tohoku.ac.jp
[4] Graduate School of Natural Science and Technology, Shimane University, Shimane 690-8504, Japan; smatsu@life.shimane-u.ac.jp
* Correspondence: tban@cc.tuat.ac.jp

Abstract: Effective phosphorus (P) management is crucial for optimal blueberry production. However, a comprehensive understanding of phosphorus distribution across soil depths and types after two decades of blueberry cultivation remains a challenge. This study examines pH, EC, SOC (soil organic carbon), Total N (total nitrogen), and phosphorus fractions in soils from Japanese blueberry fields that have been cultivated for over 20 years. The soils selected for this study represent typical soils from long-term blueberry-growing regions in Japan, ensuring the relevance of the findings to these key agricultural areas. Soil samples were gathered from depths of 0–30 cm and 30–60 cm, revealing significant variations in phosphorus content that are influenced by soil properties and fertilization history. Soil types such as KS (Kuroboku soils) and FS (Fluvic soils) show higher Total P accumulation in deeper layers, whereas BFS (Brown Forest soils) and RYS (Red-Yellow soils) accumulate more in shallower layers. Long-term cultivation has led to greater non-labile phosphorus (NLP) accumulation in shallower layers of KS, BFS, and FS soils, indicating strong phosphorus fixation. BFS soil also exhibits increased organic phosphorus (NaOH-Po) at deeper depths. NaOH-Po and $NaHCO_3$-Po, through their interactions with EC and pH, critically modulate the transformation of NLP into labile phosphorus (LP), thereby influencing overall phosphorus and nitrogen dynamics in the soil. These findings underscore the importance of tailored phosphorus fertilization strategies based on blueberry field characteristics, providing a basis for low-input phosphorus fertilization approaches.

Keywords: Japanese blueberry field; phosphorus fractions; non-labile phosphorus (NLP); labile phosphorus (LP)

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1. Introduction

Phosphorus (P) plays a crucial role in every stage of plant development. The primary source of this essential nutrient is phosphate rock, which constitutes over 90% of the majority of the world's phosphorus supply [1]. Heavily relied upon in agricultural practices, these non-renewable phosphate rock reserves are experiencing a gradual depletion [2,3]. Soil serves as the primary reservoir and supplier of phosphorus for plant uptake. Plants' availability and uptake of soil phosphorus are influenced by various factors, including plant root morphology and adaptations, climatic conditions, microbial activity, and human activities [4,5]. The indiscriminate use of phosphorus fertilizers is common, leading to an excess of phosphorus in agricultural soils and associated environmental risks [6]. The Hedley P sequential fractionation method [7] categorizes soil phosphorus into two types: plant and microbe available forms (labile P), primarily consisting of $NaHCO_3$-Pi (Sodium Bicarbonate-Inorganic Phosphorus), $NaHCO_3$-Po (Sodium Bicarbonate-Organic

Phosphorus), and Resin-P (Resin-Extractable Phosphorus); and refractory forms (non-labile P), mainly comprising NaOH-Pi (Sodium Hydroxide-Inorganic Phosphorus), NaOH-Po (Sodium Hydroxide-Organic Phosphorus), HCl-Pi (Hydrochloric Acid-Inorganic Phosphorus), and Residual-P (Residual Phosphorus). Another classification divides phosphorus into a biological fraction (organic P), which mainly includes $NaHCO_3$-Po and NaOH-Po, and a geochemical fraction (inorganic P), primarily comprising $NaHCO_3$-Pi, NaOH-Pi, HCl-Pi, and Resin-P, along with the organic and inorganic components of the Residual-P fraction [8]. In soil, phosphorus predominantly occurs in forms that are insoluble [9]. Approximately 30% to 65% of the total phosphorus exists in an organic form, which is not easily accessible for plant uptake. The rest, constituting 35% to 70%, is inorganic [10]. Within the inorganic phosphorus forms, there are various categories, including plant-available, sorbed, and mineral phosphorus. The portion of phosphorus available to plants is a limited fraction that is easily absorbable for plant utilization. Most of the phosphorus present in the soil, including both organic and part of the inorganic forms, is not immediately available to plants due to its insolubility [11]. Applied phosphorus often transforms into less available forms due to soil characteristics, organic matter content, and fertilizer usage [12]. The establishment of low-input cropping systems for phosphorus is an urgent issue, as it addresses the need for sustainable agricultural practices that optimize phosphorus use efficiency and reduce environmental impacts.

Blueberry (*Vaccinium spp.*) is globally acknowledged as one of the five most nutritious foods. Known for its delightful flavor, the blueberry is highly valued as a "superfruit" due to its rich content of health-enhancing bioactive compounds [13]. The root system development of blueberries exhibits unique characteristics compared to other common deciduous fruit trees, notably their requirement for acidic soils to sustain normal growth [14]. Blueberry plants typically have a shallow root system. In over 20 years of cultivating both highbush and rabbiteye blueberries, a common type of root system observed is the fibrous root system, with root diameters less than 0.1 mm. The sideways expansion of roots usually doesn't extend beyond 60 cm, while their downward growth rarely exceeds a depth of 60–70 cm [15].

In the cultivation of Vaccinium species, particularly blueberries, the strategic application of phosphorus fertilizer emerges as a vital element. This significance is attributed to the role of P in facilitating key physiological processes, including but not limited to leaf system development and fruit production [16]. Phosphorus fertilizer exerts a substantial influence on modifying soil pH and enhancing the mineral nutrient profile within the soil matrix. Such modification is crucial in enhancing the comprehensive growth and fruiting progression of blueberry plants [17]. Furthermore, the impact of P fertilization extends to the rhizospheric microbial dynamics. These microbial communities are crucial for maintaining soil health and, consequently, the nutritional equilibrium of the plant system. The intricacies of phosphorus management are, therefore, directly correlated with the qualitative and quantitative aspects of blueberry yield [18]. It is imperative to acknowledge that phosphorus is identified as a limiting nutrient in the context of blueberry yield and quality. Blueberries exhibit a marked sensitivity to fertilization practices, particularly to the concentration and application regime of phosphorus. Over-application of P can result in deleterious effects on yield, emphasizing the necessity of precision in fertilization methodologies [19]. Variations in soil fertility result in differential effects of P application on crop yield. Notably, in soils characterized by low levels of available phosphorus, the application of P fertilizers is more efficaciously correlated with enhanced crop production efficiency [20]. However, a significant proportion (approximately 70%) of the applied phosphorus in soils converts into fixed forms, such as iron (Fe-P), calcium (Ca-P), and aluminum phosphate (Al-P). This transformation process can lead to the accumulation of insoluble phosphorus in the soil matrix, causing a shortage of readily available phosphorus [21,22]. Blueberries, which were introduced in the United States in the 1960s, are not indigenous to Japan. Currently, they are cultivated in diverse soil types across almost all regions of the country. However, the effects of blueberry cultivation on the soil's physicochemical properties are unknown. This study hypothesizes that blueberry fields in Japan are enriched with large amounts of insoluble phosphorus. Yet, few studies have detailed the extent of insoluble phosphorus accumulation in fertilized soils and its distribution across different soil types and layers in Japan, specific to blueberry cultivation.

This research focuses on the phosphorus dynamics within blueberry fields across various dominant soil types in Japan, including Fluvic, Kuroboku, Red-Yellow, and Brown Forest soils [23]. The primary objective is to assess the potential for implementing a low-phosphorus cultivation system in the Japanese blueberry field. The scope of study encompasses the following: 1. Evaluating the phosphorus accumulation patterns in blueberry soils; 2. Conducting a comprehensive analysis of the predominant insoluble phosphorus fractions in these soils; 3. Identifying and analyzing the key determinants influencing the levels of insoluble phosphorus in blueberry soils, and 4. Investigating the phosphorus distribution and stratification within the shallow rhizosphere (0–30 cm) compared to the deep rhizosphere (30–60 cm) in blueberry cultivation areas. By addressing these aspects, this study seeks to significantly advance soil management and fertilization strategies for blueberry cultivation in Japan, ultimately boosting yield and sustainability.

2. Materials and Methods

2.1. Study Area Background

The research took place at four distinct sites: private economic cultivation plots in Shimane prefecture (35°21′02″ N 132°57′02″ E), Shimane University Biological Resources Education and Research Center (35°30′42″ N 133°06′32″ E), the Field Science Center of Tokyo University of Agriculture and Technology (35°41′00″ N 139°29′12″ E), and Tohoku University Kawatabi Field Science Center (38°44′38″ N 140°45′40″ E) in Japan. The soil types at these sites represent the typical soils for blueberry cultivation in Japan, namely Fluvic (FS), Kuroboku (KS), Red-Yellow (RYS), and Brown Forest soils (BFS). These four types of soil account for 53.4%, 15%, 1.5%, and 17.3% of Japan's total soil, respectively, as shown in (Figure 1). To better understand the characteristics of FS, KS, RYS, and BFS soils, according to the World Reference Base for Soil Resources (WRB), these soils correspond to Fluvisols, Andosols, Acrisols, and Cambisols, respectively [24].

