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Impact of Three Chainsaw Lubricants on Forest Soil Bacterial Community, Soil Respiration and Seedling Growth

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Abstract: Lubricants are applied onto chainsaw blades to achieve their optimum cutting performance; however, during logging or timber operations, lubricants may penetrate the forest soil. The persistent organic pollutants in lubricants may cause environmental damage, and different types of lubricants vary in terms of their environmental impact. Hence, selecting appropriate lubricants for timber operations is important for sustainable forest management. In this study, the effects of three lubricant types—biodegradable oil (bio-oil), petroleum-based bar-and-chain oil (mineral oil), and petroleum-based recycled oil (recycled oil)—on soil health were evaluated. The study was conducted in a controlled nursery setting, simulating post-logging reforestation. Sixteen types of polycyclic aromatic hydrocarbons and the total petroleum hydrocarbon concentrations in the soil samples were analyzed. Bio-oil facilitated faster recovery from soil contamination, whereas mineral oil exhibited slow and incomplete recovery. Recycled lubricants appear to be more environmentally sustainable options, indicating lower long-term soil contamination risks than petroleum-based lubricants. From a productivity perspective, the lubricant that supported the growth of seedlings was bio-oil. The findings of our study contribute to responsible lubricant selection for enhancing the overall health and sustainability of forest ecosystems.

Keywords: forest operation; biodegradable lubricant; petroleum-based lubricant; recycled lubricant; polycyclic aromatic hydrocarbon (PAH); total petroleum hydrocarbon (TPH)

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1. Introduction

While felling machines such as feller bunchers and harvesters are widely used in timber-harvesting operations, chainsaws are still commonly used on steep terrain. Lubricants are applied on chainsaw blades to prevent them from wearing out. In the process, however, lubricants may unintentionally penetrate the soil. Annually, more than 100 million L of lubricant that have been applied on the surfaces of harvesters and chainsaws is released into the environment during timber-harvesting operations [1].

Lubricants are petroleum-based products that contain various organic pollutants, primarily polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs). The contaminants in the soil can be converted into new toxic pollutants as they are being utilized by living organisms; therefore, certain contaminants may pose adverse effects on living organisms even when their concentrations are extremely low and almost negligible [2]. In particular, TPHs and PAHs induce various effects on living organisms, including basal and substrate-induced respiratory activities of soil microbial complexes, nitrogen fixation and denitrification activities, and toxic reactions in vegetation [2].

PAH dissipation in the soil is largely influenced by microbial activity [3]. The efficiency of the microbial degradation of PAHs and TPH depends on the soil environmental

conditions [4,5]. Certain microorganisms can effectively degrade aromatic compounds [6,7]. Thus, the bacterial communities in petroleum-contaminated soils change due to the increased or decreased activities of certain microorganisms [5,8].

Petroleum pollutants can negatively affect the soil bulk density, porosity, and nutrient availability of plants [9]. Oil pollution can also affect vegetation growth by increasing the organic matter content and electrical conductivity of the soil and decreasing the available nitrogen and phosphorus [10]. In addition, previous studies have shown that bioremediation can enhance the genotoxicity and developmental toxicity of pollutants [11–13]. The presence of contaminants and the increased toxicity of degraded contaminants affect plant growth [14]. The responses of plants to lubricant contamination depend on the type of lubricant [15].

Soil surface CO_2 efflux associated with soil respiration consists of heterotrophic soil respiration, which occurs when organic matter is decomposed by soil microorganisms, and autotrophic soil respiration, which occurs when belowground plant roots respire [16]. The proportions of autotrophic and heterotrophic soil respirations vary depending on the forest environment, weather, and climatic conditions [17,18]. Soil respiration changes when the soil becomes contaminated, reflecting the changes in the activities of soil microbes and responses of vegetation. Soil respiration increases as bacterial metabolism increases during pollutant biodegradation [19]. Moreover, the responses of different plant species in contaminated soils vary, resulting in different soil respiration rates [20,21]. Changes in soil respiration contribute to the disturbance of the global carbon cycle.

Although several studies have been published on biodegradation technologies that have been developed for petroleum product spills into the soil, studies on the effect of lubricant spills on forestry operations are scarce. The aim of this study was to evaluate the physical, biological, and organic changes in soil contaminated by the three types of lubricants. The results of this study provide essential data for selecting chainsaw lubricants for sustainable forest management.

2. Materials and Methods

2.1. Site Description and Experimental Design

The research site was a nursery field in the experimental forest of the Kangwon National University (37°46′46″ N, 127°49′42″ E; 514 m elevation) in Gangwon Province, Korea. The climate at the study site is cold, dry winter, or hot summer (Köppen Classification), with a mean annual air temperature of 11.5 °C (monthly means ranged from −9.5 °C to 27.4 °C) and mean annual precipitation of 1345.6 mm. Approximately 62% of the rainfall occurs during the summer season. During the research period (from 23 November 2021 to 28 July 2023), the mean annual temperature recorded at the meteorological observatory of Kangwon National University Experimental Forest was 8.7 °C (monthly means ranged from −7.0 °C to 23.7 °C), and the total precipitation was 2432.7 mm during the entire research period).

The nursery field was constructed after timber harvesting. The total nursery area was approximately 0.8 ha. Research plots, consisting of three treatment plots and one control plot (CP), were constructed above the nursery floor at the research site (20 m × 21 m) (Figure 1). Each of the CP and treatment plots consisted of 4 subplots (3 × 3 m) (total of 16 subplots). The terrain was uncomplicated, but the longitudinal slope was about 6% in the research area (Figure 1). Therefore, we constructed subplots of each control and treatment longitudinally, assuming that the unintended behavior of the lubricant occurs longitudinally after chainsaw lubricant scattering treatment (CLST). In addition, to prevent percolation of the surface and subsurface drainage into the control and treatment plots, a 0.3 m deep trench was dug along the outside perimeter of each plot. Finally, each research plot was spaced horizontally at least 2 m apart.

