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作品名稱　Proposal for the Restoration of Fire-Damaged Soil Using Water-Soluble Aromatic Compounds Derived from Soil Actinomycetes

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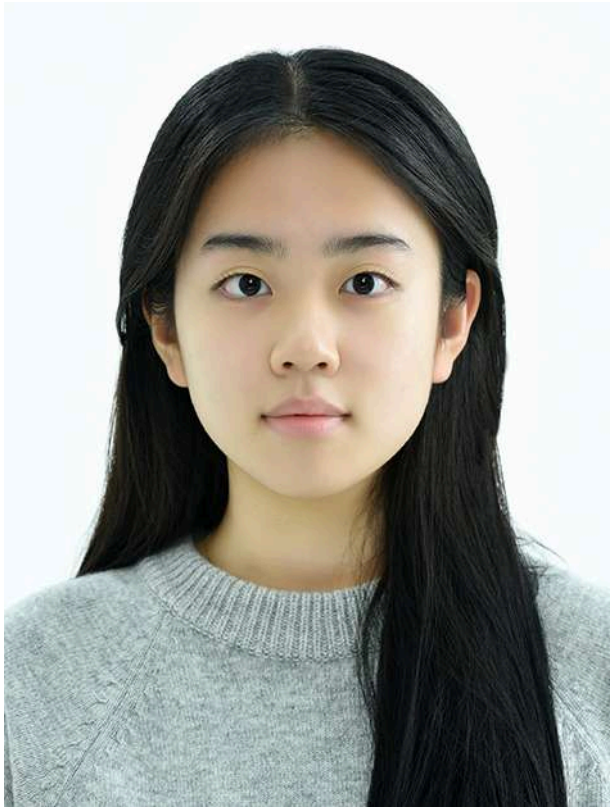
關鍵詞　actinomycetes, , anaerobic aerobic soil bacteria., Methane-producing bacteria, wildfires, watersoluble aromatic substances

# 作者照片



# Proposal for the Restoration of Fire-Damaged Soil Using Water-Soluble Aromatic Compounds Derived from Soil Actinomycetes

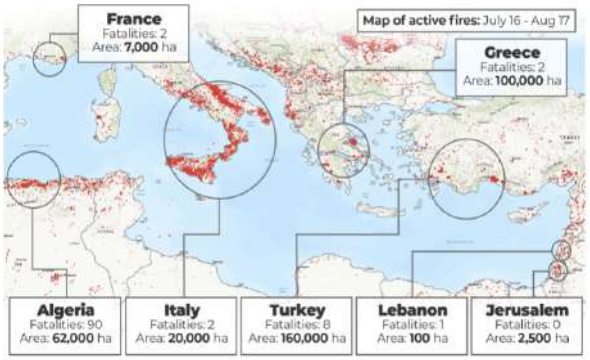
**Abstract) The following issues associated with soil affected by wildfires were identified: First, there was a significant decline in the populations of anaerobic and aerobic soil bacteria, which play a critical role in the decomposition and cycling of organic matter. This decline resulted in reduced water retention capacity and porosity of the soil, leading to poor moisture retention and increased evaporation compared to unaffected soil. Moreover, the organic matter content in the soil was significantly depleted, inhibiting plant growth. Additionally, there was a notable proliferation of methane-producing bacteria, which contribute to the greenhouse effect. It was further observed that fire-damaged soils exhibit limited natural recovery, even over prolonged periods. .An investigation into the underlying causes of these problems revealed that actinomycetes, the primary microorganisms responsible for producing water-soluble aromatic compounds in soil, are particularly sensitive to heat compared to other bacterial species. Research demonstrated that the population and diversity of actinomycetes are significantly diminished in soils exposed to wildfires. To mitigate these issues, water-soluble aromatic compounds produced by actinomycetes were extracted and introduced into wildfire-affected soil. This intervention promoted the restoration of actinomycetes populations, enabling their normal growth in the affected soil. Consequently, various wildfire-induced soil problems were effectively resolved. These outcomes were confirmed through the study...Key Words: Actinomycetes, anaerobic and aerobic soil bacteria, methane-producing bacteria, wildfires, water-soluble aromatic compounds.**