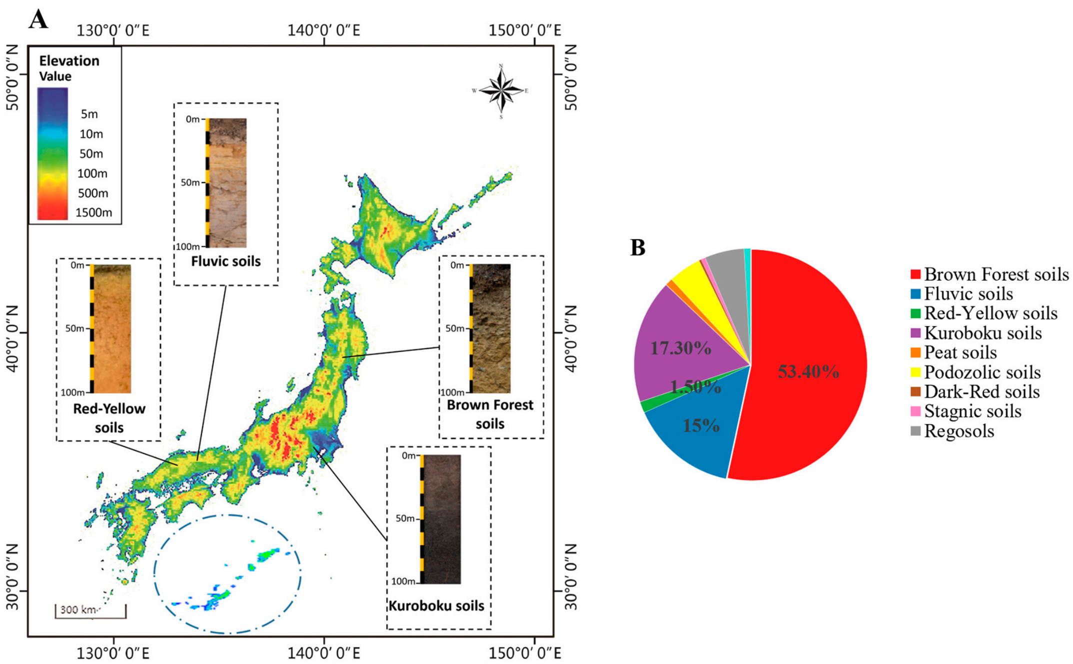

Figure 1. (**A**) Map of the study areas with Kuroboku, Brown Forest, Red-Yellow, and Fluvic soils in Japan and (**B**) proportion of soil types in Japan (Cited from Kanno et al. 2008 [23]).

2.2. Data Collection and Analysis

2.2.1. Soil Collection

In the BFS, FS, RYS, and KS blueberry cultivation areas, five blueberry trees were randomly chosen at each location. The variety of blueberries belongs to the rabbiteye series, with all trees being over 20 years old. Specifically, the BFS area was planted in 1999, and soil samples were collected on 12 December, 2022. The FS area, planted in 2001, had soil samples taken on 24 April, 2022. The RYS area, which was planted in 1986, had its soil sampled on 25 April, 2022. Lastly, the KS area was established in 1968, with soil sampling conducted on 9 June, 2022.

A 60 cm deep pit was dug 30 cm away from the center of each blueberry tree in areas that had been managed for over 20 years and similarly in adjacent native soils that had not been fertilized. Soil samples were collected from 30 cm and 60 cm depths, promptly placed in separate plastic bags, and sent to the laboratory for analysis. The collected soil samples were naturally air-dried in a glass greenhouse, with plant roots and stones removed. The samples were first sieved through a 2 mm sieve to remove large particles and gravel; then, the remaining soil was thoroughly ground using a mortar and pestle. The prepared soil samples were labeled, placed into 50 mL tubes, and stored at a temperature of 10 °C.

2.2.2. Phosphorus Sequential Fractionation and Determination

Soil phosphorus fractionation was performed using the Hedley method [7], modified by Tiessen and Moir [25]. The procedure for phosphorus fractionation of 0.5 g soil samples is as follows: First, place the soil sample in a 50 mL centrifuge tube, add deionized water to 30 mL and two anion exchange resin bags (AEM), and shake at 25 °C for 16 h. After removing the AEM, place it in 20 mL of 0.5 M HCl, shake for 2 h at 25 °C, and extract Resin-P. Second, after centrifugation, discard the supernatant, then add 30 mL of 0.5 M $NaHCO_3$ to the remaining soil and shake for 16 h at 25 °C. After shaking, centrifuge and collect the supernatant. To extract $NaHCO_3$-Pi, combine a portion of the supernatant with distilled water to make a total volume of 10 mL, then add 0.9 M H_2SO_4. For the determination of $NaHCO_3$-Pt, take a separate 5 mL aliquot of the same supernatant, add 10 mL of 0.9 M H_2SO_4 and 0.5 g of ammonium persulfate, and digest at 120 °C for 1 h. $NaHCO_3$-Po is then calculated by subtracting $NaHCO_3$-Pi from $NaHCO_3$-Pt. Third, 30 mL of 0.1 M NaOH was added to the remaining soil, shaken for 16 h at 25 °C, and centrifuged. To extract NaOH-Pi, take 10 mL of the supernatant, combine it with distilled water, and add 2 mL of 0.9 M H_2SO_4. For NaOH-Pt, take a separate 5 mL aliquot of the same supernatant, add 10 mL of 0.9 M H_2SO_4 and 0.6 g of ammonium persulfate, and digest at 120 °C for 1 h. NaOH-Po is calculated by subtracting NaOH-Pi from NaOH-Pt. Finally, add 20 mL of 1 M HCl to the remaining soil, shake for 16 h at 25 °C, centrifuge, and take the supernatant as HCl-Pi. The phosphorus content in the collected solutions and subsequent samples was measured using the molybdenum blue colorimetry method at 712 nm with a SHIMADZU UV-1280 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Total P (total phosphorus) is determined by the HNO_3-$HClO_4$ digestion method [26], adding boiling stones and 10 mL of HNO_3 and heating at 200 °C, then adding $HClO_4$ and continuing to heat at 200 °C, with phosphorus content determined by the molybdenum blue colorimetric method at 712 nm.

The content of Residual-P is calculated by subtracting the sum of $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, HCl-Pi, and Resin-P from the Total P. The fractionated phosphorus is categorized into four groups: organic phosphorus ($NaHCO_3$-Po and NaOH-Po), inorganic phosphorus (Resin-P, $NaHCO_3$-Pi, NaOH-Pi, and HCl-Pi), labile P ($NaHCO_3$-Pi, $NaHCO_3$-Po, and Resin-P), and non-labile P (NaOH-Pi, NaOH-Po, HCl-Pi, and Residual-P). Additionally, in this article, the terms organic phosphorus, inorganic phosphorus, labile phosphorus, and non-labile phosphorus are abbreviated as OP, IOP, LP, and NLP, respectively.

2.2.3. Evaluation of Soil Properties

Different techniques and soil-to-distilled-water ratios were utilized to evaluate soil pH and Electrical Conductivity (EC) [27]. For pH assessment, a HORIBA glass electrode (D-210P/220P, HORIBA Ltd., Kyoto, Japan) was used with a 1:2.5 soil-to-water ratio. In contrast, EC was measured using the AC bipolar method with a HORIBA instrument (D-210C/220C, HORIBA Ltd., Kyoto, Japan) and a 1:5 soil-to-water ratio. Following a 2-h shaking period and subsequent equilibration, measurements of pH and EC were conducted on the supernatant. The measurement of soil organic carbon (SOC) and total nitrogen (Total N) was conducted using the dry combustion technique. This procedure utilized the SUMIGRAPH NC-TR22 NC analyzer from Sumika Chemical Analysis Service, Ltd., in Osaka, Japan.

2.3. Statistical Analysis

Graphs and tables were generated and analyzed using Excel 365 and Origin Lab 2024. The data analysis software employed was SPSS software version 26. Principal component analysis was conducted using Canoco version 5.0. Two-way ANOVA was utilized to evaluate the relationships among variables from soil types and soil depths with biochemical indices and phosphorus fractions. LSD (Least Significant Difference) tested the significance of relationships between datasets. Structural equation modeling (SEM) results were obtained with Smart PLS (4.0). [28].

