



Article Effects of Fertilizers and Litter Treatment on Soil Nutrients in Korean Pine Plantation and its Natural Forest of Northeast China

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Abstract: Organic and inorganic soil fertilizer addition or removal pose significant effects on soil nutrients. As climate change and other anthropogenic factors are causing deprivation in soil nutrient profiles and altering its proper functioning, complete insight into fertilizer modification and its consequences is required for understanding the sustenance of forest ecosystems. In this regard, an experiment was conducted at Liangshui National Nature Reserve, northeast China, in which two forest soil types (i.e., Korean pine plantation and natural Korean pine forest) were evaluated for their response to external fertilizer applications and litter treatments. The litter treatments were litter application as Ck (undisturbed litter), RL (removed litter) and AL (Alter/double litter i.e., litter removed from RL was added in double litter plots), whereas the synthetic fertilizer treatments were Control (No added N and P), Low (5 g N $m^{-2} a^{-1} + 5 g P m^{-2} a^{-1}$), Medium (15 g N $m^{-2} a^{-1}$ + 10 g P m⁻² a⁻¹) and High (30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹). The outcome showed that soil organic carbon (SOC) was directly proportionate to forest litter amounts. Synthetic fertilizers affected soil total nitrogen (STN) and maximum amounts were recorded in plots with H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹, as 3.03 ± 0.35 g kg⁻¹ in AL. Similarly, altered litter/double was most effective in enhancing the quantity of soil total phosphorus (STP) (0.75 ± 0.04 g kg⁻¹). Soil sampling carried out during the start and end of the experiment showed decreases in the sixth sampling of: SOC (4-23%), STN (7.5-10.8%) and STP (8.51–13.9%). A positive correlation was observed between SOC and total nitrogen; C:N ratio also increased with SOC. Principal component analysis (PCA) on captured a total of 62.1% variability, on the x-axis (35.1%) and on the y-axis (27%). It was concluded that combined application of N and P at the level of $30 \text{ g N m}^{-2} \text{ a}^{-1} + 20 \text{ g P m}^{-2} \text{ a}^{-1}$ under AL (Alter/double litter) treatment level improved soil total N and P content. The results clearly depicted that forest litter is an important source for building up of soil organic matter, however for attaining maximum sustenance capabilities in soil, the continuity of fertilizer application in either form is a prerequisite.

Keywords: forest ecosystem; soil carbon; forest productivity; nitrogen deposition; soil fertility

1. Introduction

Forest litter significantly affects nutrient cycling, especially for carbon (C), nitrogen (N) and phosphorus (P) in forest ecosystems. Rapid urbanization and other human activities in forest vicinities, as well as climate change, have altered forest productivity, which has resulted in deliberate disturbance of aboveground forest litter [1]. Alteration in the aboveground litter layer disturbs belowground soil biogeochemical processes. Directly, litter can



Citation: Hussain, A.; Jamil, M.A.; Abid, K.; Duan, W.; Chen, L.; Li, C. Effects of Fertilizers and Litter Treatment on Soil Nutrients in Korean Pine Plantation and its Natural Forest of Northeast China. *Forests* **2022**, *13*, 1560. https:// doi.org/10.3390/f13101560

Academic Editors: Anna Zavarzina, Irina N. Kurganova, Yakov Kuzyakov, Francisco Matus, Agustin Merino and Wenhua Xiang

Received: 12 August 2022 Accepted: 18 September 2022 Published: 24 September 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). alter (increase or decrease) organic carbon (OC) content and other nutrients, while indirectly, litter can impact the biotic activities taking place mostly through microorganisms [2]. Various studies related to litter's effect on carbon built up and N cycling are presented in the literature [3,4]; on the other hand, few studies have focused on the impact of litter on soil phosphorus (P). Sayer [2] and García-Palacios et al. [5] affirm that inclusion or exclusion of litter fall affects belowground processes. Xu et al. [6] extensively studied litter application consequences in a meta-analysis comprising 70 litter management studies. They conclusively stated that in the topsoil layer (10 cm) total SOC was directly proportional to external input of litter. Whereas nitrogen was not affected with litter addition, nitrogen decreased in the top 10-cm layer if the litter was removed. Still, a study gap exists relevant to incorporation of aboveground litter on soil C and N. For example, Sayer [2] found a non-significant or minute change in surface carbon. Xu et al. [6] found a lesser impact of litter in temperate forests, while in tropical forests the soil nutrient changes due to litter fall were more visible. In addition, little attention has been given to assess P dynamics under different litter inputs than N and C, especially in temperate ecosystems. Vincent et al. [7] studied litter treatment for three years in tropical forests and revealed a reduction in P concentration by 23% while litter addition enhanced P concentration by 16% in the topsoil layer (2 cm). Similarly, another study stated that in tropical forests, litter can increase P [8]. However, a five-year study carried out in mineral content of soils of a lowland semi-evergreen tropical forest showed a non-significant effect on soil extractable P in topsoil (10 cm) under litter addition or removal [9]. Previously, it has been reported that in temperate forests, N is the main limiting factor for plant growth and development relative to that of P [10]. However, the impact of forest litter input/alteration on the availability of soil phosphorus is rarely understood in temperate forests [11]. Further, an increased indication of P limitation in temperate forests is gaining importance and researchers are more concerned about P dynamics. It is crucial to understand C, N and P dynamics in temperate forests for assessing and predicting their response toward environmental fluctuations like elevated CO₂. Litter is produced due to the metabolism that occurs during plant growth and development processes, which promotes nutrient and carbon cycles in forest ecology [12].

Fertilizers are incorporated into soil for improving soil fertility, however, excessive fertilizers decrease organic matter and increase soil acidification [1]. Agriculture sustainability is highly dependent on soil fertility, which alters soil productivity. Fertilizers can impact soil nutrients in either a positive or negative manner. Organic fertilizers increase soil biological cycles and physical structure [6] but they are relatively lower in nutrient contents. In contrast, inorganic fertilizers are rapidly available to plants due to their easy accessibility, but extensive use of inorganic fertilizers degrades soil structure, causes soil acidity and environmental pollution. Integrated use of organic and inorganic fertilizers increases soil organic C content, total nitrogen and soil nutrient availability.