**Key Words : actinomycetes, , anaerobic aerobic soil bacteria., Methane-producing bacteria, wildfires, water-soluble aromatic substances**

## 1. Introduction

The distinct differences in smell between healthy and unhealthy soil are influenced by various factors, primarily the presence and activity of microorganisms within the soil. Healthy soil typically emits a rich, earthy aroma that becomes more pronounced after rainfall. This characteristic scent is largely attributed to a compound called **Geosmin**, produced by soil-dwelling bacteria, particularly **Actinomycetes**. These bacteria thrive under moist and warm soil conditions and are known for their critical role in the decomposition of organic matter and the cycling of nutrients. When soil dries, these microorganisms release tiny spores containing Geosmin. Humans are highly sensitive to this compound, which is not only used in perfumes but also contributes to the earthy flavor of beets. This earthy scent serves as a reliable indicator of fertile soil teeming with diverse microorganisms that are vital for maintaining soil health. Conversely, unhealthy soil often emits sour or metallic odors, indicative of underlying problems such as poor drainage or oxygen deficiency. Therefore, the presence and activity of microorganisms, particularly the production of Geosmin by Actinomycetes, play an essential role in the characteristic aroma of healthy soil. In contrast, unhealthy soil typically lacks these beneficial microorganisms and their byproducts, resulting in less pleasant odors and signaling compromised soil health. Global warming is elevating average temperatures

globally, accelerating moisture evaporation from soils and vegetation. This process leaves forests and grasslands increasingly arid, creating conditions highly conducive to wildfires. Even a small spark in such environments can rapidly escalate into a large-scale blaze. In Southern Europe, heatwaves caused by hot air masses from Africa have triggered widespread wildfires across the Mediterranean. In Turkey, wildfires that began on July 28 claimed at least eight lives and devastated hundreds of locations in southern regions. Similarly, in Greece, hundreds of wildfires across areas like Evia, Peloponnese, and Attica forced thousands to evacuate. In Italy, a wildfire on July 20 in the Carso region crossed the Slovenian border, burning over 5,000 acres. Southern France also experienced wildfires, resulting in at least two fatalities. These observations highlight the complex interplay between microbial ecology, soil health, and the escalating threat of wildfires exacerbated by global climate change.




**[Fig.1] Wildfires and Affected Areas in Southern Europe, 2022 [5]**

In Russia's Siberian region, uncontrolled wildfires have devastated extensive areas of coniferous forests, spanning thousands of kilometers. According to Greenpeace Russia's forest protection representative, the largest wildfire in this region consumed approximately 1.5 million hectares (3.7 million acres). [6] These wildfire events are directly linked to climate change, driven by extreme weather conditions such as prolonged droughts, heatwaves, and strong winds. These fires have caused substantial harm to local communities and have had significant long-term environmental and ecological consequences.. Wildfires can profoundly impact soil, altering its biological, physical, and chemical properties. Recent research has demonstrated that wildfires significantly disrupt the diversity and functionality of soil microbial communities, with surface soils being particularly vulnerable. As wildfire severity increases, microbial diversity in surface soils diminishes, resulting in a loss of ecosystem complexity and reduced connectivity within the soil microbiome. Wildfires also induce structural shifts in microbial populations, with a notable rise in the relative abundance of heat-resistant microorganisms. [7].These observations underscore the intricate effects of wildfires on soil ecosystems and highlight the critical need for effective soil recovery and management strategies post-wildfire. Successful post-wildfire soil restoration necessitates a holistic approach that includes the restoration of microbial community diversity and functionality, The restoration of soil organic matter and the improvement of soil structure and water retention capacity. This study investigates the potential of water-soluble aromatic compounds produced by **Actinomycetes** in healthy soils to support the recovery of wildfire-damaged soils.