3. Results

3.1. Change of Soil Properties in Blueberry Field

Table 1 presents notable variations in soil properties between used and unused lands across different depths (0–30 cm and 30–60 cm) and soil types in blueberry fields. The BFS-MR soil at 0–30 cm exhibited the highest pH level (6.00 ± 0.02), while the KS soil at both measured depths showed the lowest pH values (4.50 ± 0.20 at 0–30 cm and 4.48 ± 0.12 at 30–60 cm), with the overall pH range spanning from 4.46 ± 0.19 to 6.00 ± 0.02. The highest electrical conductivity (EC) was recorded in KS soil at 30–60 cm depth (287.32 ± 56.03 µs cm^{-1}), contrasting with the lowest EC found in BFS-MR soil at the same depth (24.57 ± 0.40 µs cm^{-1}). Total Nitrogen (Total N) peaked in KS soil at 0–30 cm depth (5.11 ± 0.32 g kg^{-1}) and reached its minimum in RYS-MR soil at both depths (0.49 ± 0.00 g kg^{-1} at 0–30 cm and 0.81 ± 0.47 g kg^{-1} at 30–60 cm). For Soil Organic Carbon (SOC), the maximum was observed in KS soil at 30–60 cm depth (78.64 ± 5.64 g kg^{-1}), while the minimum occurred in RYS-MR soil at 0–30 cm (2.78 ± 0.01 g kg^{-1}). Additionally, almost all soils that had been subjected to long-term fertilization showed significantly higher organic matter content compared to unfertilized soils (MR soil).

3.2. Effect with Fertilized Blueberry Field Change and Distribution of P Fractions

Figure 2 and Table 2 represent different phosphorus fractions in both the 0–30 cm and 30–60 cm layers for KS, BFS, RYS, and FS soils, which are critical indicators of soil fertility and phosphorus availability for plants. KS soil stands out with notably highest phosphorus fractions, particularly in $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, HCl-Pi, Residual-P, and Total P when compared to other soils. In contrast, the Resin-P concentration in RYS soil was significantly elevated solely within the 0–30 cm layer (133.36 ± 119.22 mg kg^{-1}), in stark contrast to other soil types. Conversely, FS soil exhibited the highest levels of Resin-P (68.96 ± 34.00 mg kg^{-1} at 30–60 cm) and $NaHCO_3$-Pi (127.60 ± 81.83 mg kg^{-1} at 0–30 cm and 147.97 ± 43.22 mg kg^{-1} at 30–60 cm).

Table 1. Soil properties with both soil depths and soil types of blueberry fields.

Soil Properties	0–30 cm Soil Depth								30–60 cm Soil Depth							
	KS-MR	KS	BFS-MR	BFS	RYS-MR	RYS	FS-MR	FS	KS-MR	KS	BFS-MR	BFS	RYS-MR	RYS	FS-MR	FS
pH	4.76 ± 0.08 cd*	4.50 ± 0.20 de*	6.00 ± 0.02 a*	5.61 ± 0.38 b	4.99 ± 0.08 cd	4.46 ± 0.19 e	4.65 ± 0.02 d*	5.06 ± 0.38 c	5.16 ± 0.02 b	5.05 ± 0.23 c	5.80 ± 0.04 a	5.44 ± 0.46 b	4.99 ± 0.04 c	4.48 ± 0.12 d	5.09 ± 0.01 c	5.21 ± 0.25 bc
EC ($\mu s\ cm^{-1}$)	250.60 ± 6.95 a	217.39 ± 27.61 b*	27.07 ± 0.21 e*	81.32 ± 40.14 cd	57.53 ± 1.90 d	102.26 ± 18.36 c	55.37 ± 1.54 d*	61.97 ± 32.92 d	233.63 ± 10.69 a	287.32 ± 56.03 a	24.57 ± 0.40 d	66.02 ± 27.27 c	54.27 ± 1.18 c	112.78 ± 13.75 b	47.50 ± 0.70 c	52.81 ± 17.11 c
Total N ($g\ kg^{-1}$)	4.49 ± 0.05 b*	5.11 ± 0.32 a	2.19 ± 0.01 d*	3.28 ± 0.97 c*	0.67 ± 0.04 ef*	1.05 ± 0.36 e	0.49 ± 0.00 f*	0.81 ± 0.47 ef	4.89 ± 0.05 b	5.30 ± 0.29 a	0.48 ± 0.03 e	1.16 ± 0.44 c	0.87 ± 0.02 cd	1.27 ± 0.30 c	0.70 ± 0.01 d	0.81 ± 0.11 d
SOC ($g\ kg^{-1}$)	63.50 ± 0.03 b*	74.00 ± 4.60 a	45.29 ± 0.17 c*	65.92 ± 23.87 abc*	2.78 ± 0.01 d*	9.74 ± 5.79 d	4.72 ± 0.04 d*	7.72 ± 4.02 d	71.19 ± 0.46 a	78.64 ± 5.64 a	7.70 ± 0.16 d	21.62 ± 12.14 b	4.50 ± 0.08 e	12.64 ± 4.83 bcd	9.56 ± 0.06 c	7.68 ± 1.15 d
C/N	14.14 ± 0.14 b*	14.49 ± 0.40 b	20.71 ± 0.09 a*	19.62 ± 2.06 a	4.19 ± 0.22 d*	8.66 ± 2.08 c	9.72 ± 0.04 c*	9.77 ± 0.60 c	14.57 ± 0.07 a	14.82 ± 0.37 a	16.18 ± 0.81 ab	17.89 ± 3.78 a	5.16 ± 0.07 d	9.69 ± 2.09 c	13.56 ± 0.17 b	9.49 ± 0.19 c

Note: KS-MR: Kuroboku soils of unused land in the main road; KS: Kuroboku soils of land use; BFS-MR: Brown Forest soils of unused land in the main road; BFS: Brown Forest soils of land use; RYS-MR: Red-Yellow soils of unused land in the main road; RYS: Red-Yellow soils of land use; FS-MR: Fluvic soils of unused land in the main road; FS: Fluvic soils of land use; Total N refers to total nitrogen; SOC denotes soil organic carbon; C/N represents the ratio of soil organic carbon to nitrogen. ± values show the mean standard deviation (SD). Letters indicate significant differences among soil types at the same depths ($p \leq 0.05$). An asterisk (*) after letters signifies significant differences between soil depths within the same soil types.