In forest ecosystems, SOC is affected by litter inputs (adding/removing) along with essential nutrients bound with dissolved organic C [13]. SOC has an active organic C that is comprised of light group C, easily oxidizable soil organic C and soil particulate organic C. The easily oxidizable organic C decomposes/oxidizes during soil enzymatic activities and reactions carried out through micro-organisms. This process indicates early changes of soil organic matter. On the other hand, soil particulate C acts as temporary or transitional organic C in fresh animal and plant residues and organic matter [14] and is more responsive to external factors. Soil light group C is the organic C between animal and plant residues and organic matter, and is an important part of soil active organic C. In recent years, most of the studies on the effects of removing/adding forest litter on soil active organic C are mainly analyzed and discussed by using soil microbial C as the active organic C index and the impact of litter addition on soil nutrients (i.e., SOC, STN and STP) is different to some extent. Changes in litter treatments/application can affect soil carbon build up [15]. There are also many studies on the effect of litter input on soil respiration. However, few studies on the effect of litter input on soil respiration. Some

studies have found that litter addition has significantly increased SOC content, while a few studies have shown no impact of litter addition on SOC. Li [16] reported that litter addition has no impact on STN and STP; however, he stated that STN and C:N ratio increase with the addition of litter.

In forest regions of northeast China, Korean pine (Pinus koraiensis) is the most important conifer species as both natural forest and artificial plantation. Korean pine is the semi light demanding and relatively cold tolerant evergreen species. It is highly light demanding at the adult stage but shade tolerant in the juvenile stage. This species is most important for afforestation and timber production in the temperate forest of northeast China. In this study we selected two forest types, i.e., natural Korean pine forest and artificial plantations of Korean pine forest. Here, we examine the impact of litter application (hereafter, meaning addition or removal) on the soil's health across two forest types. Specifically, we aim to elucidate that the altered application significantly influenced the soil active organic carbon along with other main soil nutrients, such as SOC, STN and STP, across Korean pine plantation and natural Korean pine forest. Moreover, other treatments that include the addition of synthetic fertilizers (N and P) in different doses are also significant for determination of soil biogeochemical mechanisms. These results obtained from organic and inorganic inputs will provide the scientific basis and practical reference for sustainable management of these two forest types. In this study we test two questions: Does addition of forest litter improve soil nutrient contents? Do organic and inorganic fertilizer inputs enhance soil total nitrogen and soil total phosphorus? In addition, we will identify the optimal combination of organic and inorganic fertilizers.

2. Materials and Methods

2.1. Site Characteristics

The experimental site is situated at Liangshui National Nature Reserve (47°10′50″ N, 128°53′20″ E), Dailing district, Yichun city, Heilongjiang Province in northeast China. The site is located on the Dali Range (East slope) of southern Xiaoxing'an Mountains. The landform contains low mountains and hills at elevations from 280 to 707 m. In terms of environmental conditions, temperate monsoon climate, with a mean annual temperature of -0.3 °C and mean annual precipitation of 676 mm prevails. The frost-free period and snowfall period are 100–120 days and 130–150 days, respectively, and the forest soil is dark brown as classified by the Chinese classification system.

2.2. Experimental Design and Treatments

The study was carried out at two forests, viz. Korean pine plantation (KPP) and Natural Korean Pine Forest (NKPF). The Korean pine plantation comprises artificially planted pine plants and lacks other natural plant species, while the natural Korean plantation comprises naturally regenerated forest and also contains other natural plant species in the forest area. The overall experiment contained two treatment factors applied in each forest type. There were three main plots on which litter application was performed according to the treatments. Litter application was performed at three levels, i.e., Ck (undisturbed litter), RL (removed litter) and AL (alter/double litter). The plots of undisturbed soil received no litter treatment; the litter from the forest floor was removed in the RL plots, while the removed litter was added into the AL plot, thus making its quantity double. All the main plots were covered with nets so that no more forest litter was added/altered during the experimental duration. Likewise, each main plot was divided into four sub plots that comprised of fertilizer levels as treatments. Each of the four sub plots were treated as Control (no added N and P), Low (5 g N m⁻² a⁻¹ + 5 g P m⁻² a⁻¹), Medium (15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹) and High (30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹). The experimental treatments were replicated three times. The experiment follows the layout in split plot design. The nitrogen source was ((NH₄)₂SO₄) while some N and P were applied in the form of diammonium phosphate $((NH_4)_2HPO_4)$. Fertigation was used to apply the fertilizers to the plots according to the prescribed quantities. Fertilizer was applied five times in a year. The soil was analyzed

prior to the experiment and forest survey revealed a difference between KPP and NKPF soils relevant to general characteristics. KPP is situated at slope of 8° and altitude of 411.8 m whereas NKPF was situated at 15° slope and altitude of 485 m. The plantations in KPP were slightly denser (1475 trees·hm⁻²) and a canopy closure (0.75) were estimated in comparison to NKPF in which stand density was 1175 Trees·hm⁻² and canopy closure was 0.70. The soil factors revealed that KPP soils contain less total C and total N but more total P in comparison to NKPF. The detailed results are shown in Table 1.

Forest Survey Factors **Topographic Factors** Soil Factors Mean Stand Canopy Soil Total Total Total Sample Slope Altitude Avg. Slope Slope Height Density Closure Density N Р Plot Aspect Position DBH/cm (Trees · hm⁻²) (°) (g·m⁻³) m m (g ⋅ kg⁻¹) Down 2.62 KPP Half 8 411.8 21.1 184 1475 0.75 0.99 46.08 0.31 slope Up slope sunny 485 15.70.70 47.2 0.21 NKPF 15 26.4 1175 0.80 3.02 slop

Table 1. Summary of basic characteristics of the investigated forests.

KPP: Korean Pine plantation, NKPF: Natural Korean Pine Forest, C: carbon, N: nitrogen, P: phosphorus.

2.3. Soil Sampling

The soil samples were collected in May, August and October of the first year (S1: sampling-I, S2: sampling-II, S3: sampling-III), and in the same months in the second year (S4: sampling-IV, S5: sampling-V, S6: sampling-VI). Each sample was obtained by selecting three points and collecting soil from a depth of 0-20 cm. After that, the three collected samples were collectively formed into one composite sample. After being packed in sealed plastic bags, samples were tagged and immediately transported to the laboratory for further analysis.