## 2. Main Body

### 2.1. Research Methodology

#### 2.1.1 Alterations in the Composition of Soil Bacterial Communities in Wildfire-Exposed Soil

**[Fig.2] Soil Sample Collected from a Wildfire-Affected Area (Wildfire Occurred on April 11, 2023)**

① Soil samples were collected from the wildfire-affected area (wildfire occurred on April 11, 2023) and stored under refrigerated conditions (4°C) until use.

② A 0.1 g portion of the wildfire-affected soil was weighed using an electronic balance and placed into a 1.5 mL microtube. Subsequently, 1 mL of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, was added to the microtube and thoroughly mixed by shaking.

③ The 1.5 mL microtube prepared in step ② was subjected to centrifugation at 6,000 rpm for 10 minutes. This process separated a supernatant solution containing soil bacteria.

[Fig.3] Centrifugation of Soil Samples

④ The supernatant containing soil bacteria was inoculated onto an NA (Nutrient Agar) plate using 10 μL of the solution. The inoculum was spread evenly on the plate using a spreader for 2-3 minutes, followed by incubation at room temperature for 24 hours. This process enabled the cultivation of aerobic soil bacteria, which grow under oxygen-rich conditions. All procedures were conducted under a clean bench.

⑤ Similarly, 10 μL of the supernatant containing soil bacteria was inoculated onto an NA plate, spread evenly using a spreader for 2-3 minutes, and incubated. Afterward, a carbon dioxide-releasing patch, which absorbs atmospheric oxygen and converts it to carbon dioxide, was placed into an airtight zipper bag along with the inoculated NA plate. The bag was sealed and incubated at room temperature for 24 hours, facilitating the cultivation of anaerobic soil bacteria, which grow under oxygen-deprived conditions. All procedures were carried out under a clean bench.


[Fig.4] Oxygen-Absorbing Carbon Dioxide-Releasing Patch Placed in a Sealed Zipper Bag with the Culture Medium

⑥ A 10 μL aliquot of the supernatant containing soil bacteria was inoculated into 3 mL of LB (Luria-Bertani) broth and incubated at room temperature for 24 hours. This procedure facilitated the cultivation of aerobic soil bacteria, which grow under oxygen-rich conditions. All steps were conducted under a clean bench.

⑦ Similarly, a 10 μL aliquot of the supernatant containing soil bacteria was inoculated into 3 mL of LB broth. The inoculated medium was placed in a sealed zipper bag along with an oxygen-absorbing, carbon dioxide-releasing patch. The bag was sealed and incubated at room temperature for 24 hours, allowing the cultivation of anaerobic soil bacteria, which grow under oxygen-deprived conditions. All steps were performed under a clean bench.

⑧ To evaluate the growth of aerobic and anaerobic soil bacteria, 1 mL of the LB broth containing the cultured bacteria was transferred into a UV-Vis spectrophotometer. The optical density (OD) was measured at a wavelength of 600 nm to assess bacterial growth dynamics.

⑨ The same experimental procedures (steps 1–8) were performed using unexposed, normal soil (commercially available soil) as a control group.

**2.1.2 Changes in Plant Growth in Wildfire-Exposed Soil**

① **Preparation of Soil**: Four grams of wildfire-exposed soil were measured using an electronic balance and placed into a 6 cm x 6 cm plastic petri dish. The soil was completely dried to remove moisture.

② **Rehydration of Soil**: Once the drying process was complete, 10 mL of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, was added to the soil to ensure thorough rehydration.

③ **Planting Seeds**: Sixteen cabbage seeds were planted in the rehydrated soil, and the setup was maintained at room temperature for one week to observe plant growth.

④ **Control Group**: The same experimental procedures (steps 1–3) were conducted using unexposed, commercially available soil as a control group.

**2.1.3 Changes in Moisture Retention and Porosity of Wildfire-Exposed Soil**

① Four grams of wildfire-exposed soil were measured using an electronic balance and placed into a 6 cm x 6 cm plastic petri dish. The soil was completely dried to remove all moisture.

② Ten milliliters of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, were added to the dried soil for rehydration. The weight of the soil was then measured using an electronic balance.