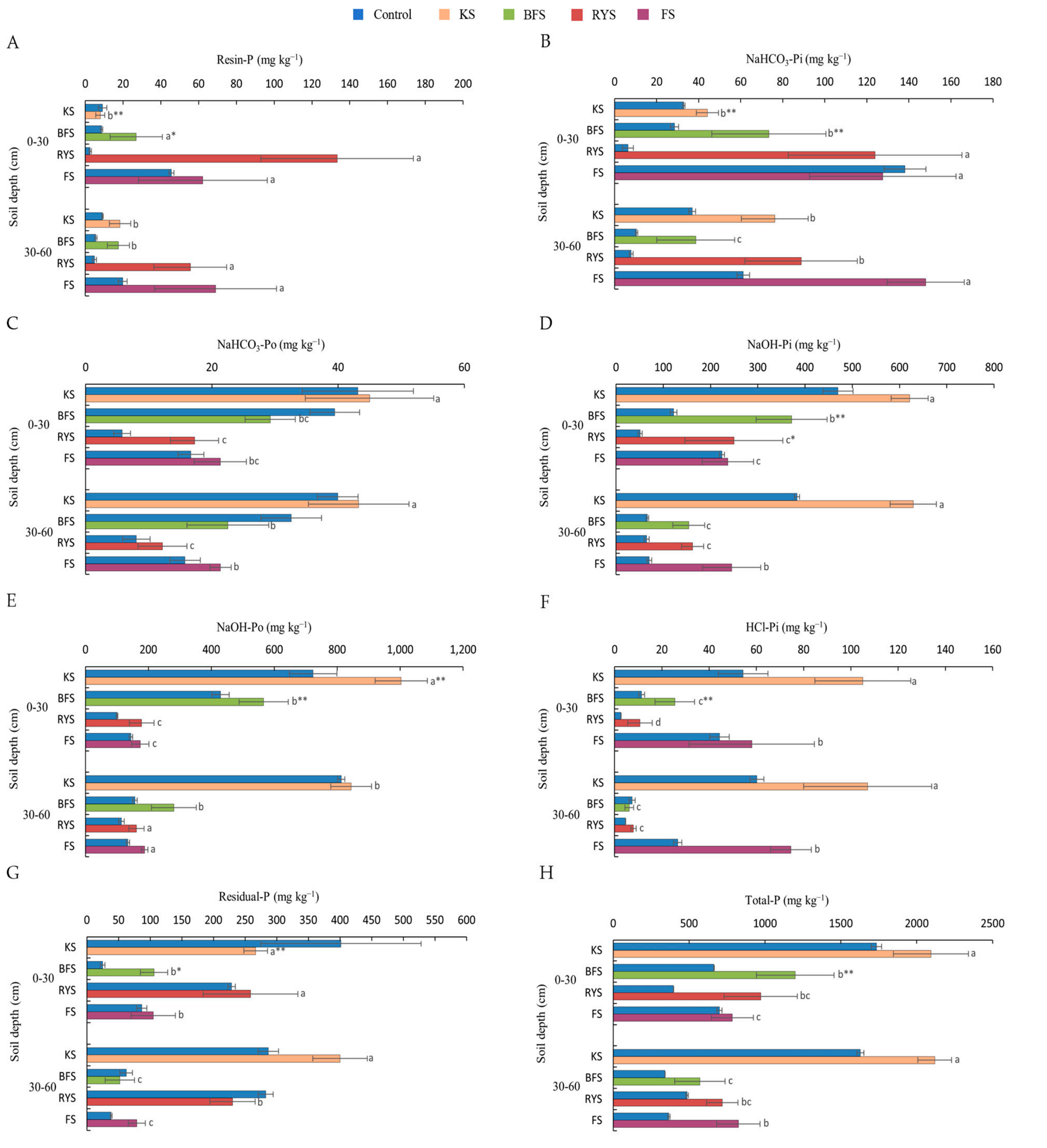

Figure 2. Phosphorus factions with soil depths and soil types of blueberry's land use. Specifically, (**A**) Resin-P, (**B**) $NaHCO_3$-Pi, (**C**) $NaHCO_3$-Po, (**D**) NaOH-Pi, (**E**) NaOH-Po, **(F)** HCl-Pi, (**G**) Residual-P, (**H**) Total-P. Note: Control: unused land on the main road. Letters denote significant differences among soil types at the same depths ($p \leq 0.05$). Asterisks (*) indicate significant differences between depths within the same soil types, with * $p \leq 0.05$ and ** $p \leq 0.01$. Total sample $n = 120$.

Table 2. Phosphorus factions with soil depths and soil types of blueberry's land use.

P Fractions	Soil Depth (cm)	Land Use Type			
		KS	BFS	RYS	FS
Resin-P (mg kg^{-1})	0–30 30–60	7.96 ± 3.01 b* 18.41 ± 5.92 b	26.97 ± 14.31 a* 17.49 ± 9.75 b	133.36 ± 119.22 a 55.62 ± 20.33 a	62.25 ± 35.97 a 68.96 ± 34.00 a
$NaHCO_3$-Pi (mg kg^{-1})	0–30 30–60	44.15 ± 14.85 b* 76.17 ± 22.98 b	73.40 ± 34.61 b* 38.60 ± 24.70 c	123.94 ± 89.02 a 88.75 ± 33.31 b	127.60 ± 81.83 a 147.97 ± 43.22 a
$NaHCO_3$-Po (mg kg^{-1})	0–30 30–60	44.98 ± 10.72 a 43.26 ± 8.40 a	29.24 ± 9.32 bc 22.53 ± 10.66 b	17.26 ± 8.97 c 12.17 ± 4.10 c	21.34 ± 11.39 bc 21.37 ± 3.10 b
NaOH-Pi (mg kg^{-1})	0–30 30–60	621.50 ± 123.51 a 629.03 ± 168.46 a	371.69 ± 137.19 b* 153.96 ± 63.16 c	249.57 ± 109.15 c* 162.18 ± 24.63 c	236.68 ± 116.00 c 244.83 ± 64.92 b
NaOH-Po (mg kg^{-1})	0–30 30–60	1003.35 ± 87.17 a* 844.41 ± 67.87 a	566.22 ± 131.33 b* 281.06 ± 73.66 b	178.44 ± 48.43 c 162.19 ± 25.86 c	174.34 ± 38.65 c 188.17 ± 14.58 c
HCl-Pi (mg kg^{-1})	0–30 30–60	105.03 ± 21.37 a 107.14 ± 28.56 a	25.50 ± 13.74 c* 6.25 ± 3.26 c	10.74 ± 5.46 d 7.96 ± 3.89 c	58.08 ± 27.92 b 74.64 ± 9.09 b
Residual-P (mg kg^{-1})	0–30 30–60	266.90 ± 41.39 a* 400.19 ± 64.44 a	106.07 ± 33.25 b* 52.13 ± 24.21 c	258.59 ± 78.74 a 230.02 ± 37.78 b	104.81 ± 36.86 b 78.81 ± 18.14 c
Total OP (mg kg^{-1})	0–30 30–60	1048.33 ± 80.23 a* 887.66 ± 64.64 a	595.47 ± 129.44 b* 303.59 ± 68.52 b	195.70 ± 53.77 c 174.36 ± 27.65 c	195.68 ± 46.32 c 209.54 ± 15.06 d
Total IOP (mg kg^{-1})	0–30 30–60	778.63 ± 151.41 a 830.75 ± 204.63 a	497.56 ± 187.82 b* 216.29 ± 85.40 d	517.62 ± 299.77 b 314.51 ± 69.78 c	484.60 ± 232.54 b 536.39 ± 127.74 b
Total LP (mg kg^{-1})	0–30 30–60	97.09 ± 17.65 a* 137.84 ± 30.99 b	129.62 ± 47.59 a* 78.62 ± 26.86 c	274.56 ± 203.37 a 156.54 ± 48.36 b	211.18 ± 115.03 a 238.30 ± 64.63 a
Total NLP (mg kg^{-1})	0–30 30–60	1996.78 ± 226.25 a 1980.76 ± 233.26 a	1069.49 ± 286.98 b* 493.39 ± 154.05 b	697.35 ± 203.42 c 562.35 ± 75.31 b	573.90 ± 145.46 c 586.44 ± 81.11 b
Total P (mg kg^{-1})	0–30 30–60	2093.86 ± 260.25 a 2118.60 ± 269.57 a	1199.11 ± 343.27 b* 572.01 ± 171.46 c	971.92 ± 420.37 bc 718.89 ± 126.67 bc	785.09 ± 273.17 c 824.74 ± 150.57 b

Note: ± values indicate mean standard deviation (SD). Letters indicate significant differences among soil types in the same soil depths ($p \leq 0.05$). An asterisk (*) after letters signifies significant differences between soil depths within the same soil types.

Regarding Total OP, Total IOP, Total LP, and Total NLP across soil depths and types (Table 2), it can be observed that BFS soil exhibits significantly higher concentrations of these parameters in the 0–30 cm compared to the 30–60 cm. Similarly, RYS soils also show higher concentrations in the 0–30 cm layer, although these differences are not statistically significant. In contrast, FS soil shows higher levels of Total OP, Total IOP, Total LP, and Total NLP in 30–60 cm than in 0–30 cm. In KS soil, Total OP and Total NLP are higher in 0–30 cm than in 30–60 cm, while Total IOP and Total LP are greater in 30–60 cm compared to 0–30 cm. Overall, KS and FS soils tend to accumulate more Total P in the deeper soil layer (30–60 cm), whereas BFS soil demonstrates more phosphorus accumulation in the shallower layer (0–30 cm).

In 0–30 cm, RYS soil has the highest sum of the most available fractions (Resin-P, NaHCO3-Pi, NaHCO3-Po), with the predominance of Resin-P, while at the 30–60 cm depth, RYS's Residual-P significantly increases to 32%, indicating that less accessible forms of phosphorus become more abundant as soil depth increases (Figure 3A,B). FS soil demonstrates high levels of $NaHCO_3$-Pi at both depths, with 16.25% and 17.94%, respectively, suggesting a greater potential for phosphorus supply to plants. In contrast, BFS soil shows a significant rise in NaOH-Po to 50.58% at 30–60 cm, reflecting that organic matter-stabilized forms of phosphorus become more prominent with increased soil depth. KS soil maintains a consistent level of NaOH-Pi at about 30% across both depths, showing the stability of its phosphorus forms.