2.4. Determination of Total Soil Phosphorus, Total Soil Nitrogen and Soil Organic Carbon

The total soil phosphorus was determined by acid digestion (perchloric acid) while the total nitrogen was determined by Kjeldahl method. For SOC, the samples were air dried and finely ground, and were passed through 0.149 mm sieve [17]. Two grams of each sample was hydrolyzed with 5 mL 1 mol·L⁻¹ HCl in a 50 mL beaker with repetitive shaking until the soil solution was free from bubbles, it was heated at 60 °C for 2 to 3 h in order to obtain dry weight [17]. We subsequently extracted 25 µg of the samples to determine the SOC content using an Element Analyzer (Elementar Vario EL, Hanau, Germany).

2.5. Statistical Analysis

All data were subjected to three-way analysis of variance (ANOVA) and treatment means were separated by least significant difference at 1.00% probability level (Fisher protected LSD). The interactive comparison was made for effect of forest litter, fertilizer application and soil samples. Further, principal component analysis (PCA) was carried out using XLSTAT software and biplots were generated to compare the correlation among the observed data. The separate correlation analysis was performed on R software.

3. Results

3.1. Effect of Different Litter Treatments at Various N and P Levels on SOC

The results of this experiment revealed that the forest litter as well as application of fertilizers, significantly influenced soil health in both forest types. In KPP, the amount of SOC was improved pertaining to forest litter. The overall SOC quantity was increased in plots where forest litter was added in double amounts (AL). However, analysis data explored that maximum ($64.42 \pm 2.14 \text{ g kg}^{-1}$) quantity of SOC was measured in M (medium) in CK plots, while maximum SOC ($72.67 \pm 3.65 \text{ g kg}^{-1}$) was obtained in control treatment (C) of RL. In the case of AL treatment, maximum SOC ($66.00 \pm 3.29 \text{ g kg}^{-1}$) was obtained in H treated plots. Apart from this observation, the second maximum quantity was 64.67 ± 3.55 observed in H treated plots of RL. The overall maximum amounts of SOC were in plots with altered litter (litter in double quantity) in H plots. As far as the sampling times is concerned, the results showed that all plots followed a trend of S1 > S2 > S3 > S4 > S5 > S6, thus depicting that SOC content decreased to some extent during each sampling in two years. For example, the maximum quantity of SOC was 66.00 ± 3.29 (AL-M) during the first soil sampling, while it decreased to 61.87 ± 1.36 in the sixth sampling. The results of all other treatments are depicted in Table 2. In NKPF the results showed that highest SOC was observed in plots where the synthetic fertilizer was applied in H. It was 64.33 ± 2.78 , 64.78 ± 3.69 and 67.19 ± 2.15 in CK, RL and AL, respectively, in the first soil samples. The addition of double litter enhanced SOC content and the amounts were affected during the sampling time. It followed the same trend as in KPP. For example, in NKPF the values were $67.19 \pm 2.15 > 66.42 \pm 3.19 > 63.76 \pm 2.58 > 60.03 \pm 2.98 > 59.93 \pm 1.89 > 59.54 \pm 1.78$ for S1 > S2 > S3 > S4 > S5 > S6 showing a decrease in SOC with the proceeding sampling (Table 2).

Table 2. Changes in soil organic matter by N and P inputs along with litter treatments across six sampling.

