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Changes in Soil-Phosphorus Fractions by Nitrogen and Phosphorus Fertilization in Korean Pine Plantation and Its Natural Forest

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Abstract: Phosphorus (P) is the restraining aspect in the forest ecosystem, particularly in temperate regions, and makes the ecosystem more liable to nitrogen (N)-derived acidification. However, it remains poorly understood how N and P fertilization together affects soil-P availability and other soil properties. To address this question, a factorial experiment was conducted with N and P additions under two forest ecosystems, i.e., Korean pine plantation (KPP) and natural Korean pine forest (NKPF). Both forests were divided in to three subplots and each subplot underwent four different treatments, i.e., C: control (no N and P addition), L: Low treatment (5 g N m^{-2} a^{-1} + 5 g P m^{-2} a^{-1}), M: Medium treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}), and H: High treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}). Results revealed that the soil-P fractions changed during N and P fertilization over time although organic-P (Po) fractions were lower than inorganic-P (Pi) fractions. The residual P was increased overall, along with N deposition in soil. Soil organic carbon (SOC) was more present in NKPF soils as compared to KPP. Principal component analysis (PCA) indicated that at medium treatment there is maximum availability of P fractions as compared to other treatments in both forests, while high treatment showed some fixation of P in soils across both forests. Furthermore, SOC showed a negative correlation with residual P, while pH showed a positive correlation. Total N in soil showed a negative correlation with soil pH and residual P. Therefore, it is recommended that application of N and P at the rate of 15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1} is suitable in these two forest types to enhance P availability.

Keywords: Korean pine plantation (KPP); natural Korean pine forest (NKPF); nitrogen; phosphorus; P fractions; (Pi); organic carbon (Po); inorganic phosphorus organic phosphorus

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1. Introduction

Soil phosphorus (P) and its conversion play a significant role in biogeochemical processes [1]. Phosphorus is the most important and essential nutrient for plants growth and development; however, its levels in soil along with its bioavailability can help in sustaining agricultural yields [2]. Phosphorus exists in two forms, i.e., organic and inorganic. According to Liu et al. [3], the P content ranges from 25 to 50 ppm, and from the total, only 0.1% of P is accessible for the plants as the remaining is present in insoluble fractions. P bioavailability is a prerequisite for estimating the plant response towards P, and it depends on the existing inorganic P (Pi) that is preferred by plants for uptake, and organic-P (P_o) contents, which act as a sink for soluble P in soil solution, hence the determination of

P fractions in soil is crucial [4]. The method previously used by Hedley et al. [5] was further modified by Sui et al. [6] in which the first extractant was H_2O instead of using an anion-exchange resin for equilibrating the soil sample for identification of P fractions in biosolid-amended mollisols. The modified process is broadly used in studies under various soil conditions as previously used by Hao et al., [7] who studied the effect of P fractions under manure-amended soils. Furthermore, Kashem et al., [8] carried out a study under organic and inorganic P-sources-amended soil while Saleque et al. [9] worked on fertilized soil. Mollisols are widely distributed in northeast China and commonly known as black soils. They are the most important reservoir of soil with regard to plant growth, as they are fertile and have superior chemical as well as physical properties. Nevertheless, during recent decades, P availability has degraded severely owing to long-term extreme and unmaintainable agronomic practices [10].

Phosphorus in its elemental form is highly reactive in nature and may influence deposition and affect P cycling if large quantities of P are applied to the soils [11]. Studies are conducted extensively related to P response towards soil condition and P fertilization input. The studies have investigated how P application to soil affects P limitations at a larger scale, though huge ambiguity is still present related to different forms of P adsorbed with various elements such as iron, aluminum, and calcium on the soil surface. Under certain circumstances, they differ in their mobility, behavior, and bioavailability and depend largely on the parent material, fertilization background, and pH [5,12]. Thus, it is a prerequisite to determine the P limitation, which occurs due to the different P-fertilizer-application rates and different mechanisms. Another important aspect for investigation is to know the impact of various P-application rates on P fractions, their availability, and their response towards the crop. Williams et al. [13] reported that the primary plant growth as well as plant development depends upon P availability in the soil, while P fractions play a significant role towards P availability to forest plants.

Phosphorus fertilization at high rates results in rapid formation of nonlabile P forms [14]. When P fertilizers are applied at a high dose, the amount exceeds the plant output limits, and ultimately the exceeded amount is accumulated in the soil with the passage of time. Phosphorus-fractionation schemes help in the identification of outcomes of P application in soil and how it is associated with plant nutrition, P forms, and processes of its uptake [15–17]. It is important to know the amounts and fractions of P and their characteristics in soils with long-term fertilization background so that maximum output with regard to P use is attainable. Soil P in inorganic form (Pi) mentions different fractions such as labile ($NaHCO_3$-Pi), moderately labile (NaOH-Pi, NaOH-Pi, HCl-Pi), and nonlabile (HCl-Pi), iron (Fe) and aluminum (Al) P (non-occluded Fe- and Al-bound P); and residual Pi (P occluded within Fe oxides) [18]. The Pi fractionation (sequential Pi fractionation and other stable forms), as with those that are absorbed on Fe, Al, and calcium (Ca-P, Al-P, Fe-P, and Residual-P) surfaces have been recognized. There are certain cases where P fractionation is not further possible, as in the case of HCl-Pi fractions, because its subfractions are relatively small in noncalcareous soils while they make up greater contribution in loess soil [19]. Different biogeochemical processes occur in the soil that affect soil after P application. For instance, Kritzler et al. [20], reported that the P application into the soil has significantly changed the microbial activities and as a result, organic P was mineralized into inorganic-Pi form. Similarly, the N and P in combined application results in nitrification and subsequently alters organic-P formation and soil pH, resulting in the acceleration of P demands of plant and primary-crop production [21]; and N application results in the mobilization of Fe and Al existing in soil and leads to P sorption on the soil surface [22]. Thus, it transforms labile P_o and Pi into moderately available P or recalcitrant P. At high P-application rates, low plant-uptake efficiency occurs, causing a substantial effect on plant growth. Phosphorus fixation takes place as a result of small P mobility into the soil under circumstances where P-fertilizer input surpasses the P output of the plant [17]. The combined application of P with N also gives rise to fixation, thus creating acidic-soil conditions [23]. Hence, it is important to know the forms and behavior of P after continuous use of P fertilization under a winter-forest ecosystem, addressing the long-term P fertilization. A few studies are present highlighting the impact of long-term fertilizer application, causes of P fertilization, along with its impact on P fixation on crop production in Loess soils. However, vast studies are still required in this field. In this regard, our study is designed with the aims of clarifying the P-fraction variations that occur due to long-term N and P application, and to know the effect of P fractions on plant growth and uptake of P in dark-brown soils of northeast China. The results will help knowing the P-cycling processes as influenced by organic- and inorganic-P fractions and their changes under long-term N and P fertilization.