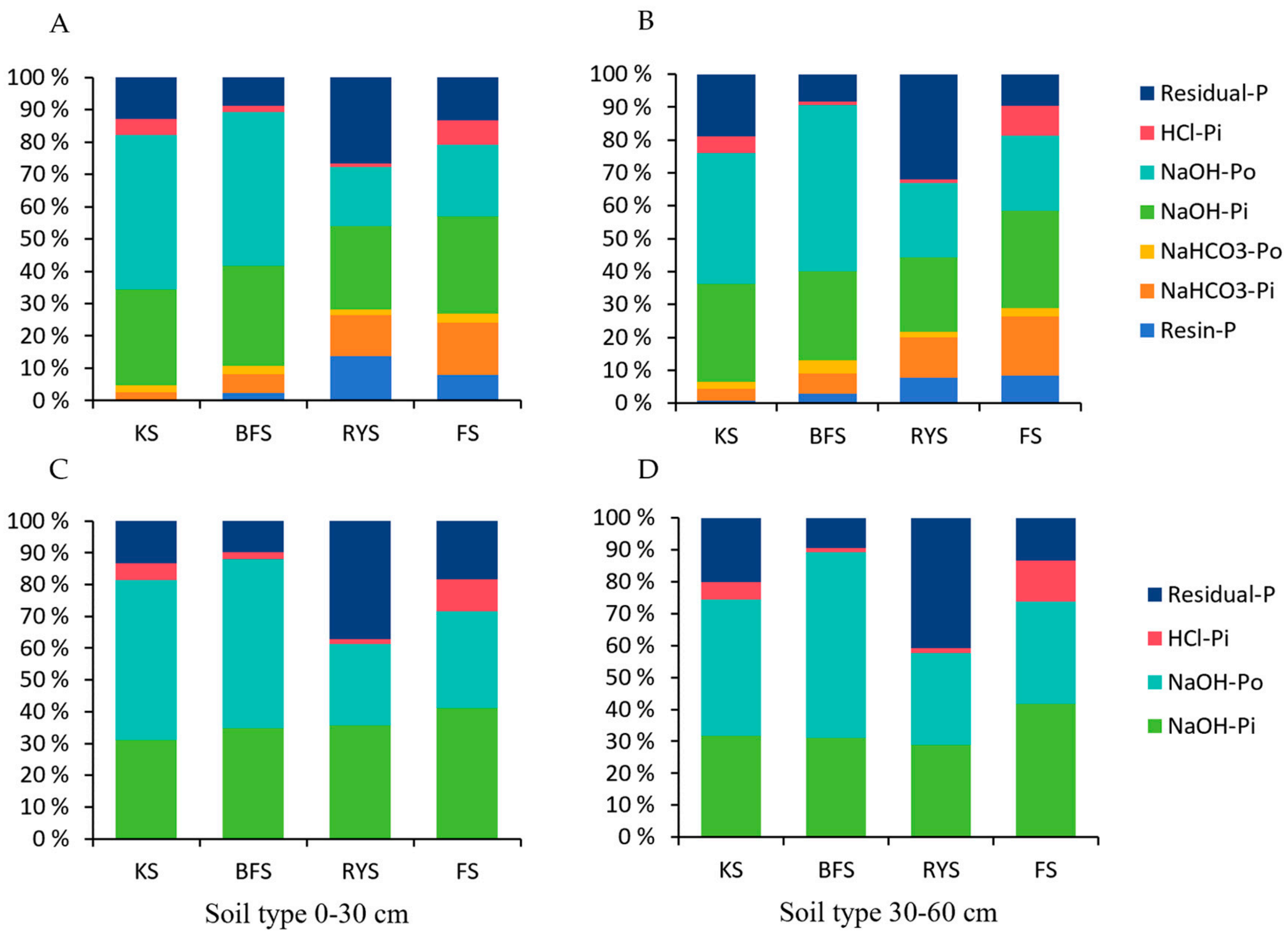

Figure 3. (**A**) Relative percentage phosphorus fractions to Total P at 0–30 cm soil depth. (**B**) relative percentage phosphorus fractions to Total P at 30–60 cm soil depth. (**C**) relative percentage phosphorus fractions to NLP at 0–30 cm soil depth. (**D**) relative percentage phosphorus fractions to NLP at 30–60 cm soil depth. Total sample *n* = 120.

At a soil depth of 0–30 cm (Figure 3C), the FS soil exhibits the highest levels of NaOH-Pi (41.24%) and HCl-Pi (10.12%), while the RYS soil has the highest Residual-P content (37.08%), indicating a substantial presence of phosphorus forms that are less available to plants. Moreover, BFS soil shows a relatively high level of NaOH-Po (53.12%). At the 30–60 cm depth (Figure 3D), the Residual-P in RYS soil increases to 40.90%, further suggesting that less bioavailable forms of phosphorus become more prevalent with increased soil depth. In contrast, FS shows a decrease in Residual-P (13.44%) despite maintaining a high level of NaOH-Pi (41.75%). BFS exhibits a notable increase in NaOH-Po (58.12%) in the 30–60 cm of the soil layer, whereas KS maintains relatively stable levels of NaOH-Pi and NaOH-Po, changing slightly from 31.12% to 31.76% and from 50.25% to 42.63%, respectively.

Moreover, as demonstrated in Table 3, blueberries of a decennial age exhibit a pronounced accumulation of insoluble phosphorus in 0–30 cm relative to the pristine soil. Concurrently, the sequestration of NLP in KS (spanning both 0–30 cm and 30–60 cm), BFS (across 0–30 cm and 30–60 cm), and FS (within 0–30 cm) were observed to reach 87–97%.

Table 3. Percentage of the NLP fractions accumulation after conversion from unused land to blueberry field.

Soil Types	Soil Depth (cm)	NaOH-Pi (%)	NaOH-Po (%)	HCl-Pi (%)	Residual-P (%)	Total NLP (%)
KS	0–30 30–60	22 (151.79 ± 123.51) 50 (246.02 ± 168.46)	32 (279.93 ± 87.17) 6 (31.55 ± 67.87)	14 (50.63 ± 21.37) 10 (46.93 ± 28.56)	0 (−134.58 ± 41.39) 23 (113.41 ± 64.44)	97 (347.76 ± 238.49) 89 (437.90 ± 245.88)
BFS	0–30 30–60	43 (250.00 ± 137.19) 32 (87.50 ± 63.16)	24 (137.19 ± 131.33) 45 (123.83 ± 73.66)	4 (14.05 ± 13.74) 3 (−1.12 ± 3.26)	15 (81.08 ± 48.10) 0 (−10.01 ± 59.37)	90 (482.34 ± 296.39) 87 (200.19 ± 159.11)
RYS	0–30 30–60	35 (198.25 ± 109.15) 25 (97.34 ± 24.63)	14 (77.94 ± 48.43) 17 (48.01 ± 25.86)	1 (8.10 ± 5.46) 9 (3.40 ± 3.89)	5 (29.91 ± 78.74) 0 (−52.42 ± 37.78)	55 (314.21 ± 214.43) 42 (96.34 ± 79.39)
FS	0–30 30–60	15 (12.62 ± 116.00) 38 (174.35 ± 64.92)	25 (29.92 ± 38.65) 12 (53.19 ± 14.58)	15 (13.65 ± 27.92) 10 (47.92 ± 9.09)	18 (18.14 ± 36.86) 9 (40.32 ± 18.14)	87 (74.32 ± 153.33) 69 (315.77 ± 85.50)

Note: Values presented in brackets indicate the accumulation of less plant-available P fractions in fertilized soils compared to unfertilized soils. These values are calculated as the difference between the P fractions in fertilized soils and the corresponding P fractions in unfertilized soils. The percentage values represent the accumulation rate of each P fraction, calculated as the difference between the P fractions in fertilized soils and the corresponding P fractions in unfertilized soils, divided by the total phosphorus content in fertilized soils. If the calculated accumulation rate is negative, it is presented as 0. ± values indicate mean standard deviation (SD).

3.3. Relationship with P Fractions Compare with Soil Properties

Figure 4 utilizes Pearson correlation coefficients to elucidate the interrelations among diverse phosphorus fractions and edaphic characteristics. The analysis indicates a substantial negative correlation between pH and the levels of Resin-P, $NaHCO_3$-Pi, Residual-P, LP, and IOP within the soil matrix. In contrast, EC, Total N, and SOC exhibit a marked positive correlation with the majority of the phosphorus fractions, with the exception of Resin-P, $NaHCO_3$-Pi, and LP. Moreover, C/N shows a significant positive correlation with $NaHCO_3$-Po, NaOH-Po, and OP.

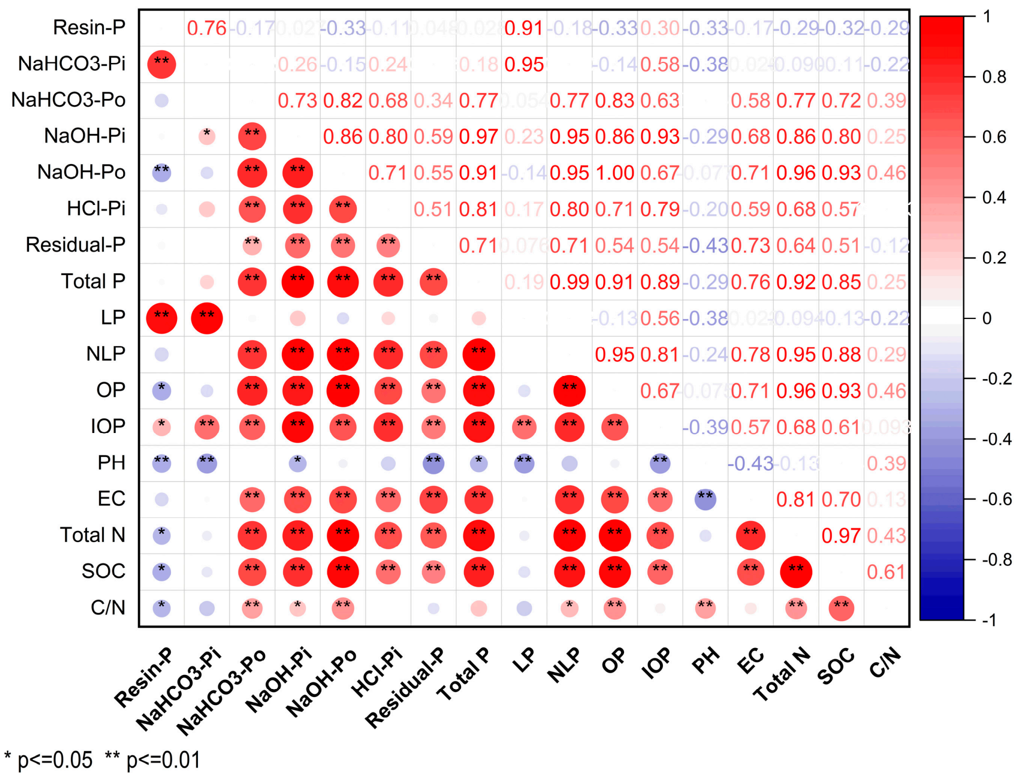

Figure 4. Person correlation analysis between soil P fractions and soil properties of blueberry land use types. Note: Asterisks (*) denote significant differences, with * $p \leq 0.05$ and ** $p \leq 0.01$. Total sample n = 120.