| Forest | Litter | N & P | SOC (g kg ⁻¹) | | | | | |
|--------|--------|-------|---------------------------------|--------------------------------|-----------------------------|--|------------------------------|-----------------------------|
| | | | S1 | S2 | S 3 | S4 | S 5 | S 6 |
| | СК | С | $58.90 \pm 1.01 \ \mathrm{k}$ | $58.32\pm1.36~\mathrm{m}$ | 57.31 ± 1.69 j,k | 56.36 ± 1.02 j,k | $56.16\pm1.15~\mathrm{h}$ | $55.87\pm1.01~\mathrm{f}$ |
| | | L | 60.93 ± 1.25 i,j | $60.35 \pm 2.81 \text{ i-k}$ | 59.34 ± 1.89 h,i | 55.03 ± 1.33 k | 54.93 ± 0.99 i,j | $54.54 \pm 1.02 \text{ g}$ |
| | | М | 64.42 ± 2.14 c–f | 63.84 ± 3.33 d,e | 62.83 ± 1.96 c,d | 58.03 ± 2.44 g–i | $57.93 \pm 1.08 \text{ e-g}$ | 57.54 ± 1.36 e |
| | | Н | $62.25\pm2.68~\text{g-i}$ | $61.67\pm3.01~\text{f-i}$ | $60.66\pm1.99~\mathrm{f,g}$ | $59.69\pm2.15\tilde{b}e$ | $59.59\pm1.23\mathrm{b,c}$ | $59.20\pm1.11~\mathrm{b,c}$ |
| | | С | 72.67 ± 3.65 a | $72.09\pm4.05~\mathrm{a}$ | $71.08\pm4.11~\mathrm{a}$ | 56.36 ± 1.36 j,k | $56.26\pm1.15~h$ | 55.87 ± 1.05 d,e |
| KPP | ы | L | 62.33 ± 2.45 g–i | 61.47 ± 3.15 g–i | 60.74 ± 3.89 f,g | 57.36 ± 2.11 h–j | 57.26 ± 1.30 f–h | $56.87\pm1.25~\mathrm{f}$ |
| ici i | KL | Μ | 64.33 ± 3.12 d–f | 63.85 ± 2.69 d,e | 62.74 ± 3.04 c,d | $59.69\pm1.88~\mathrm{b}{-}\mathrm{e}$ | 59.59 ± 1.25 b,c | 59.20 ± 1.05 b,c |
| - | | Н | $64.67 \pm 3.55 \text{ d-g}$ | $64.49\pm3.45\text{c,d}$ | $63.08\pm2.36\text{ c,d}$ | $60.36\pm1.56~\mathrm{b,c}$ | $60.26\pm1.36~\text{b}$ | $59.87\pm1.36\mathrm{b}$ |
| | AL | С | 61.68 ± 2.98 h,i | 61.09 ± 3.78 h–j | 60.08 ± 1.05 g,h | 58.36 ± 2.12 g–h | $58.26 \pm 1.25 \text{ d-f}$ | $57.87 \pm 1.21 d_{,e}$ |
| | | L | $63.00 \pm 2.78 \text{ d-g}$ | $62.42 \pm 2.63 \text{ e-h}$ | 61.41 ± 1.99 e,f | 59.03 ± 2.15 d–g | 58.93 ± 1.36 | 58.54 ± 1.25 c,d |
| | | М | 65.00 ± 3.41 c,d | 64.44 ± 2.96 c,d | 63.41 ± 2.46 b,c | $60.76 \pm 2.36 \text{ b}^{-1}$ | $60.36\pm1.25\mathrm{b}$ | $59.87\pm1.05\mathrm{b}$ |
| | | Н | $66.00\pm3.29\mathrm{b,c}$ | $65.41\pm3.45\mathrm{b,c}$ | $64.41\pm1.36~\mathrm{b}$ | $62.14\pm2.45~\mathrm{a}$ | $62.66\pm1.89~\mathrm{a}$ | $61.87\pm1.36~\mathrm{a}$ |
| NKPF - | СК | С | 59.67 ± 3.45 j,k | 59.09 ± 1.09 k-m | 57.53 ± 2.14 j,k | 56.56 ± 1.00 j,k | 56.06 ± 1.05 h,i | $55.87\pm1.36~\mathrm{f}$ |
| | | L | $59.00 \pm 3.69 \text{ k}$ | 58.42 ± 1.11 l,m | $56.86 \pm 1.88 \text{ k}$ | 54.66 ± 2.011 | 54.21 ± 1.04 j | $53.87 \pm 1.04 \text{ g}$ |
| | | Μ | 63.00 ± 2.89 f–h | 62.49 ± 2.15 e–h | 60.86 ± 1.96 f,g | 57.13 ± 1.18 I,j | 56.94 ± 1.09 g,h | $56.54 \pm 1.08 \text{ f}$ |
| | | Н | $64.33\pm2.78~df$ | 63.75 ± 1.89 d,e | $62.19\pm1.15~\mathrm{d,e}$ | $59.09\pm1.36~\mathrm{d}\mathrm{-g}$ | $58.25\pm1.63~\text{d-f}$ | 58.54 ± 1.45 c,d |
| | RL | С | $62.00\pm3.41~\mathrm{h,i}$ | 61.42 ± 1.02 g–i | 59.86 h,i \pm 1.25 | 56.64 ± 1.05 j,k | $56.19\pm1.41~\mathrm{h}$ | $56.20\pm2.05~\mathrm{f}$ |
| | | L | 59.61 ± 2.78 j,k | 59.78 ± 2.15 j–l | 57.45 ± 1.96 j,k | 56.62 ± 1.11 j,k | $56.52\pm1.03~\mathrm{h}$ | $56.20\pm1.66~{\rm f}$ |
| | | Μ | 62.45 ± 3.74 g–i | $62.45\pm3.05~\mathrm{e}{-h}$ | 60.73 ± 1.45 f,g | 59.33 ± 1.36 c–f | 59.26 ± 1.23 b–d | $58.87\pm1.96~\mathrm{c}$ |
| | | Н | 64.78 ± 3.69 c,d | $64.36\pm3.96~\mathrm{c,d}$ | 61.51 ± 1.39 e,f | $59.69\pm1.58~\mathrm{e}{-\mathrm{g}}$ | $59.59\pm1.05\mathrm{b,c}$ | $59.20\pm1.05\mathrm{b,c}$ |
| | AL | С | $6\overline{1.12 \pm 3.47}$ i,j | 61.14 ± 1.66 g–i | 58.33 ± 1.25 i,j | 58.01 ± 1.45 g–i | $57.93 \pm 1.05 \text{ e-g}$ | 57.54 ± 1.25 c,d |
| | | L | $63.67 \pm 3.15 \text{ d-g}$ | $63.09 \pm 2.89 \text{ d}{-f}$ | 60.84 ± 1.78 f,g | 58.36 ± 1.96 f–h | $58.26 \pm 1.36 \text{ b-d}$ | $57.87\pm1.36~\mathrm{c}$ |
| | | Μ | 66.06 ± 2.56 b,c | 65.42 ± 3.78 b,c | 62.81 ± 2.12 c,d | $59.69\pm1.28\mathrm{b}{-}\mathrm{e}$ | $59.59\pm1.24~\mathrm{b,c}$ | $59.20\pm1.05\mathrm{b,c}$ |
| | | Н | $67.19\pm2.15\mathrm{b}$ | $66.42\pm3.19b$ | $63.76\pm2.58~\mathrm{bc}$ | $60.03\pm2.98~\text{b-d}$ | 59.93 ± 1.89 b,c | 59.54 ± 1.78 b,c |

Values are means \pm SE, CK: no litter, RL: removed litter, AL: alter/double litter, C: control (No N & P), L: 5 g N m⁻² a⁻¹ + 5 g P m⁻² a⁻¹, M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹, H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹. SOC: soil organic carbon. S1: sampling-I, S2: sampling-II, S3: sampling-II, S4: sampling-IV, S5: sampling-V, S6: sampling-VI. The lettering shows the different statistically significant groups.

3.2. Effect of Different Litter Treatments at Various N and P Levels on C:N

Our results also exposed that NP inputs as well as forest litter impacted the C:N ratio in the soil. Similarly, the period of application of fertilizer and subsequent soil sampling and its analysis also showed that C:N ratio does not remain the same. In KPP, the results showed that fertilizer application changed the C:N ratio. The maximum values were observed in control plots of CK, RL and AL as 22.65 ± 0.78 , 26.91 ± 1.25 and 23.42 ± 1.05 , respectively, however when the fertilizer inputs changed, the C:N ratio also changed. Specifically, it decreased in L and M, but increased in H. The trend was observed as C > L > M < H in NKPF. Forest litter also impacted C:N ratio as depicted in Table 3. As far as the sampling is concerned, no linear increase or decrease was observed in C:N values, however they differ in minute quantities showing that periodic addition of fertilizer does not impact the C:N significantly (Table 3).