2. Materials and Methods

2.1. Experimental Site

This experiment was conducted at the Liangshui National Nature Reserve (47°1005000 N, 128°5302000 E), in Heilongjiang, northeast China. The site elevation ranged from 300 m to 707 m above sea level (undulating terrain). The slope ranged from 10° to 15°. Annual temperature (mean value) is −0.3 °C, annual precipitation is 676 mm (mean value). Snowfall also contributes about 10 to 20% of the total precipitation (December–April). The investigated region holds an extensive past of community development, with a diversity of forest stands. According to soil taxonomy (American), the soil of the study area was classified as dark-brown forest, equivalent to Humaquepts or cryoboralfs [24]. The regional soil is classified as Hap-Boric Luvisol, consisting of high-organic matter, and generally measured up to 50 cm deep, with 1–10 cm loamy soil, and 10–20 cm sandy loam [25]. The study area is one of the few areas in China that still support extensive natural forests. The original Korean pine plantation (KPP) covers an area of approximately 2375 hm^2. The selective cutting of KPP was regenerated naturally after selective cutting of Korean pine (intensity 30%) in 1971. The KPP was established in 1954. The second investigated forest type is natural Korean pine forest (NKPF). Natural vegetation is the deciduous broadleaf forest dominated by Korean pine. Canopy species comprise white birch, red spruce, scale spruce, Dailing spruce, fir, Xingan larch, Manchurian walnut, Amur cork-tree, Manchurian ash, and elm. Common shrub species include honeysuckle, filbert, pearl plum, and Amur lilac. The site characteristics are shown in Tables 1 and 2.

Table 1. Site characteristics of two selected forests.

Forest Type	Average DBH (cm)	Mean Height (m)	Density (Trees/hm^{-2})	Canopy Closure
Korean pine plantation	21.7	19.1	1300	0.7
Natural Korean pine forest	26.4	15.7	1175	0.7

Table 2. Basic soil-property situation before the experiment.

Forest Type	Soil pH	T-N (g kg^{-1})	T-P (g kg^{-1})	SOC (g kg^{-1})	C:N Ratio
Korean pine plantation	5.68 ± 0.21	2.3 ± 0.10	1.01 ± 0.42	69.22 ± 3	29.07 ± 1.7
Natural Korean pine forest	5.59 ± 0.20	3.5 ± 0.11	1.25 ± 0.32	71.36 ± 5	20.38 ± 1.4

Values are means of three replicates, ±SE, SOC: soil organic carbon, T-N: total nitrogen, T-P: total phosphorus, C:N carbon ratio nitrogen.

2.2. Experimental Scheme and Treatments

The experiment was laid out in a RCBD factorial design. It consisted of various species in the two forest types and four treatments. Three replications of the experiment were made, and each replication consisted of 20 m × 20 m fixed plots. Individual replication was further divided into three subplots. Further, each subplot was divided into 12 (2 m × 2 m) subplots. The whole experiment was divided into 72 experimental subunits, whereas N was applied as $(NH_4)_2SO_4$ while some N and P was applied in the form of diammonium phosphate ($(NH_4)_2HPO_4$) at three different levels. The treatment's subplots were labeled as C: Control (no

N and P applied), L: Low treatment (5 g N m^{-2} a^{-1} + 5 g P m^{-2} a^{-1}), M: Medium treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}), H: High treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}). To elucidate the impact of nitrogen and phosphorus deposition, the $(NH_4)_2SO_4$ and $(NH_4)_2HPO_4$) fertilizers were dissolved in 2 L of deionized water and sprayed on the forest floor using a backpack sprayer every month (i.e. May, July, August, September, and October) for two consecutive years (i.e. 2018 and 2019), while the control plot received the equivalent amount of water. The soil samples were taken three times a year before the application of fertilizer in the months of May, August, and October.

2.3. Soil-Sampling Plan

Soil-sampling was carried out three times a year in the months of May, August, and October, and six times overall during the whole duration of the experiment (i.e., two years). The forest floor was cleared, litter was carefully removed, and three cores were selected in each subplot. The soil from selected sites was taken (0–20 cm depth), mixed well, and put into Ziploc bags and immediately transported to the laboratory for analysis. After passing the soil samples through a 0.5 mm mesh sieve, they were subjected to pH measurement and P-fraction determination.