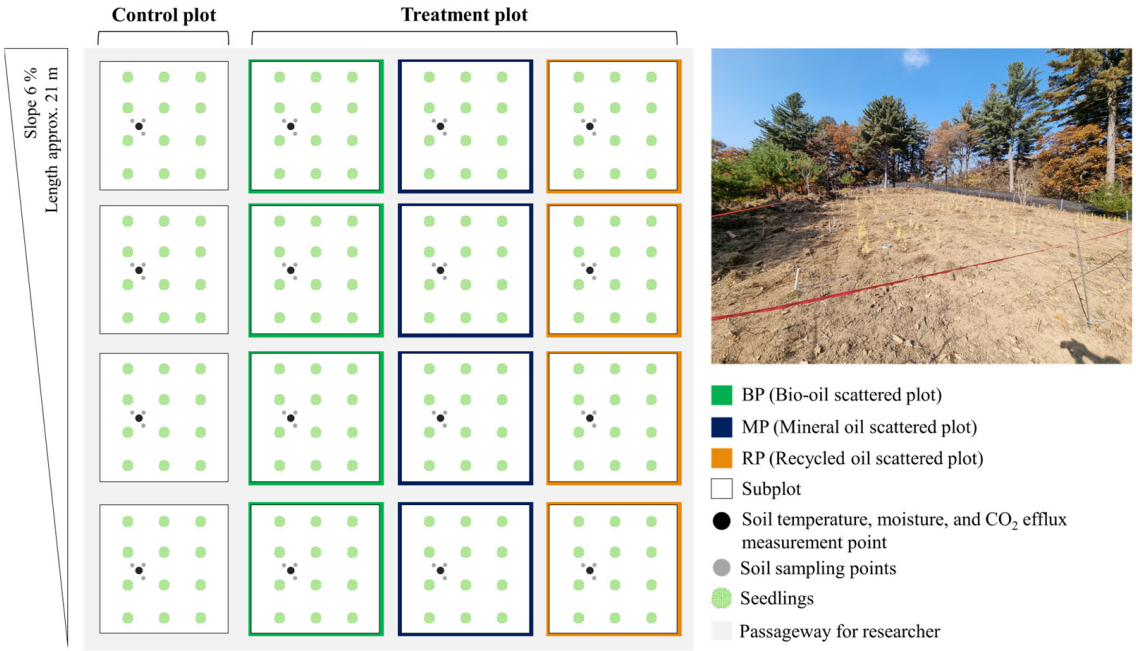

Figure 1. Experimental design to examine the effect of different chainsaw lubricants on forest soil contamination and seedling growth.

Twelve two-year-old *Larix kaempferi* (Lamb.) Carrière (Japanese larch) seedlings were planted in each subplot on 3 November 2021. On the same date, 1 PVC soil collar (210 mm diameter, 110 mm height; a total of 16 collars) for measuring the soil CO_2 efflux was installed in each subplot. To avoid measurement disturbance, the collar was inserted approximately 90 mm into the soil (the height of the above-ground exposure of the soil collar was 20 mm).

The consumption of chainsaw lubricants depends on the tree species and volume, operational environment, and type of chainsaw. For instance, Popovici [22] reported that the amount of chainsaw lubricant consumed during tree felling and landing processing in two birch-thinning stands (mean diameter at breast height was 18 and 15 cm in the two areas) was 177 mL/m^3 (m^3 represents the volume of commercial wood). Nowak et al. [1] reported that a chainsaw requires 50 mL of lubricating oil to cut 1 m^3 of wood. Antonić [23] reported that approximately 80 mL/m^3 of lubricants is consumed during beech harvesting. Furthermore, Klamerus-Iwan [24] reported that 30 million m^3 of wood is harvested and approximately 6 million dm^3 of lubricants is used in Poland each year, which translates to a lubricant consumption of 200 mL/m^3 during wood harvesting. As previous studies have shown, estimates of the amount of lubricant scattered during timber harvesting are highly variable. There is also a lack of research on the distance over which lubricants are dispersed. We have empirically assumed that wood felling results in sawdust mainly scattering within a 2 m radius. Therefore, in this study, up to 200 mL of lubricant was sprayed over a 4 m^2 area when harvesting 1 m^3 of wood. As a result, 50 mL of lubricant per 1 m^2 plot was sprayed on each treatment plot to determine changes in the soil environment, simulating extreme soil pollution caused by chainsaw lubricants scattered during bush removal, wood felling, and wood processing. The same amount of three types of chainsaw lubricants (bio-oil, biodegradable oil; mineral oil, petroleum-based oil; recycled oil, recycled petroleum-based oil) was scattered (using a motor-operated spray on 16 November 2021 to the CLST) in each treatment plot (BP, bio-oil scattered plot; MP, mineral oil scattered plot; RP, recycled oil scattered plot). Bio-oil and mineral oil are expensive typical biodegradable bar-and-chain oils, while recycled oil is a low-cost typical bar-and-chain oil.

The dominant tree species in the stand around the nursery were *Pinus koraiensis* Siebold and Zucc. (Korean pines). Soil samples for physicochemical analysis were collected on

11 November 2021. Soil samples were taken twice, 100 g each, from each subplot (for a total of eight composites from four subplots), resulting in an 800 g sample for each treatment and control for a total of four soil samples. The soil samples were composed of 12.9% sand, 64.8% silt, and 22.3% clay (Korea Forest Service). The soil pH was approximately 5.2, organic matter content was 9.08%, total nitrogen was 0.432%, and the cation exchange capacity was 20.39 (cmol/kg) at the 0–10 cm soil depth (Korea Forest Service). Moreover, the soil K^+, Na^+, Ca^{2+}, and Mg^{2+} ions were 0.45, 0.06, 5.43, and 0.83 cmol/kg, respectively, at the 0–10 cm soil depth (Korea Forest Service).

2.2. Soil 16 PAHs and TPH Concentration

Four soil samples were collected during the research period after the lubricants were scattered in the treatment plots. The soil samples were collected approximately 7 days (2021), 1 month, 6 months, and 12 months (2022) after the treatment application. During each sampling period, 300 g soil samples were collected from each subplot and placed in brown glass bottles to prevent the photolysis of organic pollutants, and the openings of the bottles were sealed with Teflon tape to prevent air circulation. Each 300 g sample was collected at a soil depth of 0–5 cm around the soil collar and composited seven times. The samples were maintained at approximately 4 °C while being transported to the laboratory. Then, the samples were immediately sent to the Korea Environment & Water Works Institute (Seoul, Republic of Korea) for soil PAH and TPH analysis. Soil PAH concentration analysis was conducted on 16 types of PAH, which are 1st-class carcinogens designated by the International Agency for Research on Cancer of the World Health Organization. Sample analyses were carried out using gas chromatography–mass spectrometry (GC–MS) in compliance with EPA method 3541/8270C. Briefly, 10 g of each sample, along with an appropriate amount of anhydrous sodium sulfate for moisture removal, was placed on a cylindrical filter paper. The decomposition vessel was then filled with a 1:1 mixture of acetone and hexane (150 mL). Subsequently, the cylindrical filter paper containing the sample was passed through a decomposition vessel, allowing the sample to come into contact with the acetone/hexane mixture. This process was facilitated using a Soxhlet extractor (Gerhardt Soxtherm, Gerhardt®, Bonn, Germany) and took approximately 3 h and 30 min. The resulting extract was concentrated to 1 mL using a GC–MS concentrator. For further analysis, an internal standard substance, Pyrene-d_{10}, was injected into the sample, along with a standard solution for the calibration curve. This combined solution was analyzed using a GC–MS system (GC 7890A/MSD 5975C; Agilent Technologies, Santa Clara, CA, USA). A DB-5MS column (30 m length, 0.25 mm inner diameter, and 0.25 µm film thickness) was also used. The oven temperature was programmed to follow a gradient of 120 °C (initial temperature) to 300 °C (final temperature), with a heating rate of 6 °C/min. The inlet and auxiliary temperatures were both set to 310 °C. A flow rate of 1.0 mL/min was employed with a split ratio of 10/1. The analysis was conducted in the selected ion monitoring (SIM) mode. The soil TPH concentration was analyzed following the "Soil Contamination Testing Standards" (according to ISO standard 16703:2004 without any technical change; https://www.iso.org/standard/39937.html, accessed on 15 November 2023) in Republic of Korea (National Institute of Environmental Research Announcement No. 2022-38), using the TPH analysis method (ES 07552.1c). Analysis was performed using gas chromatography. The lubricating oil present in the sample was extracted, purified using dichloromethane, and measured using GC.