| Forest Type | Litter Treatment | N & P | C:N Ratio | | | | | | |
|----------------|---------------------|-------|--------------------------------------|-------------------------------------|-------------------------------|--------------------------------------|-------------------------------|--|--|
| | | | S1 | S2 | S3 | S4 | S5 | S6 | |
| | | С | 22.65 ± 0.78 c–e | $22.62\pm0.89~df$ | $21.95\pm1.03~\text{d-f}$ | $24.50\pm1.15\mathrm{b}$ | 22.86 ± 0.89 b,c | $23.77 \pm 1.05 \text{ b-d}$ | |
| | CIV | L | 21.01 ± 0.88 h–k | 20.96 ± 0.99 g | $20.39\pm0.86~\mathrm{i}{-k}$ | 21.72 ± 2.12 d–h | 19.90 ± 0.69 j,k | 20.57 ± 0.59 l,m | |
| | CK | М | $19.52\pm0.69l$ | $19.47\pm0.46\mathrm{\check{h}}$ | $18.98\pm1.36~\mathrm{m}$ | $19.78\pm1.89~\mathrm{m}$ | 18.33 ± 0.781 | $18.86 \pm 0.69 \text{ n}$ | |
| | | Н | $20.30\pm0.71\text{ j-l}$ | $20.25\pm0.89~\text{g,h}$ | $19.71\pm1.89\text{ k-m}$ | $21.07\pm2.36~hk$ | $20.36\pm0.99~\text{i-k}$ | $21.01\pm1.89~\text{k-m}$ | |
| | | С | $26.91\pm1.25~\mathrm{a}$ | $26.91\pm1.26~\mathrm{a}$ | $26.22\pm1.36~\mathrm{a}$ | $21.14\pm2.15~\text{g-k}$ | $21.97\pm1.02~df$ | $22.80\pm2.15~\text{e-g}$ | |
| KPP | RL | L | $22.16 \pm 1.03 \text{ d}-\text{g}$ | $22.12\pm1.63~\mathrm{f}$ | 21.51 ± 1.89 f,g | 21.19 ± 2.36 g-k | 21.41 ± 1.36 f–h | 22.18 ± 1.39 g–i | |
| | | Μ | 20.32 ± 0.89 j–l | 20.27 ± 1.89 g,h | 19.75 ± 0.49 k–m | 20.58 ± 0.89 k,l | $19.68\pm0.78~\mathrm{k}$ | $20.29 \pm 1.66 \text{ m}$ | |
| | | Н | $23.10\pm1.03bd$ | 23.06 ± 1.36 d,e | $22.44\pm1.20~\text{c-f}$ | $22.08\pm2.15df$ | $22.65\pm1.36bd$ | $23.47\pm1.28~\mathrm{c-e}$ | |
| | | С | $23.42\pm1.05\text{b,c}$ | $23.39\pm1.78~\text{c,d}$ | $22.72\pm1.36~\mathrm{c,d}$ | $24.66\pm2.36b$ | $23.36\pm1.88b$ | $24.27\pm1.96\mathrm{b,c}$ | |
| | AL | L | 21.00 ± 0.76 h–k | 20.95 ± 1.36 g | 20.40 ± 0.89 i–k | 20.83 ± 2.14 j,k | 20.60 ± 1.98 h–j | 21.28 ± 1.78 j–l | |
| | | Μ | 20.31 ± 0.69 j–l | $20.27 \pm 1.09 \text{ g}$ | 19.75 ± 1.36 k–m | 19.90 ± 1.89 l,m | $19.69 \pm 1.24 \text{ k}$ | $20.29 \pm 0.69 \text{ m}$ | |
| | | Н | $20.63\pm0.78~\mathrm{i}{-1}$ | $20.58\pm0.89~g$ | $20.06\pm0.78~\text{j-l}$ | 20.56 ± 0.89 k,l | $20.34\pm1.35~\text{i-k}$ | $20.97\pm0.89\text{ k-m}$ | |
| | СК | С | 25.94 ± 1.63 | $25.93\pm2.10b$ | $24.89\pm0.69b$ | $26.42\pm2.69~\mathrm{a}$ | $26.04\pm2.15~\mathrm{a}$ | $27.24\pm3.16~\mathrm{a}$ | |
| | | L | $24.08\pm1.21\mathrm{b}$ | $24.05\pm2.12~\mathrm{c}$ | $23.11 \pm 2.36 \text{ c}$ | $24.52\pm2.15\mathrm{b}$ | $23.48\pm2.96b$ | $24.48\pm2.91b$ | |
| | | Μ | 23.42 ± 1.20 b,c | 23.39 ± 2.00 c,d | 22.53 ± 2.01 c,d | $23.21 \pm 1.25 \text{ c}$ | 22.32 ± 2.46 c–e | $23.16 \pm 2.96 \text{ d-f}$ | |
| | | Н | $22.47\pm1.08~\mathrm{b,c}$ | $22.43\pm0.96~\text{e,f}$ | $21.64\pm1.36~\text{e-g}$ | $22.44 \pm 2.36 \text{ d}$ | $21.63\pm2.96~\mathrm{e}{-g}$ | $22.39\pm3.88~\text{f-h}$ | |
| NKPF | RL | С | $22.14\pm1.36~\mathrm{d}{\text{-g}}$ | $22.10\pm0.78~\mathrm{f}$ | $21.30\pm1.45~\text{f-h}$ | $22.09\pm2.15df$ | $21.27\pm2.16~\text{f-h}$ | 22.03 ± 2.19 g–j | |
| | | L | 20.94 ± 0.89 h–k | $20.89 \pm 0.94 \text{ g}$ | 20.11 ± 1.25 j–l | $21.67 \pm 1.69 \text{ e}{-i}$ | 20.88 ± 0.89 g–i | 21.61 ± 1.36 h–k | |
| | | Μ | 20.22 ± 1.15 j–l | 20.17 ± 1.36 g,h | 19.46 ± 0.99 l,m | 20.71 ± 0.56 j,k | 20.01 ± 2.15 j,k | 20.65 ± 0.77 l,m | |
| | | Н | $22.30\pm1.05~\text{d-f}$ | $22.26\pm1.15~\mathrm{e,f}$ | $21.14\pm1.82~\text{f-i}$ | 22.39 ± 1.25 d,e | $21.59 \pm 2.78 \text{ e-g}$ | $22.33\pm1.56~\text{f-h}$ | |
| | AL | С | 22.56 ± 1.36 c–e | $22.52\pm0.89~\text{e,f}$ | $21.33\pm1.26~\text{f-h}$ | $23.21\pm1.36~\mathrm{c}$ | 22.33 ± 2.63 c-e | $23.16\pm0.96~df$ | |
| | | L | $21.70\pm1.24~\mathrm{e}{-h}$ | 21.66 ± 1.22 g,h | 20.56 ± 1.96 h–j | 21.35 ± 1.23 f–j | $20.85\pm0.88~\text{g-i}$ | $21.56\pm0.52~h\text{k}$ | |
| | | М | $22.76 \pm 1.20 \text{ c-e}$ | $22.72 \pm 1.59 \text{ d}-\text{f}$ | 21.60 ± 1.25 f,g | $21.84\pm1.12~dg$ | $21.59 \pm 2.45 \text{ e-g}$ | $22.33\pm0.69~\text{f-h}$ | |
| | | Н | $22.09\pm1.05~\text{d-g}$ | $22.05\pm1.23~\mathrm{f}$ | $20.98\pm1.36~\text{g-i}$ | $20.94\pm0.78~\mathrm{i}\mathrm{-k}$ | 20.71 ± 0.78 h–j | $21.38\pm0.78~\mathrm{i}{-}\mathrm{l}$ | |

