Effects of Adding Native Annual Seeds to South Korea Native Perennial Seed Mixture on Early Stage Vegetation Recovery, Soil Enzymes, and Nutrient Dynamics in Post-Fire Soils

Hyun-Gi Min¹, June Wee¹, Namin Koo² and Jeong-Gyu Kim³,*

¹ OJEong Resilience Institute, Korea University, Seoul 02841, Republic of Korea; daiamin@korea.ac.kr (H.-G.M.); dnlwns@korea.ac.kr (J.W.)
² Division of Forest Ecology, National Institute of Forest Science, Seoul 55365, Republic of Korea; kooosor@gmail.com
³ Division of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea
* Correspondence: lemonkim@korea.ac.kr; Tel.: +82-02-3920-3502

Abstract: Forests are degraded from various factors, and the first step in restoration frequently involves revegetation. One of the degradations is wildfires, which damage vegetation, affect soils, and lead to the loss of ecosystem functions. Using seed mixtures is a viable method for restoring the ecosystems. This research investigated the impacts of six perennial plant seed mixtures derived from native plants in South Korea and the addition of two types of annual plant seeds to these mixtures, both separately and combined. Cultivation of the seed mixtures was conducted by pot cultivation in a greenhouse for the early stage of vegetation (16 weeks). The seed mixture treatment enhanced plant species diversity, number, and biomass. The seed mixture treatment elevated the urease activity from 14.42 to values between 33.88 and 55.74 μg NH₄-N g⁻¹ 2 h⁻¹. A seed mixture integrated with two annual plants heightened the phosphomonoesterase activity from 482.79 to 543.75 μg p-nitrophenol g⁻¹ h⁻¹. Nitrogen leaching was reduced across all seed mixture treatments, while phosphorus leaching diminished with the addition of the annual legume. These findings illustrate the influence of seed mixture treatments and the inclusion of annual seeds on the beginning stage of revegetation, offering a basis for further ecosystem restoration.

Keywords: wildfire; seed mixture; nitrogen; phosphorus

1. Introduction

Forests and vegetation are impacted by various factors, including human activity and natural disasters [1,2]. Wildfires are a primary cause of forest destruction, leading to issues like landslides and nutrient runoff [3–5]. The subsequent changes in soil properties and removal of vegetation can cause topsoil loss and soil erosion [6]. Wildfires burn complexes of nutrients and organic matter, particularly nitrogen and phosphorus, transforming organic nutrients into their more mobile inorganic counterparts [7]. While the burning of aboveground vegetation does supply nutrients to the soil, they are often in a form more susceptible to runoff and leaching [8]. The excess nutrients that leach during wildfires can lead to eutrophication in adjacent water systems [9]. Wildfires influence nutrient cycling by first disrupting microbial communities through intense heat, then altering these communities during the recovery phase [10]. Ground vegetation destroyed by wildfire can be restored by soil seed banks, but this can take a long time and may result in the loss of some native plant species [11,12]. There is also a risk of the emergence of invasive species and loss of biodiversity in the early stages of restoration of destroyed vegetation [13]. Re-vegetation in deforested areas is designed to initially prevent runoff and soil loss [14], and
to restore ecosystems in the subsequent stages [15]. Seeding is a recognized restoration method, enabling rapid vegetation recovery over large areas, reducing soil erosion, and setting the stage for ecosystem restoration [16,17].

Typically, seeding employs seed mixtures, rather than individual plant species. These mixtures are often derived from native plants and are designed to be diverse to foster biodiverse and sustainable ecosystems [18–21]. Seed mixtures can be made up of a variety of plant species and proportions, and this composition can determine the diversity of the future vegetation, so it is important to select the right seed mix for the area to be restored [22]. It is especially important to have diversity in the functions that each plant can perform [22].