④ The soil was kept at room temperature for one week, after which its weight was measured again using the electronic balance to evaluate the moisture retention capacity of the wildfire-exposed soil.

⑤ The same procedures (steps 1–3) were conducted using unexposed, commercially available soil as a control group.

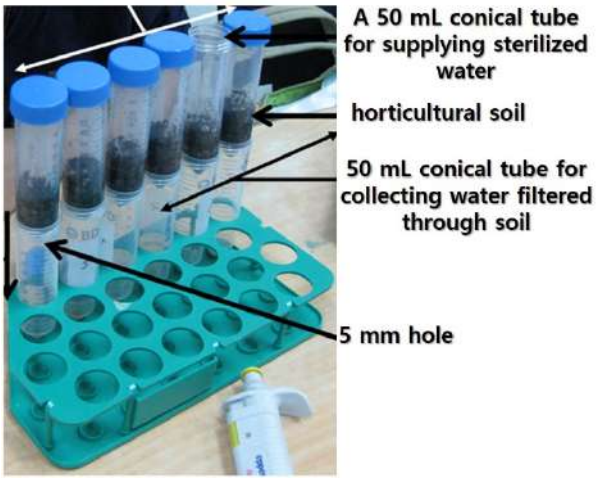
⑥ A 50 mL conical tube was prepared by drilling a 5 mm diameter hole in the bottom using an electric drill. Twenty grams of dried wildfire

⑦ exposed soil were added to the conical tube. A second 50 mL conical tube was placed underneath to collect water.

⑧ Thirty milliliters of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, were poured into the soil in the upper conical tube.

⑨ After three hours, the amount of water collected in the lower conical tube was measured to determine changes in the porosity of the wildfire-exposed soil.

⑩ The same procedures (steps 5–7) were conducted using unexposed, commercially available soil as a control group.




**[Fig.5] Apparatus for Measuring Soil Porosity**

**2.1.4 Changes in Organic Matter Content of Wildfire-Exposed Soil**

① Soil samples were collected from the wildfire-affected area (wildfire occurred on April 11, 2023) and stored under refrigerated conditions (4°C) until use.

② A 0.1 g sample of wildfire-exposed soil was measured using an electronic balance and placed into a 1.5 mL microtube. Then, 1 mL of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, was added to the microtube, and the mixture was thoroughly shaken to ensure homogenization.

③ The prepared microtube was placed in a centrifuge and spun at 6,000 rpm for 10 minutes to separate the supernatant containing soil bacteria.

④ The supernatant obtained in step ③ was passed through a 0.2 μm filter to remove any residual particulates. The organic matter content of the solution was then assessed using a testing kit capable of detecting organic matter through colorimetric changes.

⑤ The same procedures (steps 1–4) were conducted using unexposed, commercially available soil as a control group.

**2.1.5 Changes in Earthworm Growth Rate in Wildfire-Exposed Soil**

① One hundred earthworms were introduced into 500 g of wildfire-exposed soil. After one month, the number of earthworms was counted to assess their growth and survival rate.

② One hundred earthworms were introduced into 500 g of normal, unexposed soil (control). After one month, the number of earthworms was counted and used as a baseline for comparison.

**[Fig.6] Study to Assess the Impact of Wildfire-Exposed Soil on Earthworm Survival**

**2.1.6 Growth Changes of Methanogenic Bacteria in Wildfire-Exposed Soil**

[Table 1] Composition of Media for Selective Cultivation of Methanogenic Bacteria

| Reagent | Mass(g)&Volume(L,ml) |
|---|---|
| $MgSO_4$ | 1.0 |
| $Cacl_2$ | 0.2 |
| $KNO_3$ | 1.0 |
| $KH_2PO_4$ | 0.272 |
| $Na_2HPO_4$ | 0.717 |
| Agarose powder | 12.5 |
| Tertiary distilled water | 1L |
| Ferric(III)ammonium citrate | 0.1 |
| EDTA | 0.2 |
| HCl | 0.3ml |

**2.1.6 Growth Changes of Methanogenic Bacteria in Wildfire-Exposed Soil**

① A selective culture medium for methanogenic bacteria was prepared according to the composition listed in **[Table 1]**.