Table 3. Changes in carbon to nitrogen ratio by N and P inputs along with litter treatments across six samplings.

Values are means \pm SE, CK: no litter, RL: removed litter, AL: alter/double litter, C: control (No N & P), L: 5 g N m⁻² a⁻¹ + 5 g P m⁻² a⁻¹, M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹, H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹. C:N ratio: carbon to nitrogen ratio, S1: sampling-I, S2: sampling-II, S3: sampling-III, S4: sampling-IV, S5: sampling-V, S6: sampling-VI. The lettering shows the different statistically significant groups.

3.3. Effect of Different Litter Treatments at Various N and P Levels on STN

In our results, it is clear that synthetic fertilizers affected STN. Soil total nitrogen increased in M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹, and after adding more fertilizer (i.e., H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹) it started to reduce. In NKPF the highest value of STN was 3.30 g kg⁻¹, 3.17 g kg⁻¹ and 3.20 g kg⁻¹ in M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹ plots of CK, RL and AL, respectively, which reduced to 3.07 g kg⁻¹, 2.80 g kg⁻¹ and 3.20 g kg⁻¹ in H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹ treated plots, respectively (Figure 1a,b). A similar trend was observed in KPP; however, the values differ. As far as the soil samples are concerned, it was observed that generally the STN values during the last (i.e., sixth) sample was much less as compared to the first sample. For example in NKPF the value 3.30 g kg⁻¹ in S1 decreased to 3.05 g kg⁻¹ in S6 (CK- M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹ + 10 g P m⁻² a⁻¹ + 10 g P m⁻² a⁻¹ in S6 (RL- M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹ in S6 (AL- M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹), while the value 3.20 g kg⁻¹ in S1 decreased to 2.95 g kg⁻¹ in S6 (AL- M: 15 g N m⁻² a⁻¹ + 10 g P m⁻² a⁻¹). A similar trend was observed in KPP (Figure 1a,b).



Figure 1. Changes in STN during (**a**) first year and (**b**) second year of experiment affected by different litter treatments at various N and P levels under two forest types.

3.4. Effect of Different Litter Treatments at Various N and P Levels on STP

The result of our experiment showed that forest litter and NP fertilizers influenced STP in both forest types. Soil analysis in NKPF indicated that altered litter/double was most effective in enhancing the quantity of STP as the maximum value of 0.75 ± 0.04 g kg $^{-1}$ was found in medium treated plots in AL. This value decreased slightly in plots where no litter alteration was performed and gave maximum value of 0.74 ± 0.01 g kg $^{-1}$ in medium fertilizer application. However, in plots where litter was removed, the maximum values were observed in high fertilizer applied plots (0.71 ± 0.04 g kg⁻¹) but the quantity was less as compared to CK and AL (Figure 2a,b). The minimum value of STP was observed in sub plots in which no NP was added. As far as KPP is concerned, the lowest quantities were detected in control plots (with no N and P application) as 0.55 ± 0.03 g kg⁻¹, 0.44 ± 0.10 g kg⁻¹ and 0.46 \pm 0.05 g kg $^{-1}$ in CK, RL and AL respectively during the S1. A maximum value of 0.74 \pm 0.02 g kg $^{-1}$ was estimated in CK where M: 15 g N m $^{-2}$ a $^{-1}$ + 10 g P m $^{-2}$ a $^{-1}$ was applied, whereas in RL and AL both showed highest values as 0.69 \pm 0.02 g kg $^{-1}$ and 0.72 ± 0.04 g kg⁻¹ in high fertilizer treatment (H: 30 g N m⁻² a⁻¹ + 20 g P m⁻² a⁻¹) respectively (Figure 2a,b). The increase or decrease in STP was also observed during each sampling and the results revealed that at the end of experiment the STP content was reduced in comparison to sample 1. For example, in NKPF value of 0.74 S1 was reduced 0.67 in S6 (CK-M), the value of 0.71 in S1 was reduced to 0.64 in S6 (RL-H) whereas the value of

0.75 in S1 was reduced to 0.68 (AL-M). In KPP the trend in reduction of STP from S1 to S6 was similar as depicted in Figure 2a,b). The percent increase/decrease in the variables is depicted in Table 4.







(b)

Figure 2. Changes in STP during (**a**) first year and (**b**) second year of experiment affected by different litter treatments at various N and P levels under two forest types.