Such mixtures include both annuals and perennials. When the goal is to prevent immediate topsoil loss, erosion, and nutrient leaching, annual plants prove more effective than perennial plants. However, they have potential to inhibit native plant growth and negatively impact long-term ecosystem recovery [23,24]. Perennials, being more persistent than annuals, support sustainable vegetation and ecosystems. But their slower growth can be a drawback during initial establishment [25]. Research on vegetation restoration through seed mixtures typically focuses on perennials [25], but there are a number of studies that include annuals in the mixture [26–28]. However, in long-term studies, annuals often remain in small numbers compared to perennials, so they are considered to play a secondary role in the first year when perennials are not growing enough [20,26]. The growth of annuals in the early years can be pioneer for soil development and set the stage for subsequent perennial growth [29], but the impact of annual plants on soil in the early stage has mostly been studied in annuals alone, and less in seed mixtures that include perennials [30,31].

In this study, we investigated the impact of including annual plants in the seed mixture on soil collected from a wildfire-affected area in South Korea. Utilizing native seed mixtures in pots, we observed changes in soil enzyme activity and nutrients over a 16-week cultivation period. From the “Supply List of Native Plants for Forest Restoration” published by the Korea Forest Service, which includes 74 woody plants and 20 herbaceous plants deemed fit for forest restoration, we selected six of the 20 commercially available herbaceous species for our seed mixture. Since all 20 herbaceous species listed by the Forest Service are perennials, we also considered the effects of adding the annual legume Chamaecrista nomame and the annual non-legume forb Callistephus chinensis. Both these annuals are native species known to provide rapid cover in the early stages of revegetation [32,33]. Our research focused on how seed mixtures, comprising perennial native plants combined with annuals, affect revegetation and nutrient leaching in post-fire soils during the early growth phase (16 weeks). The plant growth and seeding effects observed in this study offer insights into how seeding can modify soils impacted by fire during the initial stages of revegetation.

2. Materials and Methods

2.1. Experimental Design

In June 2023, post-fire soil was collected from the wildfire that occurred in March 2023 to a depth of 20 cm from Deokgu-ri, Buk-myeon, Uljin-gun, Gyeongsangbuk-do, Republic of Korea in East Asia.

The 20-cm-diameter pots had drainage holes at the bottom and were filled with washed sand to a depth of 10 cm from the bottom. The post-fire soil was filled up to a 20 cm from the top of the sand. The drainage hole was connected by a pipe to a sterile collection bottle to collect the leachate. With control, four treatments were seeded with different seed mixtures and each treatment consisted of four pots. The treatments named Control, Seed mix A, Seed mix B, Seed mix C, and Seed mix D (Table 1). The pots were watered by regulated spraying once a week for 12 h to simulate 50 mm of rain. Leachate from the
drainage was collected every 4 weeks. The pots were grown for 16 weeks in greenhouse, and the aboveground of the plant and 10 cm of the topsoil were sampled.

<table>
<thead>
<tr>
<th>Name of Treatment</th>
<th><em>Isodon ineficus</em></th>
<th><em>Lepechinia cuneata</em></th>
<th><em>Aster yomena</em></th>
<th><em>Dendranthema boreale</em></th>
<th><em>Chrysanthemum zuanokkii</em></th>
<th><em>Cirsium japonicum</em></th>
<th><em>Gallistephus chinensis</em></th>
<th><em>Chamaecrista romane</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seed mix A</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seed mix B</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Seed mix C</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Seed mix D</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

2.2. Plant Analysis

The aboveground plant parts were counted per species before harvest. After harvesting, the plants were homogenized and dried at 60 °C for 48 h and weighed. Plant total nitrogen was analyzed using Kjeldahl digestion according to the Nelson and Sommers method [34]. Dried plants were digested in concentrated H2SO4 with a salt-catalyst mixture, alkaliized with NaOH, and the distilled solution was titrated. Total plant phosphorus was analyzed using nitrogen digestion and ICP-MS (Agilent, Santa Clara, CA, USA) [35]. Plant diversity was calculated by Shannon’s diversity index (\( H' = -\sum pi \ln pi \)) [36].