Figure 5 presents the results from a Redundancy Analysis (RDA), which indicates that Total N contributes the most to the variability of phosphorus fraction indicators in both the 0–30 cm and 30–60 cm layers, accounting for 74.3% and 81.4%, respectively. Specifically, within 0–30 cm, Total N, pH, and C/N are the factors most closely associated with phosphorus fractions. On the other hand, in 30–60 cm, Total N and pH show the most significant relationships with phosphorus fractions. Due to the close association between Total N and phosphorus fractions in blueberry soil (Figure 5), we further investigated the relationship between phosphorus fractions and Total N in 0–60 cm (Figure 6). Overall, non-labile phosphorus (NLP) and organic phosphorus (OP) exhibit the closest relationships with Total N. Specifically, NaOH-Po shows the strongest correlation (R^2 = 0.93), followed by NaOH-Pi (R^2 = 0.73), $NaHCO_3$ (R^2 = 0.50), HCl-Pi (R^2 = 0.46), and Residual-P (R^2 = 0.39). Furthermore, the Structural Equation Modeling (SEM) results (Figure 7) provide a detailed examination of the nuanced roles that different phosphorus fractions play in regulating soil properties and nutrient dynamics across varying soil depths in Japanese blueberry fields. Within the 0–60 cm soil layer (Figure 7A), $NaHCO_3$-Po is positively influenced by EC and pH, leading to subsequent effects on NLP, LP, and the accumulation of N and P. In the 0–30 cm soil layer (Figure 7B,C), NaOH-Po emerges as a critical factor, significantly impacting NLP and N and P accumulation. Meanwhile, in the 30–60 cm soil layer (Figure 7D,E), both NaOH-Po and $NaHCO_3$-Po are closely correlated with EC and pH, exerting a substantial influence on NLP and LP and thereby further modulating the accumulation of N and P.

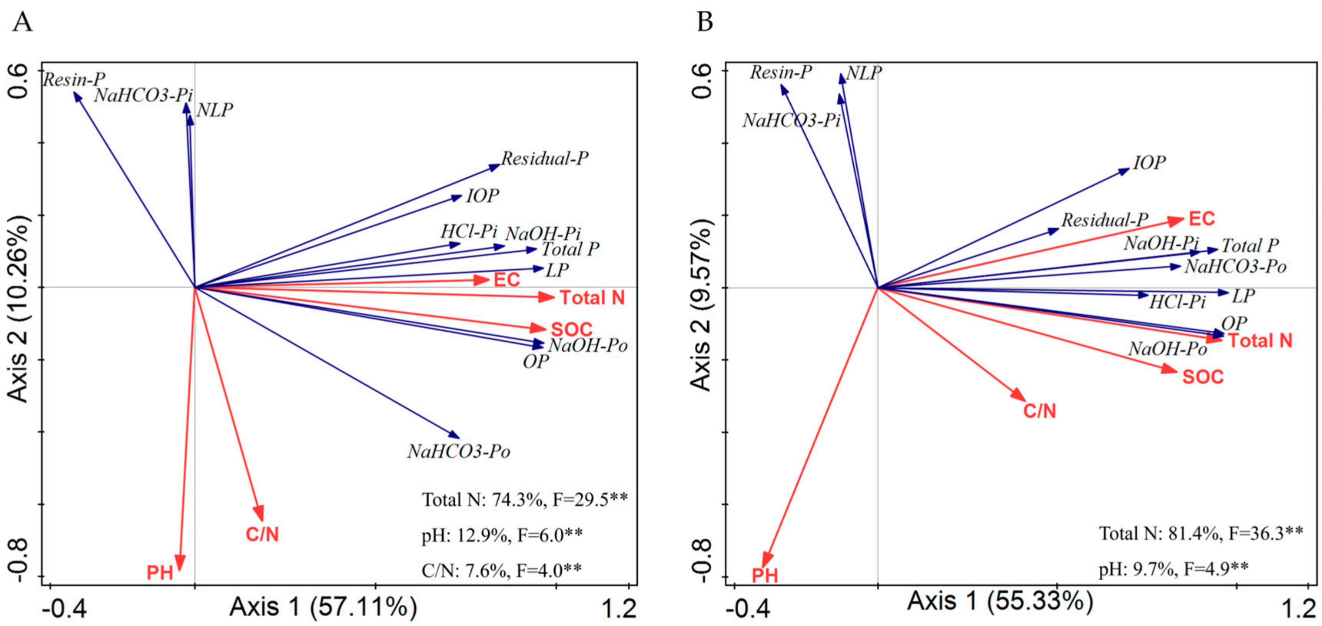

Figure 5. The association of soil properties with soil phosphorus fractions in blueberry land use type at 0–30 cm (**A**) and 30–60 cm (**B**). Note: Asterisk (*) indicates significant differences, ** $p \leq 0.01$. Total sample n = 120.

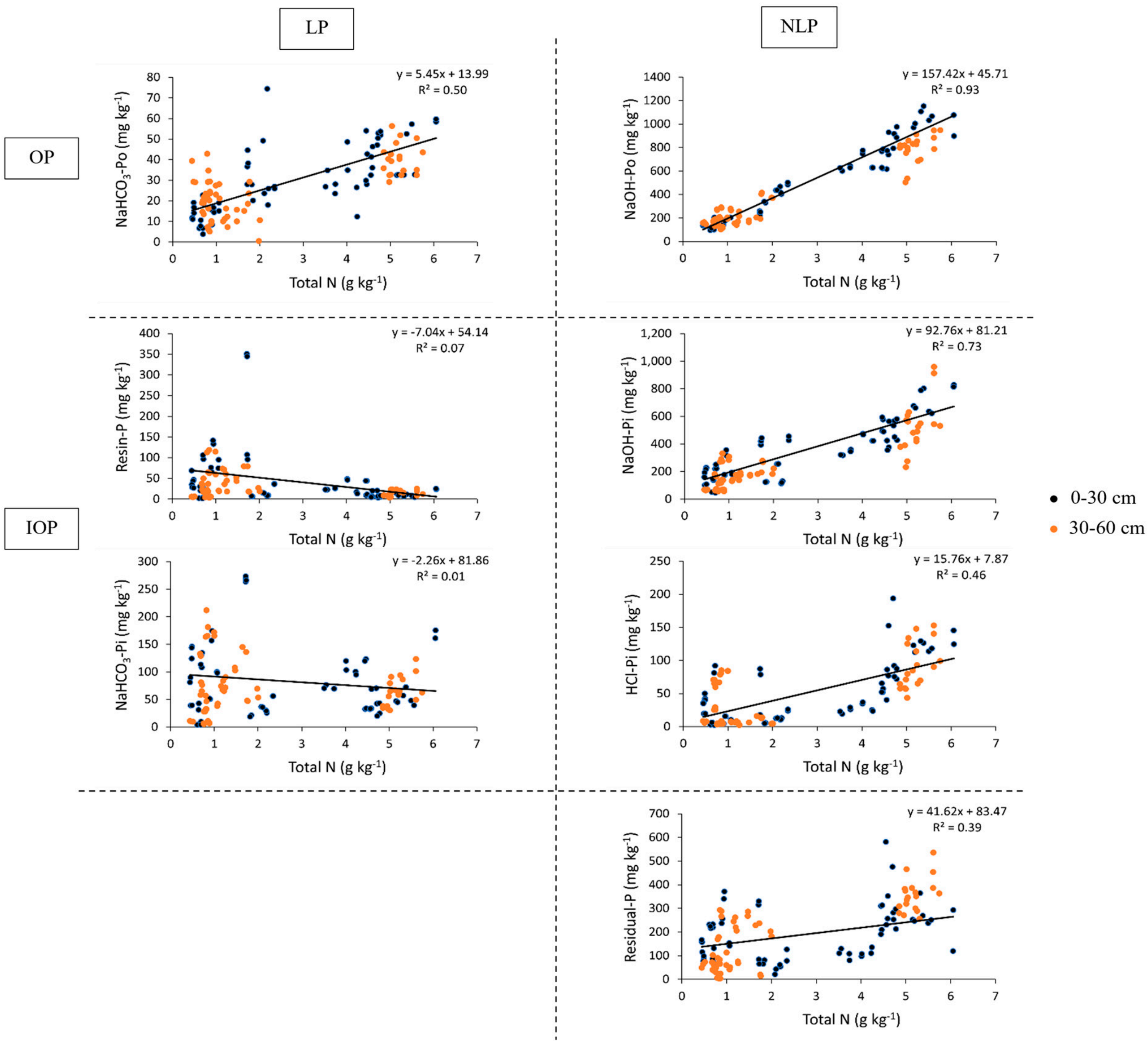

Figure 6. Relationship between LP, NLP, OP, IOP, and Total N in 0–60 cm. Note: The relationship between Total N and phosphorus fractions was statistically analyzed, $p \leq 0.01$. Total sample n = 120.