2.4. Soil-Phosphorus Fractionation

The method [6] for sequential P fractionation was applied in this study. This was conducted in order to know the changes in available P fractions that were removed on the basis of collective biochemical constancy with different labile-P fractions. A uniform soil sample of 0.5 g was taken from the thoroughly mixed soil of three replications of each treatment. The sample was subjected to sequential extraction with deionized water, and after that, additions were made of 0.5M of $NaHCO_3$ (pH = 8.2), 0.1M of NaOH, and 1M of HCL. The sample was shaken for 16 h after each addition. Lastly, digestion of residual-P was carried out by using H_2SO_4-H_2O_2. HCL, NaOH, and $NaHCO_3$ extracts divided into two aliquots in order to determine total P (TP) and inorganic P (Pi). The amount of P that was determined prior to digestion with H_2SO_4-H_2O_2 was only Pi, while the oxidation through H_2SO_4-H_2O_2 liberated Po, and the determined P was TP. The difference between the TP and Pi gave the values of Po. In addition, the molybdenum blue method was used for extracting available P (P_a) in soil by using 0.03 M NH_4F and 0.025 M HCl [7]. The P fractions and available P were determined for the four repeats. The most effective P fraction for plant growth was the H_2O-Pi fraction, while Pi and Po ($NaHCO_3$) were counted as labile P pools; this also includes some microbial P, which were weakly sorbed on the surface of iron and aluminum oxides of P. The secondary Pi was present in NaOH-P; this form was also attached to clay particles and Al and Fe compounds. Meanwhile, Po attached to fulvic and humic acid substances was also available. The HVL P and residual P were considered as stable P. The NaOH-P contained secondary Pi, which is bound to Fe and Al compounds and clay edges, and Po is associated with humic and fulvic acids; it is measured as moderately labile P. The HCl-P (Pi and Po) is associated with calcium (Ca) and is considered a stable form of P. Res-P is the most chemically stable Pi form covered by sesquioxide. Labile P included H_2O-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po fractions. Pi is the sum of H_2O-Pi, $NaHCO_3$-Pi, NaOH-Pi, HCl-Pi, and Res-P. Po is the sum of $NaHCO_3$-Po, NaOH-Po, and HCl-Po, as in the methodology mentioned by Kashem et al. [8].

2.5. Statistical Breakdown

Analysis of variance was applied statically to analyze the data regarding fertilizer, forest type, and their interaction with each other. This was conducted to understand whether N and P fertilizers had impacted P fractions. The treatments were compared under the post hoc Tukey's honest significant difference (HSD) test. The interrelations between soil chemical properties and soil P forms were determined by using the "corrplot" package [26] available in R version 3.6.1 [27]. For this, a heat map for Pearson's correlation coefficients was performed and results were evaluated. The data and their means were presented as mean ± standard error (SE).

3. Results

3.1. Changes in Soil Properties

The basic soil properties depicted differences with regard to forest types and fertilizer application. Table 3 showed the treatment effect on soil properties in which soil pH ranged from 5.1 to 5.7. The highest pH was observed in KPP at SB-I and SB-II under control treatment, even though the lowest pH was observed at high treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}) in NKPF (SB-III). Interestingly, it was also detected that the pH declined with the increment in doses of N and P in both forest types. Compared with the C (no N and P applied), all the treatments (L, M, H) showed a decrease in pH. Likewise, a significant ($p < 0.05$) decrease in soil pH was observed in medium and high N and P supplementation; however, it was not significantly affected by low treatment ($p > 0.05$) across all plantations. The total N was also affected by the applied treatments. It revealed that the total soil N ranged from 31.0 g kg^{-1} to 78.1 g kg^{-1}. The highest total soil N was found with high treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}) in NKPF (SB-III).

Table 3. Changes in soil chemical properties of the KPP and NKPF.

Forest	Subplot	Treatments	Soil pH	Total N (g kg^{-1})	SOC (g kg^{-1})	C:N Ratio
Korean Pine Plantation	SB-I	C	5.6 ± 0.2 a	2.2 ± 0.9 a	50.1 ± 4.1 a	22.7 ± 2.1 a
		L	5.5 ± 0.1 a	2.5 ± 0.7 a	51.5 ± 4.2 b	20.6 ± 1.9 a
		M	5.4 ± 0.1 a	2.9 ± 0.9 a	62.3 ± 4.2 c	21.4 ± 1.8 a
		H	5.3 ± 0.1 b	2.9 ± 1.4 b	65.3 ± 5.6 c	22.5 ± 1.9 a
	SB-II	C	5.7 ± 0.3 a	1.8 ± 0.8 a	61.2 ± 5.0 c	34.0 ± 2.5 b
		L	5.5 ± 0.2 a	1.9 ± 0.8 a	55.3 ± 4.8 b	29.1 ± 2.5 b
		M	5.6 ± 0.3 a	2.9 ± 0.7 a	56.6 ± 4.7 b	19.5 ± 1.1 a
		H	5.2 ± 0.1 b	2.7 ± 1.6 b	58.3 ± 4.9 b	21.5 ± 1.3 a
	SB-III	C	5.7 ± 0.3 a	1.5 ± 0.9 a	60.3 ± 5.0 b	40.2 ± 3.6 c
		L	5.6 ± 0.3 a	2.3 ± 0.9 a	59.1 ± 4.8 b	25.6 ± 2.0 b
		M	5.5 ± 0.2 a	2.6 ± 0.9 a	62.3 ± 4.7 c	23.9 ± 1.6 a
		H	5.5 ± 0.1 a	3.1 ± 1.1 b	65.6 ± 4.9 c	21.1 ± 1.7 a
Natural Korean Pine Forest	SB-I	C	5.6 ± 0.3 a	1.9 ± 0.8 a	60.3 ± 7.5 b	31.7 ± 1.6 b
		L	5.6 ± 0.3 a	2.3 ± 0.9 a	63.2 ± 6.8 c	27.4 ± 1.6 b
		M	5.4 ± 0.1 b	2.7 ± 0.8 b	64.3 ± 7.2 c	23.8 ± 1.6 a
		H	5.2 ± 0.1 b	3.1 ± 1.1 b	65.1 ± 8.0 c	21.0 ± 1.6 a
	SB-II	C	5.5 ± 0.1 a	2.3 ± 0.9 a	60.1 ± 4.1 b	26.1 ± 1.6 b
		L	5.4 ± 0.1 a	2.1 ± 1.1 a	61.2 ± 5.1 c	29.1 ± 1.6 b
		M	5.3 ± 0.1 b	3.2 ± 1.5 b	63.1 ± 8.7 c	19.7 ± 1.6 a
		H	5.3 ± 0.1 a	3.1 ± 14 b	66.1 ± 7.2 c	21.3 ± 1.6 a
	SB-III	C	5.6 ± 0.2 a	2.2 ± 0.6 a	65.3 ± 6.2 c	29.6 ± 1.6 b
		L	5.3 ± 0.1 b	2.1 ± 1.3 a	71.3 ± 6.2 d	33.9 ± 1.6 b
		M	5.2 ± 0.1 b	3.2 ± 1.9 b	75.3 ± 9.4 d	23.5 ± 1.6 a
		H	5.1 ± 0.1 b	3.4 ± 1.8 b	68.6 ± 9.8 c	20.1 ± 1.6 a