2.3. Soil Analysis

The sampled soil was sieved through a 2 mm sieve and dried. Soil pH was analyzed using a pH meter (Orion Star A214, Thermo Scientific, Waltham, MA, USA) after mixing deionized water and soil at a 5:1 ratio for 1 h. The soil clay content was determined using the pipette method [37]. Soil organic matter was analyzed by loss on ignition at 400 °C for 16 h [38].

The soil total nitrogen content was analyzed using Kjeldahl digestion [39]. NH4+-N and NO3−-N were analyzed using the distillation method [40]. NH4+-N was extracted with 2M KCl, and the extract was made alkaline with MgO and distilled water. After NH4+-N distillation, the soil sample was again distilled with Devarda’s alloy to extract NO3−-N. All distilled solutions were titrated to analyze the nitrogen concentration. Soil total phosphorus was extracted using aqua regia and analyzed using ICP-MS (Agilent, Santa Clara, CA, USA) [41]. Mehlich-3 phosphorus was analyzed as plant-available phosphorus [42].

Urea activity was analyzed using the method described by Kandeler and Gerver [43]. Soil was extracted from the urea solution and incubated at 37 °C for 2 h. The reaction was stopped by adding KCl and HCl, and after 30 min of stirring, the chromogenic reagent was added and measured at a wavelength of 600 nm after 30 min of incubation. Phosphomonoesterase activity was analyzed using Tabatabai and Bremner’s method [44]. The modified universal buffer and P-nitrophenyl phosphate solution were adjusted to pH 6.5 and 11, respectively, to extract the soil. It was then incubated at 37 °C for 1 h, stirred with CaCl2 and NaOH, and measured at a wavelength of 400 nm.

Ecoplates (Biolog, Hayward, CA, USA) were used to analyze soil microbial metabolic activity [45]. Five g soil and 45 mL of 0.85% sterile saline solution (W/V NaCl) were shaken for 30 min and diluted 100-fold with 0.85% sterile saline solution. The diluted solution
was inoculated into each EcoPlate well. The plates were incubated at 25 °C in the dark. The absorbance was read with a multi-detection microplate reader (Hidex, Turku, Finland) every 24 h for 168 h. Average well color development (AWCD) was calculated with the following equation: AWCD = \( -\sum(C - R)/31 \), where C is OD in each carbon source well and R is OD of the control.

2.4. Nutrient Leaching

The nitrogen and phosphorus contents of the filtered leachate were analyzed. Total nitrogen was analyzed using the Kjeldahl method [46], and phosphorus was analyzed using ICP-MS (Agilent, Santa Clara, CA, USA).

2.5. Statistical Analysis

All analyses were performed for four replicates. Significant differences were determined via Duncan’s test, and a mean \( p < 0.05 \) indicated statistical significance. Nutrient leach and EcoPlate results were statistically analyzed between results from the same time period. All data were analyzed using statistical analysis software (SAS 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Plant Analysis

3.1.1. Plant Species

The plant species that grew according to the seed mixture seeding are listed in Table 2. The total number of Control was 1.2, and in the Seed mix A, B, C, and D they were 11.7, 13.4, 18.6, and 19.5, respectively. *Isodon inflexus* was observed in the pots at week eight but was not present at the end of the experiment. *Cyperus microiria*, *Portulaca oleracea*, and *Phytolacca acinose* were grown without seeding. The plant species diversity is shown in Figure 1. Seed mix B and C had higher species diversity indices than the Control and Seed mix A; the species diversity index of the control was 0.21, and those of Seed mix A, B, C, and D were 0.79, 1.18, 1.04, and 1.29, respectively. All Seed mixture treatments had significantly higher values than the control. Seed mix B and D had significantly higher values than Seed mix A.

Table 2. Mean number of individuals per pot by species.