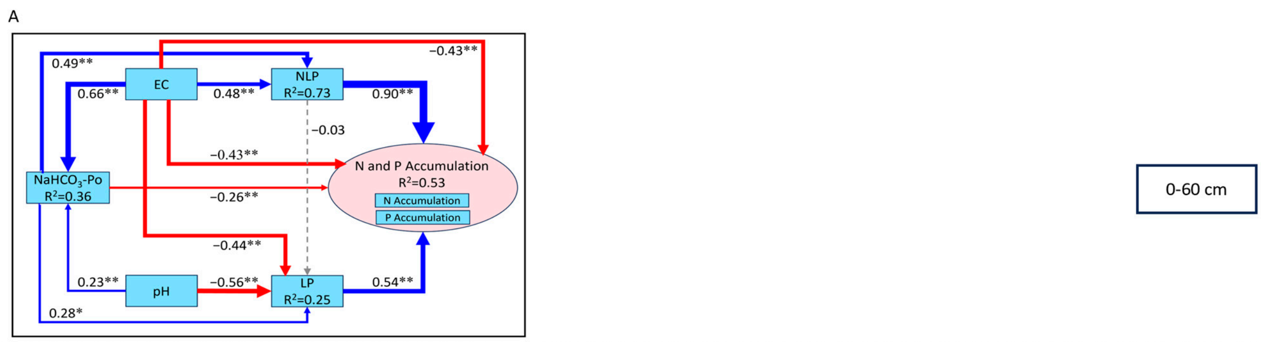

Figure 7. *Cont.*

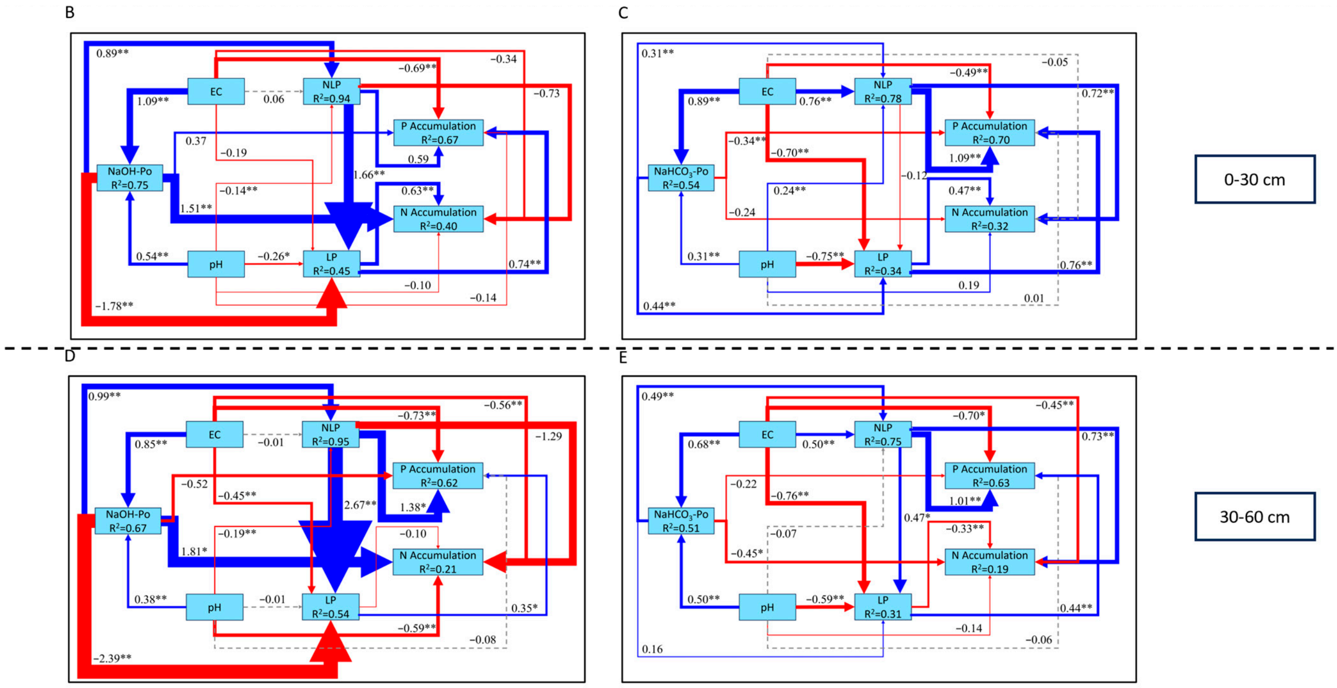

Figure 7. The SEM models illustrate the factors influencing soil properties under N and P accumulation, impacting P fractionation across different soil depths in Japanese blueberry fields. (**A**) represents the 0–60 cm soil depth SEM model, (**B**,**C**) correspond to the 0–30 cm depth, and (**D**,**E**) depict the 30–60 cm depth SEM models. Note: N and P accumulation represent the difference in Total N and Total P between fertilized and unfertilized soils. Blue and red arrows indicate positive and negative relationships, respectively. Significance is marked by * for $p < 0.05$ and ** for $p < 0.01$. Solid lines indicate significant differences, while dashed lines represent non-significant differences. The R^2 value shows the variance explained by each variable. Total sample $n = 120$.

4. Discussion

4.1. Change of Soil Properties in Blueberry Field

Blueberries are unique compared to many crops, as they have adapted to flourish in acidic soils with typically low fertility, resulting in specific nutritional requirements [29]. Blueberries require an optimal pH range of 4.0–5.5 in their rhizosphere for growth [30], and plant development is adversely affected when rhizosphere pH exceeds 6.0 [31]. As depicted in Table 1, the pH of blueberry soils is consistently below 6.0. BFS soil displays a relatively higher pH (5.44 ± 0.46 to 5.61 ± 0.38), and excluding FS soil, all non-fertilized areas exhibit higher pH than their fertilized counterparts, likely a result of long-term fertilization and varying fertilization practices altering Japan's blueberry field's pH. [32] examined the relationship between blueberry yield and electrical conductivity (EC) and found a notable decrease in productivity when EC levels in the soil layers of 0–30 cm and 30–60 cm surpassed 760 µs cm^{-1} and 291 µs cm^{-1}, respectively. In our study, the KS blueberry field (0–60 cm) presented the highest soil EC value of 287.32 ± 56.03 µs cm^{-1}, while other soil samples had comparatively lower EC values. Elevated Total N and SOC contents in the KS (0–30 cm and 30–60 cm) and BFS (0–30 cm) soils (Table 1) suggest more favorable conditions for microbes involved in organic matter transformation [33]. Furthermore, long-term blueberry cultivation has shown that yields decrease when nitrogen application surpasses recommended levels, with the accumulation of soil mineral nitrogen and its subsequent effects on pH and EC negatively impacting berry production over time [34].