2.3. Soil Physical Properties

Soil contamination from oil can change the physical properties of the soil [25]. Therefore, we measured soil temperature (ST), soil moisture (SM), soil electrical conductivity (EC) near the soil collar, and soil CO_2 efflux (F_c). An auxiliary HydraProbe (Stevens Water Monitoring System, Portland, OR, USA) equipped on an 8200-01S Smart Chamber was used to monitor the ST and SM. We measured the ST, SM, and EC simultaneously (for 2 min) at one point near the soil collar, and the average value of each parameter was calculated.

2.4. Soil Bacteria

Soil sampling for soil bacterial analysis was performed simultaneously as sampling for the soil organic pollutant concentration analysis described in Section 2.3. The collected soil samples were stored at −20 °C before analysis. Soil bacterial analysis was conducted via DNA extraction using the DNeasy Power Soil Kit (Qiagen, Hilden, Germany) and next-generation sequencing (NGS). The concentration of the extracted genomic DNA from each sample was measured using Quant-IT PicoGreen (Invitrogen, Carlsbad, CA, USA), and only samples with a final concentration of 1.0 ng/u or more were used for the experiment through QC (quality check). A sequencing library was constructed by the Illumina 16S metagenomic sequencing library preparation method. For bacterial cluster analysis in the extracted DNA, the first PCR was performed using a universal primer set (V3-Foward: 5′–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3′; V4-Reverse: 5′–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–3′) targeting the V3–V4 region of the 16S rRNA gene.

The primary PCR products were purified using AMPure beads (Agencourt Bioscience, Beverly, MA, USA). The refined primary PCR product produced a sequencing library with a bar code attached through secondary PCR using the Nextra XT index kit (Illumina, San Diego, CA, USA). The secondary PCR products were subjected to QC by measuring the length and concentration of the amplification products using a TapeStation D1000 ScreenTape (Agilent Technologies, Waldbonn, Germany). The sequencing of amplicon libraries was sequenced using a MiSeq platform (Illumina, San Diego, CA, USA) at Macrogen (Seoul, Republic of Korea) according to the protocol of the manufacturer.

Index sequences were used to sort the Illumina MiSeq raw data and create paired-end FASTQ files for each sample after sequencing was completed. Sequencing adapters and forward and reverse primers were removed using Cutadapt software (version 3.2) [26]. Amplicon sequence variants (ASVs) were obtained after assembling error-corrected paired-end sequences into one sequence and removing the chimera using DADA2 [27]. BLAST+ (ver. 2.9.0) in the NCBI 16S microbial database was used for the comparative analysis of bacterial communities and taxonomic information for the bacteria with the highest similarity [28].

2.5. Soil Respiration

Soil respiration is the result of heterotrophic respiration related to microbial activity and autotrophic respiration related to root respiration [16]. In general, soil respiration is estimated by measuring the F_c on the surface [26]. We monitored the F_c twice a month (22 measuring days) during the research period (23 November 2021 to 28 July 2023). All measurements were conducted during the daytime (between 11:00 and 14:00, GMT +9). One soil collar per subplot was permanently installed in a Smart Chamber (LI-8200-01S; LiCOR Biosciences, Lincoln, NE, USA). A PVC soil collar with an inner diameter of 20 cm and height of 12 cm was installed at a depth of 8 cm in the soil. The F_c was analyzed using a gas analyzer (LI-870 CO_2/H_2O Analyzer; LiCOR Biosciences, Lincoln, NE, USA) connected to an 8200-01S Smart Chamber. The gas analyzers measured the CO_2 concentration in the 8200-01S Smart Chamber during an average of 2 min at 1 s intervals to calculate the F_c (μmol m^{-2} s^{-1}). Soil respiration is strongly influenced by temperature, and annual measurements of soil respiration may include the effects of daily and seasonal temperature variations. Therefore, it is necessary to normalize and minimize the effects of temperature on soil respiration. For the normalization of soil respiration, the temperature coefficient (Q_{10}) was calculated according to Lloyd and Taylor [29] using Equation (1):

$$Q_{10} = exp(10 \cdot \beta_1) \quad (1)$$

where β_1 is the fitted parameters to assess the effect of soil temperature on soil respiration using first-order exponential Equation (2).

$$F_c = \beta_0 \times \exp\ (\beta_1 \cdot ST) \quad (2)$$

where β_0 and β_1 are the fitted parameters, F_c represents the measured soil CO_2 efflux, and ST is the measured soil temperature.

Normalized soil respiration was calculated according to Lloyd and Taylor [29] using Equation (3):

$$F_{norm} = F_{10} \times Q_{10}^{((ST_{daymean} - ST)/10)} \quad (3)$$

where F_{10} is the soil respiration (μmol m^{-2} s^{-1}) normalized to a soil temperature of 10 °C, ST is the measured soil temperature (°C), and $ST_{daymean}$ is the average soil temperature during measuring time.

2.6. Vegetation Growth

We monitored seedling and herbaceous growth to assess the environmental implications of soil contamination using chainsaw lubricants for forest management. The girth and stem height of seedlings (a total of 192) were measured on 15 November 2021 and 18 November 2022 to compare the changes in seedling growth by treatment plot. Seedling girth was measured using a digital vernier caliper (Mitutoyo, Kanagawa, Japan) for the root collar. We calculated the stem height: stem diameter (height: diameter) ratio to assess the morphological quality of the seedlings after soil contamination [30,31]. Aboveground herbaceous biomass (AGB), which is also used to estimate carbon sequestration changes that can affect seedling growth, was measured in a research plot in December 2022. The herbaceous samples for AGB estimation were manually collected from the 16 subplots using pruning scissors, transferred to the laboratory, dried at 40 °C for 1 week in a dry oven, and their dried weights were recorded.

2.7. Data Analysis

The Anderson–Darling normality test and Bartlett's test were used to evaluate normality and homogeneity of variance, respectively. To assess the effects of CLST on the ST, SM, EC, F_c, and seedling quality, repeated-measures analysis of variance was performed. A post hoc test was conducted using Tukey's pairwise comparisons. All analyses of variance and normality were conducted in IBM SPSS Statistics for Windows (Version 25.0, IBM Corp., New York, NY, USA), and significance was set at p-value ≤ 0.05. Principal component analysis (PCA) was performed to understand the effects of soil contamination caused by CLST on the soil environment. PCA was performed using 'Factoextra' packages in R (Kassambara and Mundt, published on 1 April 2020; https://cran.r-project.org/web/packages/factoextra/index.html; accessed on 15 November 2023). The obtained experimental data were visualized using the 'ggplot2' package (Wickham H., 2016; https://cran.r-project.org/web/packages/ggplot2/citation.html; accessed on 15 November 2023). All statistical analyses were performed using R statistical software (version 4.2.3.) in RStudio 2022.12.0 + 353. The significance level was set at p-value ≤ 0.05.