| Forest | Littor | N & P - | Percent Increase/Decrease | | | | |
|--------|--------|---------|---------------------------|-------|--------|--------|--|
| rolest | Litter | | SOC | C:N | STN | STP | |
| | | С | -5.14 | -4.91 | -9.58 | -12.23 | |
| | CV | L | -10.48 | 2.07 | -8.59 | -10.90 | |
| | CK | М | -10.68 | 3.38 | -7.55 | -8.63 | |
| | | Н | -4.89 | -3.51 | -8.12 | -9.93 | |
| | | С | -23.11 | 15.29 | -9.22 | -11.92 | |
| KPP | DI | L | -8.76 | -0.10 | -8.85 | -11.28 | |
| | KL | М | -7.97 | 0.11 | -7.86 | -9.88 | |
| | | Н | -7.41 | -1.63 | -8.9 | -8.99 | |
| | | С | -6.15 | -3.65 | -9.46 | -11.56 | |
| | ΔT | L | -7.08 | -1.33 | -8.30 | -9.49 | |
| | AL | М | -7.89 | +0.11 | -7.78 | -8.51 | |
| | | Н | -6.25 | -1.65 | -7.78 | -9.53 | |
| | | С | -6.36 | -5.01 | -10.83 | -11.59 | |
| | CV | L | -8.69 | -1.64 | -10.17 | -9.04 | |
| | CK | Μ | -10.25 | 1.09 | -9.26 | -8.59 | |
| | | Н | -9.01 | 0.33 | -8.70 | -10.09 | |
| | | С | -9.34 | 0.49 | -8.9 | -14.56 | |
| NKPF | DI | L | -5.80 | -3.22 | -8.74 | -12.23 | |
| | KL | М | -6.05 | -2.15 | -8.03 | -10.59 | |
| | | Η | -8.44 | -0.15 | -8.59 | -9.17 | |
| | | С | -6.69 | -2.66 | -9.11 | -13.92 | |
| | ΔŢ | L | -9.10 | 0.66 | -8.49 | -11.49 | |
| | AL | Μ | -10.29 | 1.86 | -8.59 | -8.91 | |
| | | Н | -11.13 | 3.18 | -8.21 | -8.87 | |

Table 4. Increase/decrease in SOC, C:N, STN and STP at the end of experiment as compared to initial stages.

3.5. Effect of Forest Litter and NP Fertilizer on Correlation of Various Variables

The results from our study reveal some interesting aspects regarding correlation among the observed variables. The highest *R* value, nearest to 1, revealed a highly positive correlation. In our study R = 0.99 was highest for TP1 and TP2 revealing that Total P in the first year is associated with increased amount during the second year of experiment revealing a buildup of TP in the soil. Nitrogen for years one and two show a positive correlation (r = 0.95), SOC2 and TN2 showed a positive correlation as well (R = 0.76), thus showing that if total N increase in soil, it positively impacts SOC and escalates its quantity. SOC also showed a positive correlation with C:N (R = 0.57) during the first year indicating that as SOC increased, C:N ratio also increased. There are also variables which showed a negative correlation with each other. For instance, TN and C:N were highly negatively correlated with one another during the second year (R = -0.95), thus showing that with increase in total nitrogen, the C:N ratio of soil decreased. *R* values of studied variables for both years are depicted in Figure 3.



Figure 3. Correlation analysis between the analyzed parameters during the two years of investigation.

3.6. Principal Component Analysis

The studied variables were subjected to principal component analysis for capturing the maximum variation at x and y axis in order to interpret the results relevant to suitability of applied treatments in forest ecosystem. Figure 4a depicts those two principal components on axis X and Y captured a total of 62.1% variability on dimension one (35.1%) and dimension two (27%). The initial variables are projected in the factor space and variables are read on the basis of angles and distance from the origin point. The variables that share a small angle were positively correlated to each other, whereas those with larger angles were significantly negative correlated. In our results under litter treatment, the PCA revealed that STN and C:N were negatively correlated to each other. The vectors in lower left quadrant showed three vectors and among them STPC year one and STPC year two were highly correlated. Figure 4b represents PCAs with reference to fertilizer application and reveals the relationship among the observed variables during both years. For instance, the angle between STNMO year two and STNMF year one was small, thus showing a positive correlation between them. A similar case can be seen with STPC year one and STPC year two vectors present in the lower left quadrant showing that if soil total P increased in the first year, then it was also increased during the second year. It can also be seen that STNMF year 2 and STPC year 2 share a relatively moderate angle, thus depicting that these factors share a moderate positive correlation among them. Similarly, Figure 4crepresents the relationship with reference to the two forests. Clusters of the similar groups



are also formed in PCA analysis and show that values in each individual cluster possess highly homogenous characteristics.

Figure 4. Cont.



Figure 4. Biplots showing principal component analysis. (a) Biplot showing the two principal components and the loading vectors in a single display between litter treatments during the two years of the experiment. The principal component analysis (PCA) for measured parameters shows portioning and grouping of correlated variables. (b) Biplot showing the two principal components and the loading vectors between applied treatments (N & P). (c) Biplot showing the two principal components and the loading vectors in a single display between two forests.

4. Discussion

Forest litter, as an essential element of the ecosystem, expedites the formation and cycling of nutrients. Our results have depicted that forest litter changed the nutrient dynamics in the soil. Numerous studies have reported positive effects of organic amendments on soil health. Li et al. [18] showed that forest litter can control SOC in addition to influencing transport of nutrients. Likewise, Peng et al. [19] discovered that litter application and its subsequent decomposition is among the imperative causes of SOC, thus justifying our results, as an increase in SOC was estimated in double litter treatments. Our results agree with the results of Wang et al. [20], who elaborated an enhancement in SOC content under litter application. However, there are studies which show no positive results on SOC quantities under forest litter application [21,22].

Our experiment revealed an elevated soil N in forest litter (double/altered) additional plots. Previously, it has been observed that STN and carbon showed a positive linear relationship with litter application [23], however Li [16] showed no effect on total soil nitrogen and phosphorus. Our results are also in line with Vincent et al. [7] who affirms a 23% and 36% increase in soil nitrogen and carbon, respectively, when litter was added into the soil. They also showed that soil organic phosphorus was decreased by 23% when litter was removed.

Our results clearly illustrate that forest litter and NP fertilizer effects were similar in both forest types, however the quantities of SOC, TN and TP differ. This is due to dissimilar plant communities in regard to natural and artificial plantations. Zhao et al. [24] and Deng et al. [25] stated that the concentration and distribution of C, N and P differ in plant communities because of litter characteristics and its chemical traits. Moreover, soil moisture, pH and texture also contribute to N, P and SOC concentration and prevalence in soil [26,27]. Burton et al. [28] asserted that naturally regenerated forests are always superior regarding nutrient cycling and soil quality.