Values are means of three replicates, C: Control (no N and P applied), L: Low treatment (5 g N m^{-2} a^{-1} + 5 g P m^{-2} a^{-1}), M: Medium treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}), H: High treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}), Significant differences between treatments indicated by different alphabetical letters (post hoc; $p < 0.05$; mean ± SE).

The soil organic carbon (SOC) was significantly affected by the applied treatments, ranging from 50.1 to 75.3 g kg^{-1}. The SOC increased with the increment in N and P application. It was also noted that overall, KPP showed lower SOC values as compared to natural Korean pine forest with the same application treatments. The total soil N was directly proportional to N and P doses positively. Similar drift was found in all treatments.

3.2. Effects of Fertilizations on Soil Inorganic P, Organic P, and Total P

The changes in soil-P fractions during N and P fertilization at different times are shown in Figures 1–6 (each figure shows one time sample). There were significant differences among treatments within time. Total P increased with the higher application of N and P and declined in control treatment where no N and P was applied. The total P ranged from 1300 to 1683 mg kg^{-1}, 1180 to 1563 mg kg^{-1}, 1012 to 1395 mg kg^{-1}, 1262 to 1645 mg kg^{-1}, 1092 to 1475 mg kg^{-1}, and 710 to 959 mg kg^{-1} after the 1st (Figure 1), 2nd (Figure 2), 3rd (Figure 3), 4th (Figure 4), 5th (Figure 5) and 6th (Figure 6) fertilization, respectively.

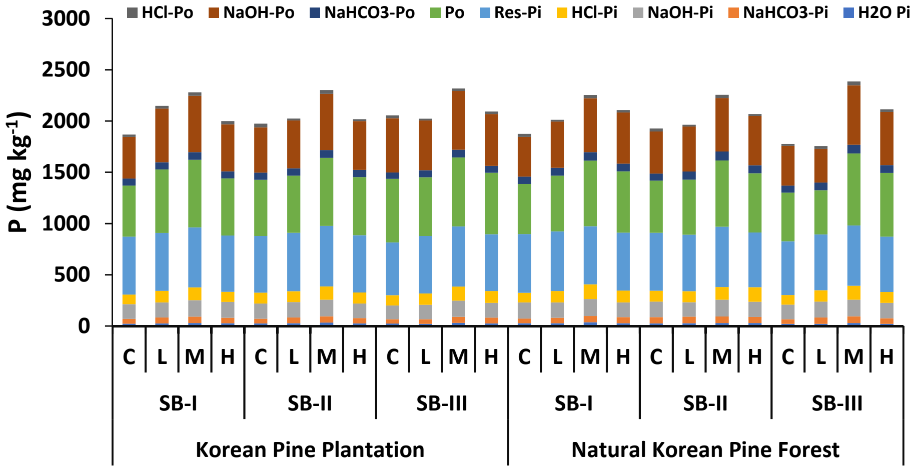

Figure 1. Changes in soil P fractions affected by N and P addition after 1st fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

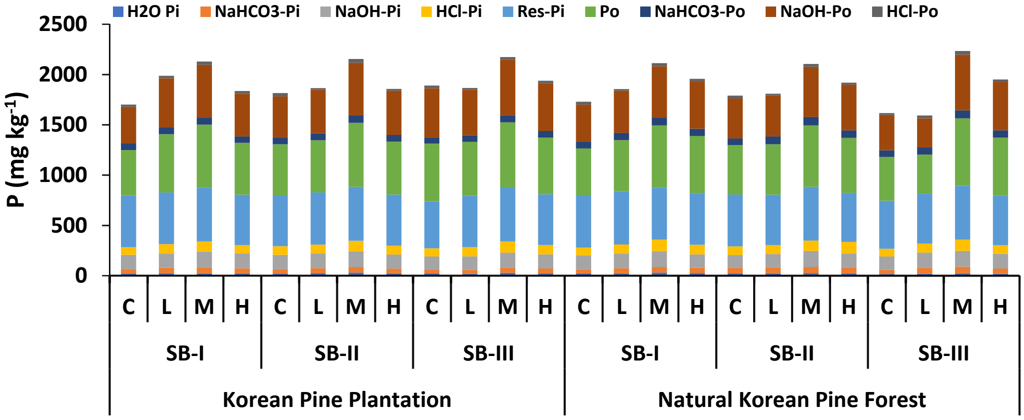

Figure 2. Changes in soil-P fractions affected by N and P addition after 2nd fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