3. Results

3.1. PAH and TPH Persistence in Forest Soil Following CLST

During the research period, the MP (0.50–0.90 mg/kg) had the highest average soil concentration of 16 PAHs (Figure 2). However, 1 year after CLST, the concentration of 16 PAHs in the soil similar to or lower than in the CP was observed in all treatment plots (Figure 2). In particular, the soil concentration of the 16 PAHs was always lower in the RP (average 0.20 mg/kg) than in the CP (average 0.36 mg/kg) (Figure 2). Meanwhile, the average soil concentration of the 16 PAHs in the BP during the research period was 0.34 mg/kg, which was always similar to the CP (average 0.36 mg/kg) (Figure 2).

There was a slight difference in the distribution of PAH by type according to CLST elapsed time and treatment plot (Figure 2). Six months after CLST, the concentration of dibenzo (a,h)anthracene in the MP remained at 0.27 mg/kg, however the concentration decreased to 0.02 mg/kg a year after CLST (Figure 2). In contrast, the composition and distribution of the 16 PAHs in the other treatment plots were similar a year after CLST; the

dominant species were benzo (g,h,i)perylene, benzo (a)pyrene, benzo (k)fluoranthene, and chrysene (Figure 2).

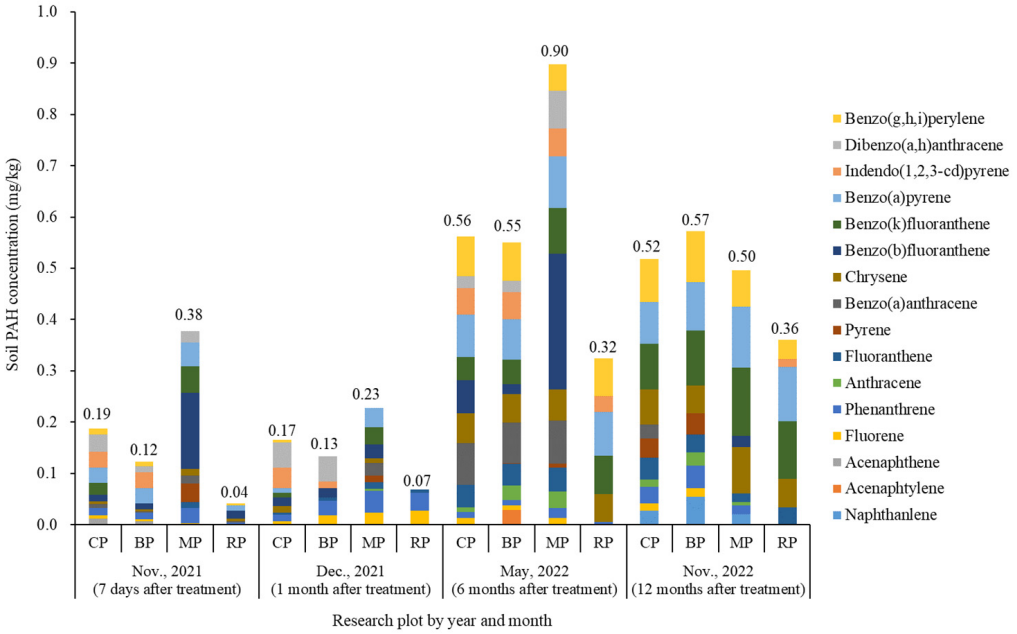

Figure 2. Average of soil polycyclic aromatic hydrocarbon (PAH) concentration by research plot (CP—control plot; BP—bio-oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot) and elapsed time after chainsaw lubricant scattering treatment (CLST). The values at the top of the bar represent the sum concentration of 16 PAHs (n = 3).

The soil concentration of TPH was the highest in the MP (2583–6215 mg/kg) during the research period (Figure 3). However, in contrast to the 16 PAHs, the TPH concentrations in the MP and RP did not fully recover to concentrations similar to the CP, even after a year of CLST. Specifically, a year after CLST (2583 mg/kg), the soil TPH concentration in the MP decreased by approximately 58% compared with 1 week after CLST (6215 mg/kg). However, the soil TPH concentration in the MP remained approximately 7.5 times higher than in the CP (344 mg/kg) (Figure 3). A similar trend was observed in the RP: 1 year after CLST, the TPH concentration in the RP (2258 mg/kg) was approximately 6.5 times higher than in the CP (344 mg/kg) (Figure 3). In contrast, 1 year after CLST, the soil TPH concentration in the BP was 355 mg/kg, indicating that soil contamination had recovered to a level similar to that in the CP (344 mg/kg) (Figure 3).

3.2. Changes in Soil Physical Properties Following CLST

The average ST during the research period was higher in all treatment plots (ST increased by 5.1% in the BP, 4.6% in the MP, and 2.8% in the RP) than the CP. However, no significant differences were observed among research plots ($p < 0.05$) (Table 1). In contrast, the average SM increased by 6.1% in the BP compared with in the CP and decreased by 3.5% and 2.8% in the MP and RP, respectively. However, there were no significant differences among research plots ($p < 0.05$) (Table 1). Similarly, there was no significant difference in soil EC among the research plots ($p < 0.05$) (Table 1).

3.3. Changes in Soil Bacterial Community Following CLST

The observed frequencies of soil bacterial genera detected in the research plots are shown in Table 2**.** During the research period, *Chrhoniobacter*, which is related to organic carbon decomposition [32,33], was the most abundant soil bacterial genus in all the treatment plots. *Chrhoniobacter* was detected approximately 8.9% higher in the BP than in the CP and MP (which only differed by approximately 0.2%), however it was approximately

23% lower in the RP than in the CP. *Bradyrhizobium*, which is the second-most frequent soil bacterial genus, and *Rhizobium*, which is a group of nitrogen-fixing bacteria that play an important role in plant growth [34,35], were the most frequently detected genera in the MP, however they were the least frequent in the RP (Table 2).

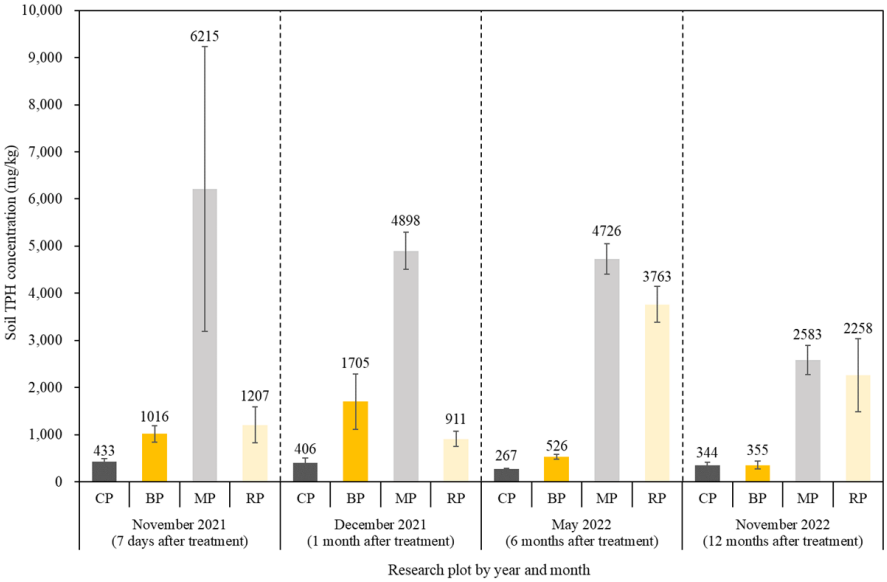

Figure 3. Average and standard error of soil total petroleum hydrocarbon (TPH) concentration by research plot (CP—control plot, dark grey filled bars; BP—bio-oil scattered plot, yellow filled bars; MP—mineral oil scattered plot, light grey filled bars; RP—recycled oil scattered plot, light yellow filled bars) and elapsed time after chainsaw lubricant scattering treatment (CLST) (n = 3).