Jourgholami et al. [29] affirmed that leaf litter (1.31 kg m⁻²) altered and boosted soil quality. Others stated that soil physical and chemical properties can recover if forest litter is retained on forest floor [30], however, they found that C:N ratio was higher when organic manure was applied. This is contrary to our results as C:N ratio decreased under litter treatment in our experiment. Studies affirm that litter on soil surface determines C storage $(3.70-4.36 \text{ Mg ha}^{-1})$ more so than the litter incorporation treatments $(2.89-3.33 \text{ Mg ha}^{-1})$ [31]. Soil P is greatly affected by litter amounts. Similar to our results, Schreeg et al. [8] stated that an increased P concentration was found in soil solution experiments conducted in tropical forests. Litter manipulation (addition) increased TOC in the uppermost soil layer in mineral soils [6]. However, certain studies are also present which are not consistent with our results. For instance, Sayer [9] and Hoosebeek and Scarascia-Mugnozza [32] elaborated that in temperate forests, soil carbon did not differ with litter alterations. The positive influence of fertilizer (organic and synthetic) is defensible because litter addition tends to augment soil microbial respiration since litter acts as easily decomposable substrate for the microbes consequently liberating nutrients into the soil [33–35]. Another factor which helps in nutrient mineralization is the presence of fine roots. Studies are present that state that roots play a significant role in P mineralization in comparison to aboveground litter [36,37]. Koranda et al. [38] detailed in their study that beech roots swiftly caused P mineralization. However, in our experiment, we lack information regarding root biomass, hence, any conclusion made on this factor is not satisfactory. Yet, there are reports of Spohn and Kuzyakov [39], Spohn et al. [40] and Tang et al. [41] which highlight that P mineralization transpires through soil microorganisms, and root exudates work by stimulating microorganism activity thus resulting in release of P. As for NP fertilizer addition, like our results, it has been previously confirmed that added N can help in C storage in temperate forests [42,43]. However, Harrington et al. [44] and Ostertag [45] revealed that soil N did not increase in N-rich tropical forest when external N fertilizer was applied. These facts affirm that fertilizer application is only responsive in cases where soil is already deprived of nutrients and the buildup only occurs up to a certain limit. The increase in TN and TOC is attributed to both biotic as well as abiotic factors. N fertilizers affect C storage as it governs litter decomposition, C loss is via respiration and C stabilization [46–49]. Nitrogen fertilizer stimulates microbial activity and litter decomposition [50,51]. Nitrogen application stimulates C deposition through respiration [49,52]. Moreover, Kalbitz et al. [34] and McDowell et al. [53] affirm increased production and transportation of OC, similar to our results.

The addition of fertilizers enhances the SOC, STP and STN in our study and mostly the maximum quantities were identified in medium treated plots. Previous research has also recommended that an adequate number of fertilizers is beneficial for the soil health, however, excessive P addition can result in P fixation which can further deteriorate soil health. Our results are in agreement with Ma et al. [54] and Diez et al. [55] who suggested well-managed and optimum amounts of fertilizers for minimum soil environment impacts. The variability that occurred in soil nutrient amount is justified because litter acts as substrate for microorganisms and external fertilizer application helps decomposers to ignite their mechanisms. More substrate availability means more mineral N production and if C:N ratio is less then more mineral consumption will occur [3]. The nutrient cycling actually takes place with the action of soil enzymes involved in C, N and P cycling. The N addition was involved in increased activity of BG enzymes which enhanced C cycling while

decreased NAG and LAP activity were responsible for N cycling. Previously, Li et al. [56], Shi et al. [57] and Xiao et al. [58] have reported that exogenous fertilizer can influence extracellular enzyme activities which in turn alter soil N, P and C cycles. They attributed their results to resource allocation theory of enzyme production, i.e., N addition impedes the activity of N-cycling enzymes and increases the activity of other enzymes [59]. However, N addition had no significant effect on soil ACP enzyme activity, which is inconsistent with other studies showing that N addition enhances soil phosphatase activity [60–62]. The periodic application of NP fertilizer also impacted soil chemical properties. As in our case, the changes were observed during each sampling and at the end of the experiment the maximum change was observed. The same was reported previously that long term fertilizer application impacted alteration in nutrients and chemical properties due to buildup of exchangeable ions which further impact soil pH and P fractionation [63]. Ahmed et al., [64] stated that under periodic or continuous NP application, the P accumulation in soil increases. However, in our study the STP was found to be less in the sixth sample in comparison to the first. Similarly, SOC was reduced at the end of the experiment, i.e., at the sixth soil sampling as compared to the first. However, there are studies which report that periodic application of N and P had no significant impact on OC [65]. The authors further agreed with a study carried out in calcareous soils in China which showed that varying fertilizer quantities did not impact SOC [66]. However, they attributed the small difference because organic carbon change always occurred in minute quantities which are not detectable if soils have heterogenous fertility status as well as varying precipitation [67].

5. Conclusions

It was concluded that N fertilizer application along with P fertilizer significantly increased the total N and P contents in soil while litter treatment also plays a vital role to improve the soil organic carbon contents and to maintain nutrient balance in pool. Synthetic fertilizers affected soil total nitrogen and the highest quantity was determined in plots with H: $30 \text{ g N m}^{-2} \text{ a}^{-1} + 20 \text{ g P m}^{-2} \text{ a}^{-1}$ under an altered litter treatment combination (AL). The soil organic carbon showed positive correlation with soil total nitrogen and soil total phosphorus while total soil nitrogen showed a strong negative correlation with carbon to nitrogen ratio. This study is important for understanding the complex mechanisms which occur during the decomposition process, and our knowledge regarding natural and anthropogenic factors governing nutrient cycles in forest soils. Moreover, the literature lacks studies relevant to temperate forests, their sustenance abilities and the factors which impact the soil nutrient profile buildup, hence well-structured studies need to be carried out on a regular basis for fulfilling the research gaps regarding temperate forests.

Author Contributions: Conceptualization, A.H., K.A. and W.D.; methodology, A.H.; software, K.A.; validation, A.H., M.A.J. and W.D.; formal analysis, K.A.; investigation, A.H.; resources, M.A.J.; data curation, A.H.; writing—original draft preparation, A.H.; writing—review and editing, A.H., W.D. and K.A.; visualization, C.L.; supervision, W.D.; project administration, L.C.; funding acquisition, W.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Fundamental Research Funds for the Central Universities (2572021DT04) and the National Natural Science Foundation of China (31770656, 31670627).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

Acknowledgments: We are highly grateful to our lab mates for their support and help in the fieldwork. We are also indebted to Muhammad Rizwan and Umair Riaz for their statistical advice and valuable suggestions and their appreciation and support.

Conflicts of Interest: The authors declare no conflict of interest.

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