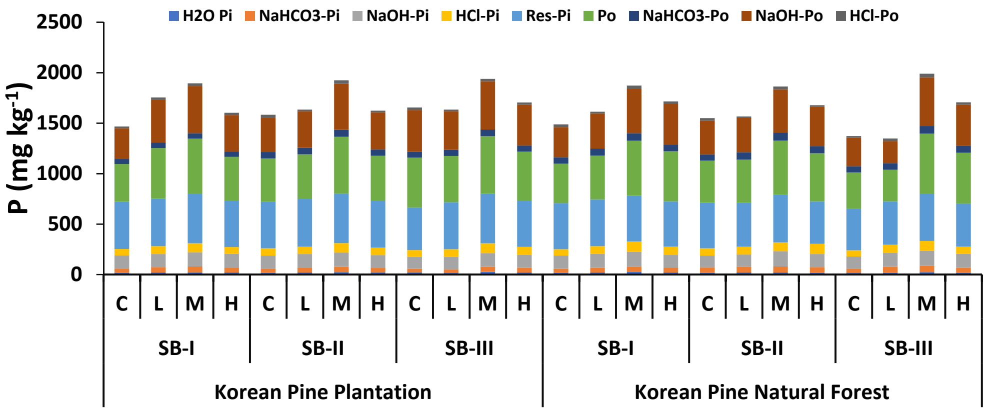

Figure 3. Changes in soil P fractions affected by N and P addition after 3rd fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

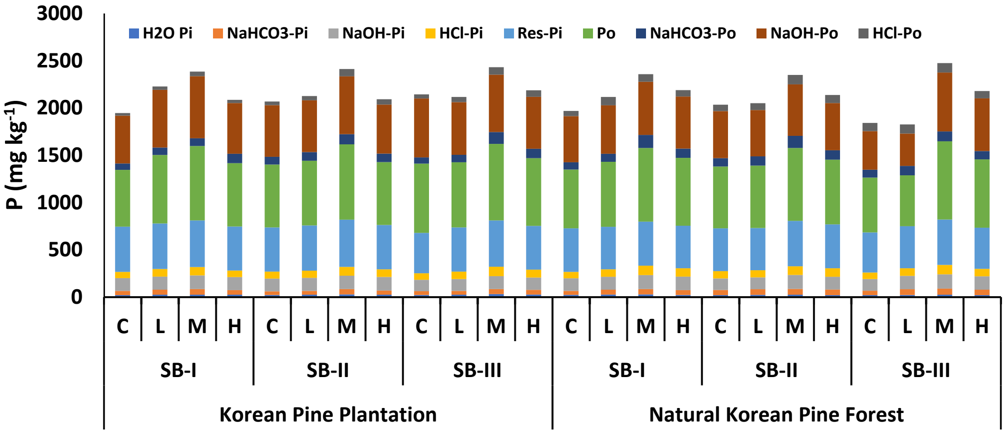

Figure 4. Changes in soil-P fractions affected by N and P addition after 4th fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

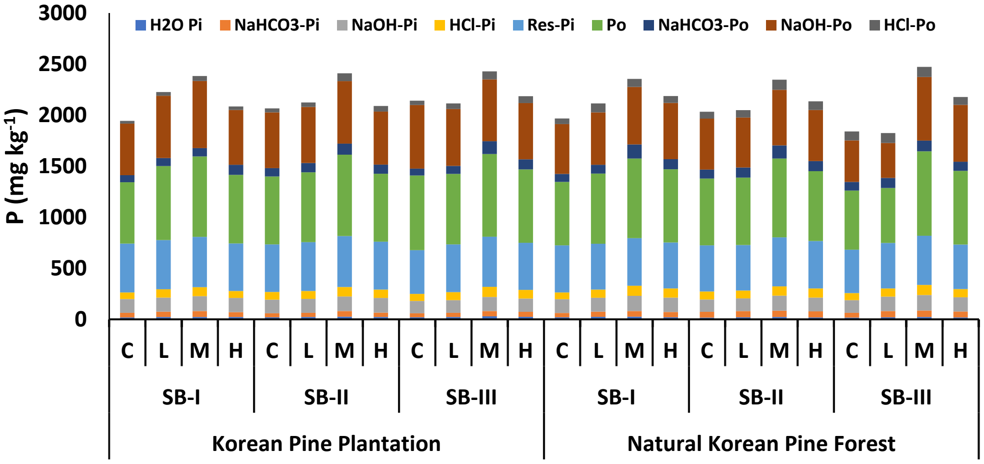

Figure 5. Changes in soil P fractions affected by N and P addition after 5th fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

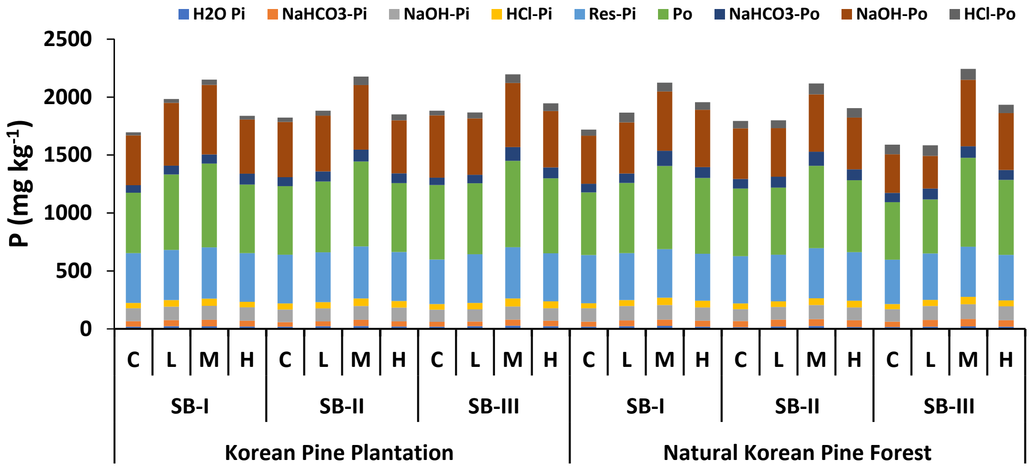

Figure 6. Changes in soil-P fractions affected by N and P addition after 6th fertilization. C: Control (no N and P applied), L: Low treatment, M: Medium treatment, H: High treatment, SB-I: subplot 1, SB-II: subplot 2, SB-III: subplot 3, Tukey's HSD post hoc; $p < 0.05$.