Table 1. Average ± standard error of soil temperature (ST), soil moisture (SM), and soil electric conductivity (EC) for each research plot (CP—control plot; BP—bio-oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot) from November 2021 to July 2023. Different letters represent significant differences among the treatments.

Treatment	Variable (n = 116)		
	ST (°C)	SM (%)	EC (µS/cm)
CP	23.9 ± 0.8 [a]	21.62 ± 0.74 [a]	39.92 ± 2.18 [a]
BP	25.12 ± 0.81 [a]	22.94 ± 0.76 [a]	39.67 ± 1.82 [a]
MP	25.01 ± 0.76 [a]	20.86 ± 0.63 [a]	40.84 ± 2.87 [a]
RP	24.57 ± 0.76 [a]	21.01 ± 0.77 [a]	38.13 ± 2 [a]

The soil bacterial genera that are related to PAH degradation, such as *Mycobacterium*, *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Rhodococcus*, *Methylobacterium*, *Pseudomonas*, *Flavobacterium*, *Burkholderia*, *Novosphingobium*, *Sphingomonas*, and *Streptomyces* [36–40], were detected in the research plots at lower frequencies than those of *Chthoniobacter* and *Bradyrhizobium*. The observed frequencies of *Mycobacterium* and *Sphingomonas* were higher than those of the other genera. The highest frequency of *Mycobacterium* was observed in the RP, whereas *Sphingomonas* had the highest observed frequency in the CP. Among the 15 genera detected, *Rhodococcus* had the lowest observed frequency in the CP, nevertheless it still had a higher abundance than the other genera in the BP, MP, and RP (Table 2).

In general, the observed frequencies of soil bacterial genera differed per treatment plot. All the treatments affected the presence of bacteria in the soil compared with the CP. The BP and MP improved the frequencies of *Chthoniobacter* and *Bradyrhizobium*, the two most abundant bacterial genera in the research plots, but the frequency decreased in the treatment. *Mycobacterium*, *Rhodococcus*, and *Streptomyces* were more abundant in all the

treatment plots than in the CP. In contrast, *Sphingomonas*, *Arthrobacter*, *Methylobacterium*, and *Novosphingobium* were less abundant in all the treatment plots than in the CP. The frequencies of *Rhizobium* and *Beijerinckia* only increased in the MP, while the frequencies of *Bacillus* and *Pseudomonas* increased in the MP and RP. *Flavobacterium* was more abundant in the MP and RP, whereas treatment RP had an equal frequency to the CP. *Burkholderia* was reduced in the BP, however it increased in the MP and RP compared with the CP.

Table 2. Average ± standard error of observed number of reads for the soil bacteria genus related to organic carbon degradation, nitrogen fixation, and polycyclic aromatic hydrocarbon (PAH) degradation by research plot (CP—control plot; BP—bio-oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot) following chainsaw lubricant scattering treatment (CLST) (n = 12).

Major Role		Representative Soil Bacteria (Genus)	Observed Frequency/Treatment			
			CP	BP	MP	RP
Nutrient cycling	Nitrogen fixation	*Rhizobium*	72 ± 3	71 ± 11	90 ± 16	43 ± 3
		Bradyrhizobium	1283 ± 44	1313 ± 99	1349 ± 56	1198 ± 63
	Nitrification	*Nitrobacter*	5 ± 2	2 ± 1	6 ± 3	6 ± 3
	Subtotal		1381 ± 58	1406 ± 120	1472 ± 86	1273 ± 80
Bioremediation	Organic carbon degradation	*Chthoniobacter*	1717 ± 186	1870 ± 154	1721 ± 180	1305 ± 137
	PAH degradation	*Mycobacterium*	275 ± 25	318 ± 32	343 ± 21	358 ± 24
		Arthrobacter	192 ± 49	156 ± 50	167 ± 60	188 ± 67
		Bacillus	108 ± 8	106 ± 9	143 ± 24	135 ± 22
		Beijerinckia	85 ± 32	37 ± 5	86 ± 29	67 ± 15
		Rhodococcus	3 ± 2	52 ± 40	114 ± 54	60 ± 19
		Methylobacterium	33 ± 8	28 ± 11	26 ± 14	11 ± 4
		Pseudomonas	21 ± 9	20 ± 9	27 ± 11	26 ± 11
		Flavobacterium	12 ± 6	23 ± 11	23 ± 8	12 ± 3
		Burkholderia	14 ± 5	10 ± 4	13 ± 6	21 ± 5
		Novosphingobium	16 ± 5	13 ± 5	6 ± 4	4 ± 2
		Sphingomonas	375 ± 27	242 ± 46	218 ± 32	266 ± 36
		Streptomyces	25 ± 6	30 ± 10	42 ± 13	84 ± 19
	Pollutant degradation	*Deinococcus*	14 ± 3	10 ± 2	4 ± 1	4 ± 1
	Subtotal		2890 ± 371	2915 ± 388	2933 ± 457	2541 ± 365

3.4. Changes in Soil Respiration Following CLST

The average of F_{norm} during the research period was observed to be high in the order of the BP (11.5 µmol m^{-2} s^{-1}), RP (10.8 µmol m^{-2} s^{-1}), MP (10.2 µmol m^{-2} s^{-1}), and CP (8.4 µmol m^{-2} s^{-1}). For approximately 4 months after CLST, the F_c values of the research plots were similar (Figure 4). However, F_{norm} variability following the CLST was distinct at the start of the growing period (April 2022) (Figure 4). The first increase in F_{norm} was in the MP, followed by a similar increase in the BP a month later (Figure 4). However, for approximately 9 months until July 2022, the F_{norm} in the BP was higher than in the other treatment or CP (Figure 4). Subsequently, the F_{norm} of the treatment plots were similar, and the RP had the highest value (Figure 4). The MP and RP had higher F_{norm} values than the CP for most of the research period (Figure 4). Eleven months post-CLST, there was no significant difference of F_{norm} between BP and CP, whereas RP continued to have a higher F_{norm} compared with CP during the second half of the research period (Figure 4).