4.2. Effect with Fertilized Blueberry Field Change and Distribution of P Fractions

Our study reveals that compared to unfertilized soil, blueberry fields with long-term fertilization accumulate more insoluble phosphorus significantly, ranging from 42% to 97%

(Table 3), supporting our hypothesis. Specifically, the 0–30 cm soil layer accumulates more NLP than the 30–60 cm layer. Typically, phosphate fertilizers are used to tackle the issue of low available phosphorus in soil. However, it is noteworthy that plants absorb merely 5% to 25% of the phosphate content in these fertilizers [35]. The greater accumulation of NLP in the 0–30 cm layer may be due to long-term fertilization in the shallow solum of blueberry fields. Most phosphate ions in fertilizers quickly become fixed in the soil through reactions with Fe^{3+}, Al^{3+}, and Ca^{2+}, rendering them predominantly inaccessible to plants [36]. Figure 3A,B show that the proportions of Residual-P, HCl-Pi, NaOH-Pi, and NaOH-Po, which are difficult for plants to absorb and utilize, are significantly higher than other phosphorus forms in the 0–30 cm soil layer. The order of NLP from highest to lowest in the 0–30 cm layer is KS > BFS > FS > RYS, while the 0–60 cm layer shows a little difference, which is KS > BFS > RYS > FS. Figure 3C,D indicate that NaOH-Po and NaOH-Pi constitute a larger proportion of insoluble phosphorus; however, in RYS soil, Residual-P significantly exceeds other P fractions. These NLP variations suggest different distributions and deposition of insoluble phosphorus in various soil layers, possibly related to soil fertilization management, root structure, and microbial activity [37–39]. Phosphate deposition and release also relate to soil type and environment. In dry environments, long-term fertilization mainly increases organic phosphorus through phosphate precipitation, while in flooded environments, it reduces organic phosphorus, further transforming it into active phosphorus. Phosphorus release in flooded conditions is triggered by NaOH-Pi release caused by $NaHCO_3$-Po release [40]. FS soils are frequently replenished with new sediment and organic material due to periodic flooding, which might explain why, in blueberry fields, only FS soil in the 0–60 cm layer has lower SOC than uncultivated soil and higher Resin-P and $NaHCO_3$-Pi than other layers (Tables 1 and 2).

In Japan, KS soil, mainly derived from volcanic ash, is characterized by a loose surface, lightweight, high humus content, and a strong ability to fix phosphorus. BFS soil, a relatively younger soil, is widely found in temperate and subtropical regions with high precipitation. RYS soil, characterized by its red or yellow color, has low organic matter accumulation, low alkalinity saturation, and strong weathering. Fluvic soil, developed from recent alluvial deposits, covers the largest area (47%) in cultivated land. Japanese agricultural soils face challenges like nutrient imbalance, organic matter reduction, heavy metal contamination, and soil sealing [41]. KS soil in (Tables 1 and 2) contains high levels of OP and NLP, indicating a significant presence of organic matter and insoluble phosphorus, consistent with previous studies. The elevated levels of Residual-P in RYS soil are attributed to phosphorus that is attached to iron and aluminum oxides, contributing to the higher concentration of Residual-P observed in this study (Figure 3C,D) [42]. Interestingly, BFS and RYS soils show higher OP, IOP, LP, NLP, and Total P in the 0–30 cm than in the 30–60 cm, while FS soil displays the highest levels of these phosphorus indicators in the 30–60 cm layer, likely due to frequent replenishment with new sediment and organic material from flooding in the shallow plow layer.

Soil adsorption and desorption of phosphorus is a continuous and relatively fast process, while phosphorus precipitation is slower and nearly irreversible [43]. Regardless of the type of phosphate fertilizer used, crops absorb a significant amount of phosphorus at a depth of 10–30 cm over a long period of fertilization [44]. Table 3 indicates the importance of studying and monitoring phosphorus, especially Residual-P, NaOH-Po, and NaOH-Pi representing NLP soil, in the 30–60 cm layer of blueberry fields. Phosphorus can move to deeper soil layers in both dissolved and particulate forms. The degree of vertical movement in soil is significantly affected by factors like soil type and structure, the physical disruption from topsoil cultivation, the dynamics of adsorption and desorption, and the mechanisms of water transportation [45]. The accumulation rates of NaOH-Pi and NaOH-Po in soil depths and soil types were opposite (Table 3), reflecting high and low-value changes from the upper-side and lower side of soil, possibly related to vertical transport of P between different soil layers. In addition, a large amount of evidence shows that mycorrhizae and acid phosphatase secreted by the blueberry rhizosphere may be involved in the reuse of organic phosphorus pools in the vertical soil layer by converting organic phosphorus into inorganic phosphorus or transfer of available phosphorus from root's senescent tissues to young tissues [31,46–48]. However, the patterns and mechanisms of vertical transport in soil phosphorus pools require further in-depth study.

4.3. Relationship with P Fractions Compare with Soil Properties

Our study revealed significant negative correlations between some phosphorus fractions and pH and positive correlations with EC, Total N, SOC, and C/N (Figures 4 and 5). This suggests that suboptimal blueberry growth could be linked to elevated soil pH levels and is intricately connected to factors like soil organic matter content, microbial diversity, and enzyme activities such as catalase and acid phosphatase [49]. In different soil layers, phosphorus fractions show varied relations with soil properties. In the RDA analysis (Figure 5), Total N, pH, and C/N contribute differently to soil P in the 0–30 cm (74.3%, 12.9%, 7.6%) and 30–60 cm layers (81.4%, 9.7%). This indicates Total N and pH are the most influential soil properties on P fractions during blueberry cultivation. Soil pH, SOC, and C/N ratio are key factors influencing soil enzyme activity and microbial community composition across various soil types and climatic zones [50]. In different soil depths, factors affecting phosphorus availability vary. In the 0–30 cm layer, a close relationship between C/N and phosphorus may indicate more active microbial communities and organic matter decomposition, while in the deeper soils, less organic matter decomposition affects phosphorus availability. Moreover, in phosphorus-deficient soils, labile carbon can promote the transformation of non-available phosphorus into available forms by enhancing specific bacterial and fungal groups and interactions among phosphorus-solubilizing bacteria [51].

Moreover, the enhancement of soil phosphatase activity due to nitrogen tends to decrease with time. The introduction of nitrogen into the soil initially increases phosphatase activity, helping to overcome the phosphorus scarcity caused by nitrogen, thereby maintaining a steady supply of phosphorus for plant development [52]. Our research shows a negative correlation between Total N and P fractions within the LP and IOP zone, while a significant positive correlation in the LP and OP zone, NLP and OP zone, IOP and NLP zone, as well as Residual P ranges (Figure 6). This indicates a strong correlation between Total N and most P fractions, except for Resin-P and $NaHCO_3$-Pi, which possess both LP and IOP characteristics and appear to be insensitive.

The Structural Equation Modeling (SEM) results presented in Figure 7 provide critical insights into the complex interactions governing P dynamics and nutrient accumulation in the soils of Japanese blueberry fields. In the 0–30 cm soil layer, NaOH-Po emerges as a key modulator, intricately influencing the transformation of non-labile phosphorus (NLP) to labile phosphorus (LP) through its interactions with soil EC and pH. This finding underscores the significant role of NaOH-Po in enhancing nutrient availability at shallower soil depths, where biological activity is more pronounced. In contrast, in the 30–60 cm soil layer, both NaOH-Po and $NaHCO_3$-Po are found to be critical in regulating the NLP to LP conversion, again mediated through EC and pH. This dual involvement at greater depths highlights the intricate and depth-dependent mechanisms by which P fractions influence nutrient dynamics.

However, the observed transformations of P in these soils are likely more complex than can be fully captured by SEM alone. The long-term fertilization practices characteristic of these fields may invoke additional chemical and biochemical processes, including the activities of soil microorganisms, that contribute to the conversion of NLP to LP. These potential microbial interactions, although not explicitly quantified in this study, warrant further investigation to elucidate their role in P cycling. Indeed, the interplay between chemical, biochemical, and microbial processes could provide a more comprehensive understanding of P dynamics, particularly under long-term land use and fertilization regimes [50,53,54]. Future research should aim to disentangle these complex interactions, particularly focusing on the roles of microbial activity and other biochemical pathways in P transformation, to enhance our understanding of soil fertility management in blueberry cultivation.

5. Conclusions

This study on Japanese blueberry fields highlights a notable distribution of P fractions in the soil, providing a foundational basis for low-input phosphorus fertilization strategies. The significant presence of non-labile phosphorus (NLP) offers an excellent opportunity for its precise utilization, transforming it into labile phosphorus (LP) for blueberry fields. Our research demonstrates that soil type and depth significantly impact phosphorus fractions, with higher NLP accumulation in shallower layers, particularly in KS (0–30 cm, 30–60 cm), BFS (0–30 cm, 30–60 cm), and FS (0–30 cm) soils, indicating strong phosphorus fixation capacity. The correlation between C/N ratio and phosphorus in 0–30 cm suggests microbial involvement in phosphorus cycling. Differences in phosphorus accumulation across soil types and depths reveal that KS and FS soils accumulate more Total P in deeper layers (30–60 cm), while BFS and RYS soils accumulate more in shallower layers (0–30 cm). Notably, NaOH-Po plays a crucial role in the 0–30 cm soil layer by influencing the transformation of NLP to LP, primarily through its interactions with EC and pH, while in the 30–60 cm layer, both NaOH-Po and $NaHCO_3$-Po are key modulators of this process. These findings underscore the importance of soil-specific characteristics in optimizing fertilization strategies, enhancing soil resource management in Japanese blueberry cultivation, and providing insights into the efficient utilization of NLP to improve soil fertility.

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