<table>
<thead>
<tr>
<th>Seed Mixture Type</th>
<th><em>Isodon inflexus</em></th>
<th><em>Leptadenia zonarea</em></th>
<th><em>Aster yomena</em></th>
<th><em>Dodonaea borrascoides</em></th>
<th><em>Chrysanthemum zawadskii</em></th>
<th><em>Cirsium japonicum</em></th>
<th><em>Callistephus chinensis</em></th>
<th><em>Chamaecrista biflora</em></th>
<th><em>Cyperus microiria</em></th>
<th><em>Phytolacca oleracea</em></th>
<th><em>Phytolacca acinosa</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td>Seed mix A</td>
<td>0</td>
<td>2.8</td>
<td>7.8</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.7</td>
</tr>
<tr>
<td>Seed mix B</td>
<td>0</td>
<td>1.1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
<td>5.8</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>13.4</td>
</tr>
<tr>
<td>Seed mix C</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>18.6</td>
</tr>
<tr>
<td>Seed mix D</td>
<td>0</td>
<td>1</td>
<td>2.8</td>
<td>0.8</td>
<td>0</td>
<td>0.5</td>
<td>5.8</td>
<td>8</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>19.5</td>
</tr>
</tbody>
</table>
Figure 1. Shannon diversity index ($H'$) of seed mixture seeded pots. Different letters indicate significant differences at the 5% level determined via Duncan’s test.

3.1.2. Biomass and N, P Accumulation of Plants

The dry weights of the plants and the amounts of N and P are described in Figure 2. All seed mixture treatments resulted in an increase in aboveground plant weight compared to the control (Figure 2a). The plant weight in the Control was 3.91 g and 11.84–14.76 g in the seed mixture treatments. There were no significant differences in the concentrations of nitrogen and phosphorus within the plants, but the total amount of nitrogen was higher in all treatments than in the control due to differences in biomass (Figure 2b).

Figure 2. Biomass and N, P accumulation of plants. (a) is dry weight of plants in each pot; (b) is the amount of plant nitrogen and phosphorus in each pot. Different letters in same color bar indicate significant differences at the 5% level, determined via Duncan’s test.
3.2. Soil analysis

3.2.1. Soil Physico-Chemical Properties including Nutrients

Soil N, P, and other physicochemical properties are listed in Table 3. Soil pH in the Control was 6.48, and Seed mix A, C, and D had lower pH values than the Control. The soil pH of Seed mix C was 6.27, which was the lowest. The clay content of Control soil was 11.2%. The range of total nitrogen content was 3766–4025 mg kg⁻¹, NH₄⁺-N was 148.58–182.70 mg kg⁻¹, and NO₃⁻-N was 15.93–26.78 mg kg⁻¹. The concentration of total P in the Control was 466.48 mg kg⁻¹ and that of Seed mix C and D were 575.09 and 573.63 mg kg⁻¹, respectively. The total P concentration in Seed mix C and D was significantly higher than that of the Control. Mehlich-3 phosphorus did not differ significantly between treatments.

Table 3. Soil physico-chemical properties *.

<table>
<thead>
<tr>
<th>Seed Mixture Type</th>
<th>pH</th>
<th>OM (%)</th>
<th>Clay (%)</th>
<th>TN (mg kg⁻¹)</th>
<th>NH₄⁺-N (mg kg⁻¹)</th>
<th>NO₃⁻-N (mg kg⁻¹)</th>
<th>TP (mg kg⁻¹)</th>
<th>M3-P (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.48 aa</td>
<td>11.2 a</td>
<td>5.8 ba</td>
<td>3969 a</td>
<td>182.70 a</td>
<td>26.78 a</td>
<td>466.48 aa</td>
<td>99.76 a</td>
</tr>
<tr>
<td>Seed mix A</td>
<td>6.37 ba</td>
<td>12.6 a</td>
<td>6.3 ab</td>
<td>3766 a</td>
<td>168.53 a</td>
<td>15.93 a</td>
<td>485.93 ab</td>
<td>100.59 a</td>
</tr>
<tr>
<td>Seed mix B</td>
<td>6.39 ab</td>
<td>12.1 a</td>
<td>5.7 ba</td>
<td>3801 a</td>
<td>162.23 a</td>
<td>21.70 a</td>
<td>492.51 ab</td>
<td>100.15 a</td>
</tr>
<tr>
<td>Seed mix C</td>
<td>6.27 ca</td>
<td>11.6 a</td>
<td>6.6 aa</td>
<td>4025 a</td>
<td>148.58 a</td>
<td>24.15 a</td>
<td>575.09 ba</td>
<td>104.76 a</td>
</tr>
<tr>
<td>Seed mix D</td>
<td>6.30 bc</td>
<td>11.1 a</td>
<td>5.9 ba</td>
<td>3962 a</td>
<td>159.08 a</td>
<td>20.83 a</td>
<td>573.63 ba</td>
<td>105.06 a</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences at the 5% level determined via Duncan’s test. ¹ Soil pH was extracted via 1:5 D.I. water extraction. ² OM is organic matter analyzed with loss on ignition. ³ TN is total nitrogen analyzed with Kjeldahl extraction. ⁴ TP is total phosphorus. ⁵ M3-P is phosphorus extracted by Mehlich-3 method.