The treatment plots in F_{norm} identified after CLST led to an increase in the variance of F_c with respect to temperature in all treatment plots (Figures 4 and 5). Compared with BP (R^2 = 0.47), MP (R^2 = 0.32), and RP (R^2 = 0.35), ST was a single independent variable in CP (R^2 = 0.56), which better explained F_c (Figure 5). Notably, R^2 was the lowest in the MP, which had the highest PAH and TPH concentrations during the research period; it was also low in the RP, which was the second-most contaminated treatment plot (Figures 2, 3 and 5). Moreover, there was a difference in the Q_{10} of F_c for each research plot following CLST. The Q_{10} value of F_c increased in all treatment plots compared with in the CP. Compared with in

the CP (Q_{10} = 1.78), the Q_{10} value increased the most in the MP (Q_{10} = 1.99), followed by those in the BP (Q_{10} = 1.98) and RP (Q_{10} = 1.85) (Figure 5).

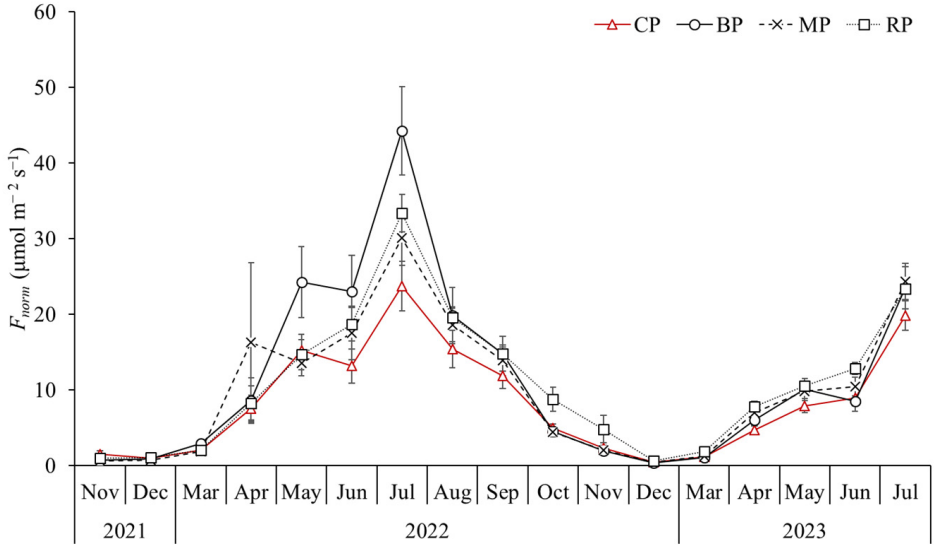

Figure 4. Monthly average and standard error of soil CO_2 efflux (F_c) by research plot (CP—control plot; BP—bio oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot) during research period (from 23 November 2021 to 28 July 2023) after chainsaw lubricant scattering treatment (CLST) (n = 4 or 8).

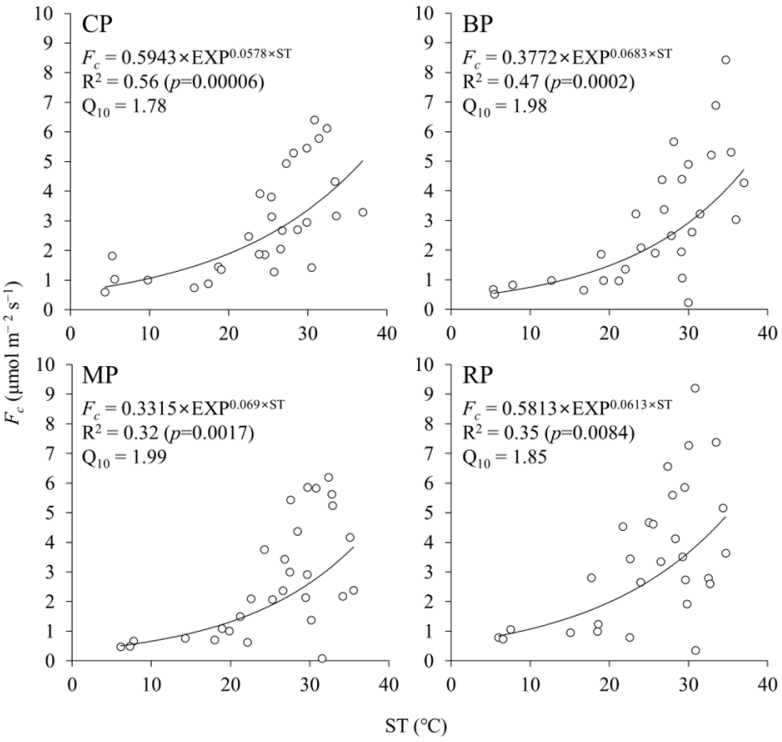

Figure 5. Soil CO_2 efflux (F_c) for research plots (CP—control plot; BP—bio oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot) from November 2021 to July 2023, as a function of soil temperature (ST). Q_{10} values represent temperature sensitivity of soil respiration by research plot.

3.5. Seedling Quality Following CLST

Healthy seedlings should exhibit balanced growth between the stem height and diameter. For stem height, the stem diameter ratio is the sturdiness ratio of the seedlings. A

high ratio indicates a relatively slender seedling, whereas a lower ratio indicates a stouter seedling [31]. Moreover, this ratio can be used to predict if seedlings would have a stable long-term growth trajectory [30]. In this study, the CP had the lowest height: diameter ratio (82.6) 1 year after CLST. However, all treatment plots except BP had increased height: diameter ratios compared with the CP ($p < 0.05$); MP (96.4) had the largest increase (Figure 6). However, there was no significant difference between the height: diameter ratios of the BP (86.9) and the RP (92.8) ($p < 0.05$) (Figure 6).

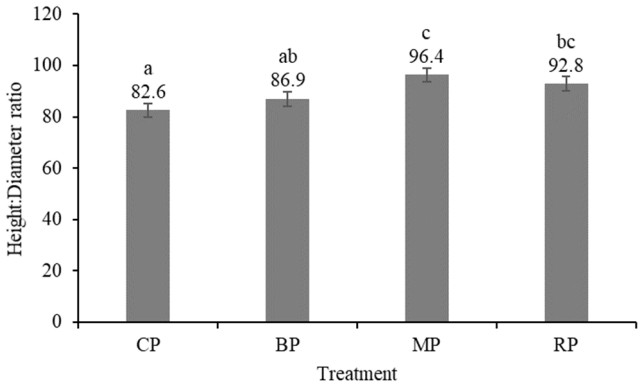

Figure 6. Ratio of height to stem diameter indicates the sturdiness of seedlings (n = 48). Different letters represent significant differences among the research plots (CP—control plot; BP—bio oil scattered plot; MP—mineral oil scattered plot; RP—recycled oil scattered plot).

The PCA result shows that only MP is grouped into a distinct cluster (Figure 7). In addition, soil 16 PAH is the first most influential variable, and height: diameter is the second most influential variable among all variables (Figure 7). Therefore, the sturdiness of the seedlings seems to change significantly after soil contamination (Figure 7). However, since AGB is the closest to the PC2 axis and AGB is strongly correlated with height: diameter, we cannot exclude the influence of understory vegetation on seedling growth (Figure 7).