3.3. Inorganic-P (Pi) Fractions

The inorganic-P (Pi) fractions of H_2O-Pi, $NaHCO_3$-Pi, NaOH-Pi, and HCl-Pi showed significant variation by the application of N and P with different doses (Figures 1–6). The maximum H_2O-Pi was determined at NKPF {SB-1(M)} with M treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}). It was also noted that the trend for H_2O-Pi increment was as follows: M > H > L > C. Overall, H_2O-Pi declined in two years' experimentation. NKPF experienced a greater reduction in H_2O-Pi as compared to KPP. A similar trend was found with $NaHCO_3$-Pi. The $NaHCO_3$-Pi was detected more in NKPF as compared to KPP. The control treatment showed lower values as compared to low-, medium-, and high-treatment combinations. The highest values of $NaHCO_3$-Pi after the 1st, 2nd, 3rd, 4th, 5th, and 6th fertilization were observed in NKPF of M (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}) treatment (SB-III(NKPF)). The NaOH-Pi ranges were (135–166 mg kg^{-1}) after 1st fertilization, (130–155 mg kg^{-1}) after 2nd fertilization, (117–146 mg kg^{-1}) after 3rd fertilization, (120–149 mg kg^{-1}) after 4th fertilization, (104–125 mg kg^{-1}) after 5th fertilization, and (68–81 mg kg^{-1}) after 6th fertilization. At the end of experiment, 50% NaOH-Pi remained in the soil unchanged while 50% was up taken by plants or changed into unavailable form. The HCl-Pi ranged from 93 to 143 mg kg^{-1} (after 1st fertilization), 76 to 117 mg kg^{-1} (after 2nd fertilization), 63 to 104 mg kg^{-1} (after 3rd fertilization), 65 to 99 mg kg^{-1} (after 4th fertilization), 46–69 mg kg^{-1} (after 5th fertilization), and 30–45 mg kg^{-1} (after 6th fertilization).

3.4. Residual-P Fractions

The recalcitrant-P fraction, which is highly bonded with soil particles, is termed as a residual-P fraction. In our case, the residual P showed high values as compared to all P fractions. The control treatment showed lower values while medium treatment showed highest values as compared to all treatment combinations. A similar trend was found in both forest types.

3.5. Organic P (Po) Fractions

The changing trend for organic P (Po) in response to different N and P rates is shown in Figures 1–6. The changing trend of Po followed the order of NaOH-Po > $NaHCO_3$-Po > HCl-Po. After 1st fertilization, the NaOH-Po ranged from 328 to 582 mg kg^{-1}, while after 6th fertilization, it ranged from 183 to 373 mg kg^{-1}. Overall, the Po declined in two years, but the maximum decline was observed with C (control), L (low), and H (high) treatments, while at M (medium) treatment the Po was quite stable.

3.6. Correlation between Soil-P Fractions and Chemical Properties

A correlation coefficient (Pearson) was used for assessing the relationship between various P fractions and soil properties (Figure 7). The organic P fractions were found to be significantly positively corelated with soil properties (i.e., N, SOC and pH). Interestingly, it was found that both the forest types showed a more or less similar pattern of correlations to their trait's interrelations. The SOC showed significant negative correlation with residual P, while pH showed significant positive correlation. Total N in soil showed negative correlation with soil pH and residual P.