3.2.2. Soil Enzyme Activity

Soil enzyme activities are described in Figure 3. The urease activity of Control soil was 14.42 µg NH₄⁺-N g⁻¹ 2 h⁻¹ and that of Seed mix A, B, C, and D soil were 33.88, 44.72, 40.59, and 55.74 µg NH₄⁺-N g⁻¹ 2 h⁻¹, respectively. All seed mixture-treated soils had significantly higher urease activity than the Control. In particular, Seed mix D had the highest urease activity, which was significantly higher than that of Seed mix A and C. Phosphomonoesterase activity under acidic conditions did not show significant differences. Phosphomonoesterase activity in alkali of Control soil was 482.79 µg p-nitrophenol g⁻¹ h⁻¹ and in Seed mix A, B, C, and D soil, it was 531.05, 505.31, 523.21, and 543.75 µg p-nitrophenol g⁻¹ h⁻¹, respectively. Only Seed mix D showed a significantly higher phosphomonoesterase activity than the Control. The AWCD with Ecoplates showed significant differences at 24, 48, and 96 h. The AWCD values in the Control and Seed mix A, B, C, and D at 24 h were 0.12, 0.17, 0.19, 0.23, and 0.23, respectively and those at 48 h were 0.56, 0.71, 0.70, 0.74, and 0.76, respectively. That of 96 h was 1.12, 1.19, 1.23, 1.24, and 1.37, respectively. At 24 h and 48 h, the AWCD of Seed mix C and D was significantly higher than that of the Control. At 96 h, only the AWCD of Seed mix D was significantly higher than that of Control.
Forest 2023, 14, 2281

Figure 3. Soil enzyme activity and AWCD (average well color development) analyzed by Ecoplate: (a) is activity of urease and phosphomonoesterase; (b) is AWCD analyzed by Ecoplate. Different letters in the same color bar indicate significant differences at the 5% level, determined via Duncan’s test.

3.3. N, P Leaching

Nutrient leaching is described in Figure 4. The leached nitrogen in the Control and Seed mix A, B, C, and D at week four were 97.99, 87.21, 63.49, 68.46, and 66.12 mg pot⁻¹, respectively and week eight were 35.65, 6.24, 4.04, and 4.19 mg pot⁻¹, respectively. The values at week 12 were 7.07, 2.59, 2.21, and 3.15 mg pot⁻¹, respectively, and at week 16 they were 12.34, 6.30, 8.53, 9.93, and 12.32 mg pot⁻¹, respectively. There was a significant difference in leached nitrogen compared with the Control in all experimental periods, except for week zero, which was the start of the experiment. At week four, nitrogen leaching from the Control was significantly higher than that from Seed mix B, C, and D but not significantly different from Seed mix A. At weeks 8 and 12, nitrogen leaching from the Control was significantly higher than from Seed mix A, B, C, and D. At week 16, only Seed mix A showed significantly lower nitrogen leaching than the Control; the other treatments were not significantly different from the Control. There was a significant difference in leached phosphorus compared with the Control at weeks 8, 12, and 16. The leached phosphorus in the Control and Seed mix A, B, C, and D at week 8 were 0.23, 0.28, 0.14, 0.13, and 0.13 mg pot⁻¹, respectively. At week 12 they were 0.18, 0.13, 0.05, 0.06, and 0.05 mg pot⁻¹, respectively. At week 16 they were 0.20, 0.08, 0.04, 0.04, and 0.06 mg pot⁻¹, respectively. At week 8, Seed mix C and D were significantly lower than the Control, and at weeks 12 and 16, Seed mix B, C, and D were significantly lower than the Control.