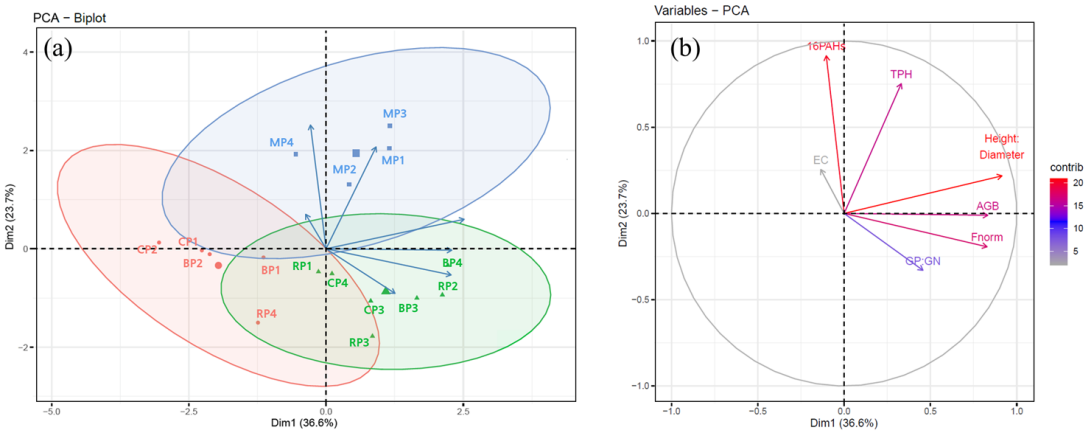

Figure 7. (**a**) Biplot and (**b**) contribution of variables from Principal component analysis (PCA) in the research plots (CP1-4—subplot 1-4 of control plot; BP1-4—subplot 1-4 of bio-oil scattered plot; MP1-4—subplot 1-4 of mineral oil plot; RP1-4—subplot 1-4 of recycled oil scattered plot over the above one-year following chainsaw lubricant scattering treatment (CLST). In biplot, ellipses filled with different colors are clusters grouped by k-means (The unlabeled, largest shape in the ellipse represents the center of the cluster). Variables PCA: girth growth and height growth represent seedling growth rate measured one-year after CLST, EC (soil electric conductivity), F_{norm} (normalized soil CO_2 efflux at daily mean soil temperature), 16 PAHs (sum of soil 16 PAH concentration), TPH (soil TPH concentration), height: diameter (ratio of height to diameter of seedlings), AGB (above-ground biomass), and GP:GN (ratio of Gram-positive bacteria genera to Gram-negative bacteria genera).

4. Discussion

4.1. Environmental Impact of Different Types of Lubricants

Choosing a chainsaw lubricant—whether biodegradable, petroleum-based, or recycled—has a substantial influence on the environmental health of forest soils. Our study revealed a dynamic interplay between the lubricant type and soil contamination over time.

Initially, the concentrations of the 16 PAHs and TPH were significantly higher in the MP, indicating a greater risk of soil contamination upon spillage. However, what sets our findings apart is the observation that, 1 year after CLST, all treatment plots exhibited 16 PAH and TPH concentrations either similar to or lower than those in the CP. This suggests that the soil can potentially recover from the initial contamination events.

Soil contamination by petroleum-based organic pollutants can be mitigated through photodecomposition and biodegradation [41]. Because this study was conducted in a nursery with an open tree canopy within a forest, we assumed that the sunlight conditions for each treatment were similar. Therefore, we focused on the biodegradation of contaminants using bacteria.

Several studies have recognized the ability of soil microorganisms, particularly certain bacteria, to degrade PAHs and TPHs; hence, studies have been actively conducted to develop bioremediation technologies using such useful bacteria [42–45]. Petroleum hydrocarbon degradation is commonly attributed to indigenous microorganisms [46]. Several studies have been published on the degradation of PAHs and TPH by indigenous bacteria.

Bacterial degradation of organic pollutants can also be observed in soil respiration changes [19,47,48]. In this study, all treatment plots showed an increase in average soil respiration compared with the CP. These results are similar to those reported by Silva et al. [19], who observed an increase in soil respiration in PAH-contaminated soils over a 12-week monitoring period. However, the BP showed a significant increase in soil respiration 8 months after CLST; its soil respiration value was similar to that of the CP thereafter. In addition, the BP had the second-highest Q_{10} of soil respiration, supporting the idea that the biodegradation process of microorganisms, including bacteria, is active early during soil contamination. This indicates that the use of bio-oil could be a favorable option for the early remediation of contaminated soil. However, although the MP had the highest increase in the number of degrading bacteria, the recovery of 16 PAH contamination was slow, and TPH contamination did not recover after 1 year. These results suggest that the use of mineral oil for deforestation should be reconsidered. In particular, a lower level of soil contamination was observed in the RP than in the MP. Moreover, according to the PCA results, the CP, BP, and RP were grouped into the same cluster (Figure 7). These findings indicate that recycled lubricants, which are more cost-effective, are a more environmentally sustainable option with lower long-term soil contamination risks than petroleum-based lubricants. This finding underscores the importance of considering not only the immediate impact but also the lasting consequences of lubricant choices in forest management. However, the PC2 axis is believed to describe the response of the biosphere after soil contamination, therefore the environmental implications of lubricant types should be considered in selecting lubricants for timber operations (Figure 7).

4.2. Effects of Soil Contamination on Soil Physical Properties and Respiration

The impact of the lubricant type extends beyond the contamination levels, affecting key soil physical properties, microbial activity, and plant growth. Our study sheds light on the critical aspects of soil health.

In this study, EC did not show significant differences among the treatment plots. However, the approximately 2.3% increase in EC in the MP compared with in the CP was similar to the findings of Torimiro et al. [49], who found that EC was 2.9% higher in oil-contaminated soil than in the CP. He suggested that the electrical conductivity was also higher than that of normal soil because of soluble salts, as it has a higher organic

content. No significant changes were observed in the ST and SM; however, the ST and SM are determinants of soil respiration [50] and may have been influenced by treatment.

Changes in soil respiration were also observed. Soil respiration increased in all treatment plots, suggesting a higher microbial metabolic rate. This increase may be attributed to shifts in soil bacterial communities (discussed below) and their influence on organic matter decomposition.

Soil respiration is defined as the release of carbon dioxide from the soil to the atmosphere as a product of microbial activity and root respiration and has been widely used to understand microbial responses to contaminated soil and to monitor contaminant degradation [51,52]. Slight fluctuations in soil respiration rates can be attributed to the physiological responses of microbial communities to changes in environmental factors [53,54]. Zhu et al. [55] reported that the average soil respiration rate during a 59-day incubation period in soil contaminated with crude oil was 38.8% higher than in the CP. During the study period, the average soil respiration increased by 36.9% in the BP, 28.6% in the RP, and 21.4% in the MP compared with in the CP. However, soil respiration was not consistently higher in the RP than in the CP throughout the study. Rather, an increase in soil respiration was observed in the BP during the first 8 months of contamination; afterwards, it was similar to that of the CP. These differences suggest that the increased soil respiration observed in this study was correlated with the rate and timing of PAH removal [19]. In addition, the PCA results showed that F_{norm} was correlated with AGB, height: diameter, and Gram-positive bacteria: Gram-negative bacteria (GP:GN) ratios (Figure 7). These results may be due to changes in soil microbial activity and vegetation respiration after soil contamination. Our findings suggest that soil contamination caused by forestry operations can have long-term global effects on the carbon cycle.