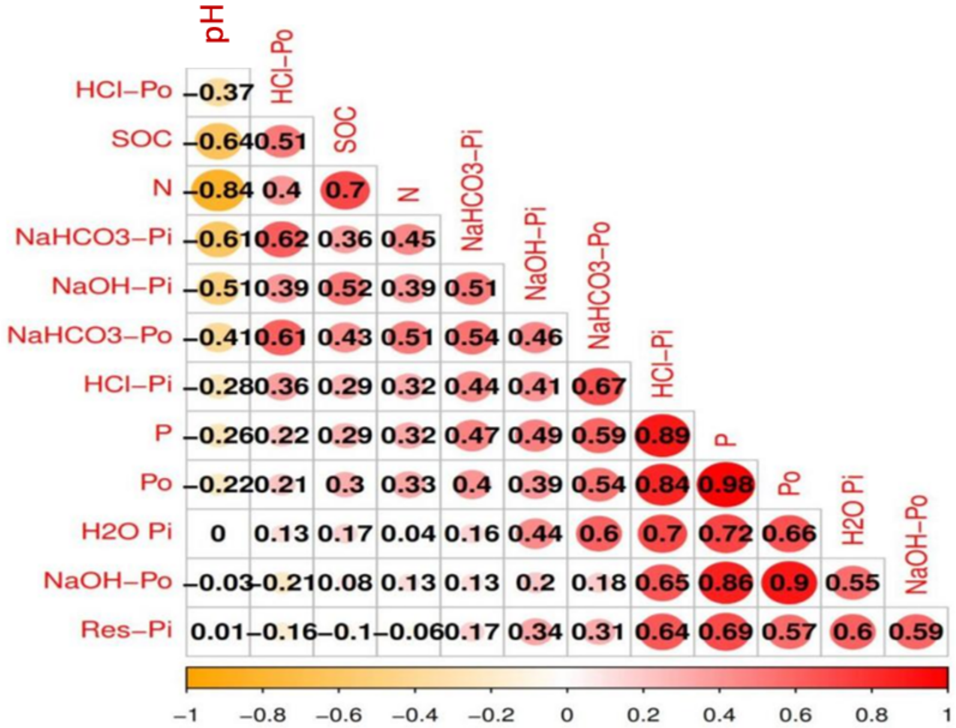

Figure 7. Heat map for Pearson's correlation coefficients of soil-P fractions and soil chemical properties. Abbreviations: phosphorus (Pi and Po) fractions (extracted by H_2O, $NaHCO_3$, NaOH, and HCl); residual P (Resi P); total nitrogen (N); soil organic carbon (SOC); soil pH (pH); and total phosphorus (P).

3.7. Principal Component Analysis (PCA) of P Fractions

Principal component analysis (PCA) produced two principal components (PC/factor) with an eigenvalue >1 (Figure 8a). The scatter plots depicted the positions of each P fraction in the soil after experiment. The HCl-Po, $NaHCO_3$-Po, $NaHCO_3$-Pi, and NaOH-Pi lie on 0.20, 0.30, 0.40, and 0.35, respectively, but all showed positive relationships. The HCl-Pi, H_2O-pi, Res-P, and NaOH-Po also lie on the positive side. Figure 8b shows a biplot graph between two principal components and the loading vectors between applied treatments (Control, Low, Medium, and High) which clearly depict that at medium treatment there is maximum availability of P fractions as compared to other treatments in both forests.

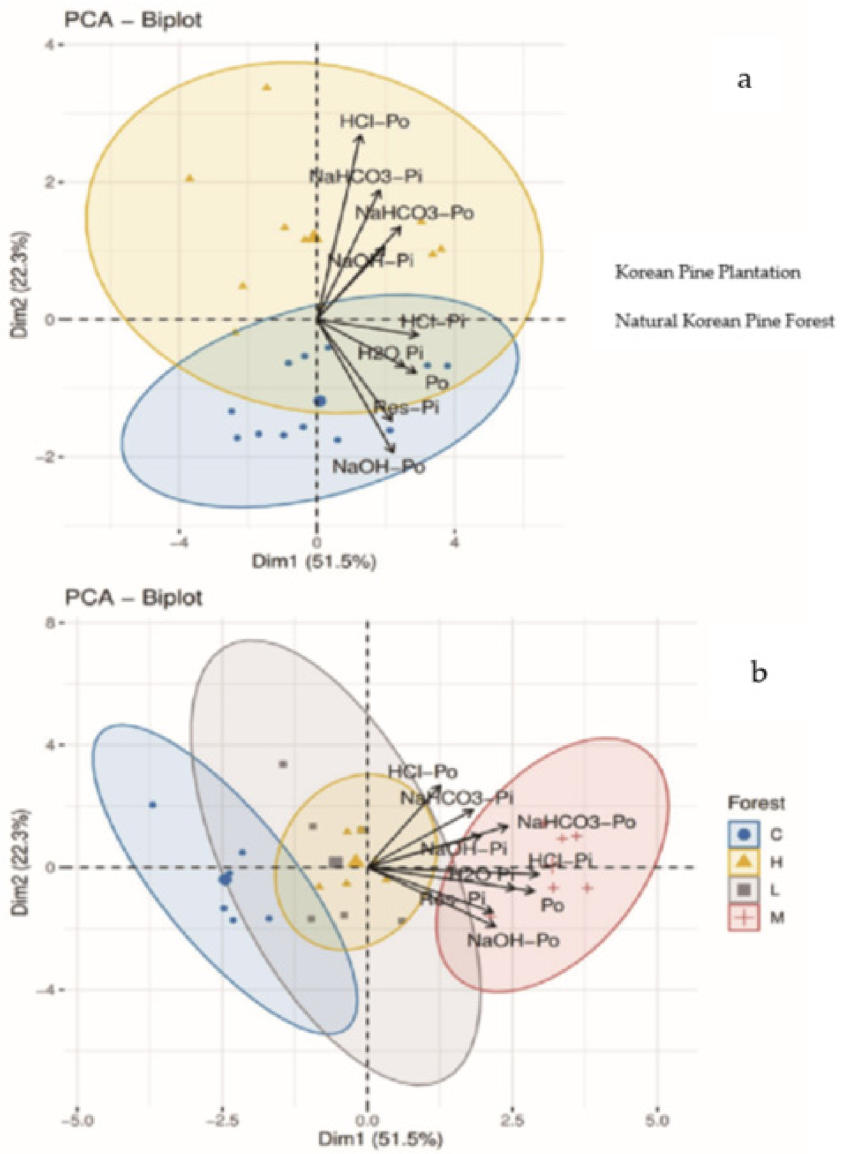

Figure 8. Biplot showing the two principal components and the loading vectors in a single display between two forests. The principal component analysis (PCA) for measured parameters shows portioning and grouping of correlated variables. Biplot showing the two principal components and the loading vectors between applied treatments.