Figure 4. Nitrogen and phosphorus leaching: (a) is nitrogen leaching for 16 weeks; (b) is phosphorus leaching for 16 weeks.
4. Discussion

Three species of plants grew that were not included in the seed mixture, and these were assumed to be plants included in the soil seed bank (Table 2). Every seed mixture increased plant diversity and the number of plants (Table 2 and Figure 1). Fewer plants without seed mixture treatment might be attributed to the loss of the soil seed bank from wildfires [47]. In areas with a rich soil seed bank, artificial revegetation can actually hinder the establishment of on-site plants [48]. A reduced number of plants emerging from a soil seed bank suggests that the soil was suitable for restoration with seedling [48]. Although the collected soil was close to the topsoil, they were homogenized during the pot-making process, possibly decreasing vegetation from the soil seed bank. Incorporating annual plants in the seed mixtures increased both plant number and diversity (Table 2 and Figure 1). The impact of annuals might be overemphasized since this study focused on the initial 16-week period when annuals typically grow more robustly than perennials. Notably, the number of perennials decreased when annuals were included in the seed mixtures (Table 2). Annual plants have a low rate of regrowth from seeds the following year, whereas perennials are more sustainable because they grow over multiple years and also reproduce by rhizomes [32]. As perennials are more sustainable than annuals for vegetation restoration [16], introducing annuals could potentially impact long-term vegetation sustainability. However, the growth of native annuals can also suppress invasive annuals and help to establish vegetation [25]. The addition of annual plant seeds enhanced the biodiversity of the initial vegetation. However, further studies need to identify the optimal ratio and sowing methods for seeds that minimize the inhibition of perennial plant growth by annuals, considering the later stages of regeneration.

The seed mixture acidified the treated soil, with the greatest acidification occurring in the pots where the highest numbers of *Chamaecrista nomame* were grown (Tables 2 and 3). Acidic root exudates can acidify the rhizosphere [49]. Increased plant number and biomass can cause the soil acidification (Table 2 and Figure 2a).

Urease activity changes with the treatment were similar with the plant diversity index (Figures 1 and 3a). Alkaline phosphomonoesterase activity and plant diversity index were both highest in pots with two types of annual plants in the treatment (Figures 1 and 3a). In the rhizosphere, plant roots significantly affect soil’s microbial and enzyme activities, releasing enzymes, exudates, and forming symbiotic relationships with microorganisms [50]. An uptick in enzyme activity is often associated with increased plant diversity and symbiotic relationships [51]. Both phosphorus and nitrogen are essential for plants and microbes; moreover, enzymes like urease and phosphomonoesterase are also impacted by plant diversity [52–54]. In this study, the urease activity rose with the inclusion of a seed mixture, and the addition of annual plants increased this value further (Figure 3a). However, treatment with a seed mixture containing the annual legume *Chamaecrista nomame* did not have a greater effect on urease activity than treatment with a seed mixture containing *Callistephus chinensis* (Figure 3a). This contrasts with past studies indicating legume nitrogen fixation’s impact on urease activity [55,56]. High nitrogen might inhibit nitrogen fixation [57]. Increased inorganic nitrogen from wildfires could be a reason why legume species didn’t significantly influence urease activity.