4.3. Changes in Soil Bacterial Communities

One of the most significant findings of our study was the impact of the lubricant type on soil bacterial communities. These communities play essential roles in nutrient cycling and organic matter decomposition, making them crucial components of forest ecosystems. We observed shifts in the composition and frequency of various bacterial genera in response to the different types of lubricants. The soil bacterial genera detected in the research plots could be divided into three major groups based on their roles in the soil: organic carbon degradation, nitrogen fixation, and PAH degradation (Table 2). Only *Chthoniobacter* can degrade organic carbon [32,33]; for example, *Chthoniobacter flavus* plays an important role in the transformation of organic carbon compounds in the soil [33]. *Bradyrhizobium* and *Rhizobium* are nitrogen-fixing bacteria; specifically, *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* form nitrogen-fixing root nodule symbioses with soybean plants [34,35]. Twelve other bacterial genera belong to the PAH degradation group [36–39]; some species within this group are *Mycobacterium vanbaalenii* [40], *Rhodococcus opacus*, and *Pseudomonas migulae* [38]. Most of the detected bacteria belonged to the PAH degradation group; however, the observed frequency of these bacteria was lower than that of organic carbon-degrading bacteria and nitrogen-fixing bacteria (except for *Rhizobium*). *Chthoniobacter*, which is associated with organic carbon decomposition, was the most frequent genus in the BP, followed by the CP and MP. Interestingly, lower levels of *Chthoniobacter* were found in the RP, suggesting its potential influence on carbon cycling.

The frequencies of *Bradyrhizobium* and *Rhizobium*, which are important nitrogen-fixing bacteria, varied across the treatment plots. This variability suggests that different lubricants may affect nitrogen availability in the soil, which, in turn, affects plant growth and ecosystem productivity. The bacterial genera associated with organic carbon degradation and nitrogen fixation decreased in the RP, suggesting that plant growth is negatively affected by chainsaw lubricants made of recycled oils. It appeared that PAH-degrading bacterial communities usually increased after treatment with chainsaw lubricants to remove soil pollutants. However, the results of this study could open further research avenues to evaluate potential bacterial isolates in culture-based experiments.

4.4. Implications for Forest Ecosystem Health and Management

Our findings have direct implications on the health and management of forested ecosystems. The choice of chainsaw lubricants can influence not only soil quality, but also aboveground vegetation and, by extension, overall ecosystem health.

Although there are various physical and chemical approaches for removing harmful toxic contaminants from soils, these remediation methods are not suitable for large areas and are not cost-effective [56]. Therefore, it is worth considering the accompanying bioremediation methods for lubricant-contaminated soils because they are cost-effective and environmentally friendly. For example, the genus *Bacillus* that increased in the MP and RP in this study includes plant growth-promoting rhizobacteria [57,58]. The efficiency of plant growth-promoting rhizobacteria in hydrocarbon degradation and plant growth promotion, accompanied by the use of various biofertilizers, has been demonstrated [56,59]. However, they must possess a wide range of characteristics, including a variety of enzymes, to form suitable microbial populations [60]. However, the most important aspect in forest management is minimizing pollution; in this respect, the use of bio-oil is a promising option. The results show that, when contaminated with the same amount of lubricant, the use of bio-oil resulted in a lower degree of contamination and an active natural biodegradation reaction.

Another reason to minimize soil pollution is the response of the biosphere to pollution. In this study, a weak positive correlation between the TPH concentration and GP:GN ratio was identified (Figure 7). In addition, the growth patterns of seedlings change during reforestation, which is a major consideration from the economic aspect of forest management. In this study, the height: diameter ratio of the seedlings appeared in the following order: MP > RP > BP > CP (Figure 6). This result indicates that seedling sturdiness was not good in the MP and RP. Inhibition of seedling growth in lubricant-contaminated soil has been proven, although the degree of inhibition varies depending on the environment [15]. The TPH and PAH in lubricants induce the formation of active oxygen species, including peroxide, hydrogen peroxide, and free radicals [14], which interfere with normal plant metabolism [61]. In addition, oxidative stress can occur in soil contaminated with petroleum-based lubricants, causing damage to the lipid membrane, which can affect the initial growth, including the height of seedlings, number of leaves, and biomass [15].

Even if the short-term non-recovery of TPHs is excluded, the fact that PAHs were found to have dissipated one year after CLST does not mean that soil contamination from chainsaw lubricants has completely recovered, because mineralization and detoxification of PAHs and TPHs are separate processes [3]. Bioremediation of PAHs can potentially enhance soil toxicity [11–13,62] due to the production of toxic intermediates such as oxygenated and nitrated PAHs [63]. Zeng et al. [63] reported that 28 °C is a suitable temperature for the mineralization of PAHs, and 50 °C is the optimum temperature for the detoxification of PAHs. However, it is difficult to control microbial metabolism and temperature at forest sites contaminated with chainsaw lubricants.

5. Conclusions

Our study highlights the complex interplay between lubricant type, soil contamination, soil physical properties, microbial activity, and vegetation growth. Through a careful analysis of these aspects, we arrived at several significant conclusions.

First, we determined the capacity of the soil to recover from initial contamination events, primarily through biodegradation by indigenous microorganisms. Notably, the use of biodegradable lubricants (bio-oil) facilitated faster recovery from soil contamination, whereas petroleum-based lubricants (mineral oil) exhibited slow and incomplete recovery. Recycled lubricants appear to be more environmentally sustainable options, indicating lower long-term soil contamination risks than petroleum-based lubricants.

Second, changes in soil physical properties, specifically soil respiration, revealed a heightened microbial metabolic rate in all treatment plots compared with the CP. Thus, soil contamination from forestry operations can have enduring impacts on the global carbon cycle, making responsible lubricant selection for forest management more important.

Third, various bacterial genera responded differently to different lubricants, affecting their roles in organic carbon fixation, nitrogen fixation, and PAH degradation. These changes in bacterial communities can have far-reaching consequences on nutrient cycling and organic matter decomposition, making them crucial aspects of forest ecosystem health.

In conclusion, our study highlights the multifaceted effects of lubricant choice on forest ecosystems. The health and growth of seedlings are not only influenced by soil contamination but also by the type of lubricant used in forestry operations. Responsible lubricant selection, such as favoring biodegradable options, not only promotes soil recovery, but also contributes to robust seedling growth, thus enhancing the overall health and sustainability of forest ecosystems. As forest management practices evolve, it is essential to consider the broader ecosystem dynamics and long-term impact of lubricant choices on the growth and vitality of forest vegetation.

This study is novel because it is a rare research in which the response of soils contaminated with different lubricants is observed in a forest site. However, the small area of the study site and the small sample size limit the generalizability of the results. Furthermore, the results of this study should be validated by studying the effects of lubricant contamination on the soil environment in chainsaw-harvested forests.

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