4. Discussion

The repeated application of chemical fertilizer may alter soil properties such as electrical conductivity, soil reaction (pH), etc. In the current investigation, a significant ($p < 0.05$) decrease in soil pH was observed in results to chemical (N and P) fertilization. The same was reported by Qaswar et al. [28], who stated that when soils are subjected to long-term fertilization (chemical inputs), it results in buildup of exchangeable acidity and reduced soil pH, as well as a significant effect on soil-P fractionation. Constant application of N and P can significantly make increments in P accumulation in soils [29]. Continuous or long-term fertilization leads to increases in different forms of P such as $NaHCO_3$-Pi (bioavailable form), NaOH-Pi and HCl-Pi (temporary available Pi) and residual-Pi [30]. Our results depicted that the application of N and P directly influence the P fractions in different forest-type soils. The soil chemical properties were also affected by the applied treatment combinations (Table 3). As a comparison with the control treatment, the medium treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}) showed the highest P accumulation in soil among other treatments (Low and High). This was attributed to the fact that different Pi rates can influence P forms, their buildup and subsequent movement in the soil layer (0–20 cm) [31]. The $NaHCO_3$-Pi (bioavailable form) decreased during the first year of experiment and become stable in the second year, this might be attributable to activities of microbes in the soil. Previously, it was reported that $NaHCO_3$-Pi significantly correlated to microbial activity [23]. However, our results confirmed the previous findings of Sardans et al. [32], who reported that under evergreen Mediterranean forest, the phosphorus uptake was significantly decreased due to less available P in soil. In our case, at high treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}) the P was more uptaken by trees and showed lower values as compared to medium treatment (15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1}). Therefore, the medium treatment performed better as compared to all treatments in terms of available P forms and plant uptake in both type of forests. The main portion of P was bound as residual P, but the combined application of N and P improved the availability of labile P in both forests, and large values were determined in NKPF.

The labile-Pi concentrations clearly depict that tree species are important factors in nutrient uptake, specifically under the applied treatments relative to that of control. This is also in line with the previous experimentation results, which exhibit that the NaOH-P fraction (intermediate) was not readily available to the plants but resulted in the formation of chelates, with Al and Fe oxides also acting as P-desorption sites [25,33]. However, an increase in NaOH-Pi under the influence of high N deposition gave rise to a significantly enhanced nitrification process; resultantly, soil acidification by the aid of microbial mediation processes of ammonia oxidation H+ ion occurred. Moreover, the soil pH decreased in N-addition plots and remained stable in other plots, indicating that an increase in Fe and Al solubilization results in increasing the P binding with soil [25,34]. However, we observed that N and P deposition might increase the available P in soil by changing the inorganic P solubilization, P uptake, and microbial and enzymatic activities; but at high application of N and P, the nitrification process might lead to acidification, and as a result, enhanced soil-P binding. The total P declined in the initial stages of the experiment and went towards restoration at the end of second year of the experiment; this might be because of the balance of acquisitions through fertilization, recycling of P through biological processes, redistributions and transformations, soil mixing, organic-matter decomposition and mineralization, eluviation, and leaching and site-restoration processes [35].

In our study, a significant ($p < 0.05$) variation was observed in nutrient concentration among the forest types. Specially, high nutrient (most of the studied elements) concentrations were observed in NKPF as compared to the KPP. This was because of the significantly higher nutrient concentration in hardwood (birch) in NKPF as compared to softwood (pine). Similar supportive work conducted by Harmon and colleagues [36] outlined the two major reasons for this commonly cited pattern. There are studies presented which state that P fractions in soils are highly dependent on their biogeochemical properties, fertilization, and different microbial activities and their management strategies [37,38]. In this study, the P-fraction concentration was significantly greater in N and P treatment but considered the balance of a positive approach of fertilizer application. Regarding the increase in residual-P fractions, high P-fertilizer application resulted in a high fixation of HCl-P fractions and decreased the other fractions. Our results further confirmed the previous findings of Wang et al. [39]. Different studies are presented that elaborate that excessive P application can result in P fixation, which further deteriorates the soil environment. In this regard, Ma et al. [40] and Díez et al. [41] stated that for minimum soil-environmental impacts, along with better crop production, a well-managed fertilizer-application-rate plan is required under long-term fertilization conditions. Figures 1–6 depict that under long-term P application, soil P significantly increased as compared to the control. According to Si et al. [42], this was because the long-term addition improves P availability to plants in soil, and the change in substrate required by plants during the grain-filling stage was an important aspect identified in this study. This study also helped in understanding the behavior of different forest species and how these are transformed under the long-term application of N and P fertilization. The P uptake was enhanced and yield was improved under a balanced rate of P application along with N, and L: Low treatment (5 g N m^{-2} a^{-1} + 5 g P m^{-2} a^{-1}) as well as H: High treatment (30 g N m^{-2} a^{-1} + 20 g P m^{-2} a^{-1}) had similar effects on the P uptake and fractionation distribution in soil. Long-term N and P fertilization of a balanced amount of fertilizer, with an application of 15 g N m^{-2} a^{-1} + 10 g P m^{-2} a^{-1} (M: Medium treatment), was found to be the best strategy to optimize the availability of soil P for plants at their critical stage when the P requirement must be particularly needed in this region. Zhang et al. [43] suggested that inherited total P contents are the principal traits affecting concentration of P fractions in the soil, along with applied treatments. In the current study, it was also confirmed by the PCA results that the forest types and applied treatments respond differently to the P fractions in soil, while it was also explored that the positive correlation among soil organic carbon and P fractions indicated that organic matter controls the release of phosphorus.

5. Conclusions

Conclusively, the long-term balanced application of P fertilizer in combination with N fertilizer results in better and enhanced levels of total phosphorus, as well as its organic and mineral fraction and soil fertility in general. The results generated after statistical analysis clearly revealed that differentiation among the studied variants is mainly due to the P accumulation and its distribution in the soil. In addition, other soil characteristics such as soil reaction, organic-C content and total N content in soil were significant in posing the observed differences across the treatments. The application of N and P at high rates causes fixation of P, and less availability to trees in NKPF as well as KPP. The results revealed that maximum P was retained as residual P, while all other fractions showed positive correlations with each other and negative correlations with pH.

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