AWCD, an indicator of microbial metabolic activity in the soil, was highest in the two treatments where the highest numbers of *Chamaecrista nomame* grew (Table 2 and Figure 3b). The urease and phosphomonoesterase levels increased with the addition of annual plant seeds, but individual species didn’t make a difference, while AWCD was influenced more by *Chamaecrista nomame* than *Callistephus chinensis* (Figure 3). Legume rhizospheres elevate microbial activity and improve soil structure [58,59]. Specifically, rapid growth of annual legumes can stimulate the nitrogen cycle and foster microbial communities [60]. Reintroducing microorganisms lost to wildfires assists vegetation establishment [16]. Enhanced microbial community also makes the soil structure better [61–63], aiding in long-term ecosystem restoration. Increasing the activity of soil enzymes and microbial activity in the early stages of re-vegetation may provide the basis for extended ecosystem restoration.
Using seed mixtures and increasing vegetation lowered nitrogen and phosphorus leaching (Table 2 and Figure 4). Nitrogen leaching with seed mixture treatments having annual plants decreased early but not later, while mixtures without annuals saw a later decreasing (Figure 4a). Soil nitrogen levels remained consistent between treatments (Table 3). Nitrogen amounts in plants in each pot increased with seed mixture treatment (Table 3). Plant roots are pivotal in soil nutrient cycling [64]. Nitrogen uptake by plants is typically faster than other nutrients, hence leaching often reduced for nitrogen more than phosphorus [65,66]. The wildfire might have led to nitrogen being leached from the Control and taken up by plants in the treated pots. Different seed mixtures’ leaching timings could be because of varied initial growth rates of annuals and perennials [30,67].

Phosphorus leaching was reduced in pots grown with annual plants, especially in pots grown with the annual legume *Chamaecrista nomame* (Table 2 and Figure 4b). Phosphorus leaching can increase with root growth because of increased drainage and root exudate mobility [68–70]. However, post-wildfire, large phosphorus amounts are often leached, which vegetation can prevent [71,72]. The phosphorus amount in plants and soil Mehlich-3 didn’t alter with the seed mixture treatment (Figure 2b and Table 3), but total soil phosphorus increased with annual legume treatment (Table 3). The treatments with the least phosphorus leaching throughout were Seed mix C and D with annual legumes. Those treatments also had higher AWCD values. Microbes absorbing phosphorus reduce its leaching [73]. Enzyme activity can hint at a microbial community’s presence [74,75]. While phosphomonoesterase releases phosphorus, alkaline phosphomonoesterase, produced by microbes, can indicate microbial phosphorus intake [75]. The rise in microbial activity due to plant interaction could decrease phosphorus leaching while increasing soil phosphorus concentration.

In this study, we observed that introducing seed mixtures to post-wildfire soils boosts enzyme and microbial activity, lessens nutrient leaching in early plant growth stages, and annual legumes amplify these effects. These preliminary changes are foundational for subsequent ecosystem restoration, offering insights into early restoration phases for wildfire-impacted soils.

5. Conclusions

This study assessed the impact of seed mixtures over an initial 16-week period on soil enzyme activity and nutrient dynamics (including leaching, soil concentration, and plant uptake) in post-fire soil. The seed mixtures comprised six perennial native species in South Korea, with an examination of the added effects of two native annual species. Seeding with seed mixtures increased plant diversity and urease activity and reduced nitrogen leaching, regardless of the type of seed mixture. The inclusion of annual plants in perennial mixtures increased the phosphomonoesterase activity and AWDC of the ecolate and decreased phosphorus leaching; these effects were greater in seed mixtures containing annual legumes, *Chamaecrista nomame*. Increased plant diversity, soil microbial activity, and reduced nutrient losses are the foundation for future plant growth and ecosystem health. The results of the present study showed that in seed mixture-based vegetation restoration, the growth of early annual plants, especially the growth of annual legumes, improves the soil environment, supporting the growth of future vegetation.

**Author Contributions:** Material preparation, H.-G.M. and N.K.; data analysis, H.-G.M. and J.W.; writing—review, H.-G.M. and J.-G.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2021R1A6A1A10045235) and partly granted by National Institute of Forest Science (Management Number: 22-00-51) and Korea university (k230031).

**Data Availability Statement:** Data are contained within the article.
Conflicts of Interest: The authors declare no conflict of interest.

References


Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.