② A 0.1 g sample of wildfire-exposed soil was measured using an electronic balance and placed into a 1.5 mL microtube. Then, 1 mL of sterilized triple-distilled water, filtered through a 0.2 μm pore-sized filter, was added to the microtube. The mixture was thoroughly shaken to ensure homogenization.

③ The prepared microtube was centrifuged at 6,000 rpm for 10 minutes to separate the supernatant containing soil bacteria.

④ Ten microliters (10 μL) of the supernatant containing soil bacteria were inoculated onto NA (Nutrient Agar) media. The inoculum was evenly spread using a spreader for 2–3 minutes. The NA media were then placed in a sealed zipper bag containing an oxygen-absorbing and carbon dioxide-releasing patch, along with the selective medium for methanogenic bacteria. The bag was sealed and incubated at room temperature for 24 hours to cultivate anaerobic soil bacteria, including methanogenic bacteria, which grow under oxygen-deprived conditions. Methanogenic bacteria are classified as anaerobic soil bacteria. All procedures were performed under a clean bench.

⑤ Similarly, 10 μL of the supernatant containing soil bacteria were inoculated into 3 mL of LB broth. The inoculated broth was placed in a sealed zipper bag containing an oxygen-absorbing and carbon dioxide-releasing patch. The bag was sealed and incubated at room temperature for 24 hours to cultivate anaerobic soil bacteria. Methanogenic bacteria, being anaerobic, thrive under such conditions. All procedures were conducted under a clean bench.

⑥ To assess the growth of methanogenic bacteria, 1 mL of the culture from the selective medium was transferred into a UV-Vis spectrophotometer. The optical density (OD) was measured at a wavelength of 600 nm to evaluate the growth dynamics of the methanogenic bacteria.

⑦ The same procedures (steps 1–6) were conducted using unexposed, commercially available soil as a control group.

**2.1.7 Assessment of Soil Recovery After One Month of Storage Under Greenhouse Effect Conditions Induced by Global Warming and Normal Conditions**

① Based on the experimental procedures, soil samples were stored for one month under greenhouse effect conditions induced by global warming and normal conditions. The degree of soil recovery was then evaluated.

**2.1.8 Design and Development of Research Equipment for Collecting Water-Soluble Aromatic Compounds Produced by Soil, and the Collection of Such Compounds**

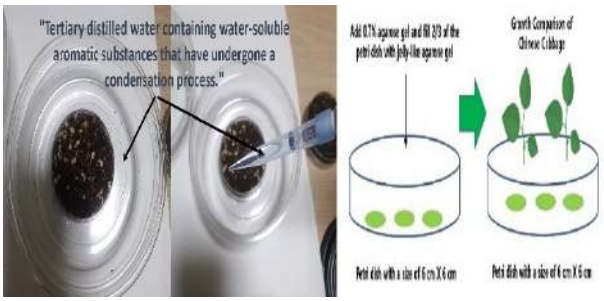
① Research equipment capable of collecting water-soluble aromatic compounds generated by soil was developed, as illustrated in the figure below. Soil known to support optimal plant growth was placed in the research apparatus and stored for one month. After one month, the lid of the sealed plastic container was opened, and the tertiary distilled water located between the petri dish containing the soil and the sealed container was collected. The collected water was transferred into a 15 mL conical tube and stored under refrigeration at 4°C.


**[Fig.7] Apparatus for Collecting Water-Soluble Aromatic Compounds from Soil**

**2.1.9 Evaluation of Growth Changes in Plants (Cabbage) Exposed to Water-Soluble Aromatic Compounds Collected from Soil**

① A disk filter was soaked in a solution containing water-soluble aromatic compounds extracted from healthy soil.

The soaked filters were placed into a 6 cm x 6 cm petri dish, with a total of 10 filters per dish. Subsequently, 0.7% agarose gel was poured into the dish, filling two-thirds of the dish with jelly-like agarose gel. Twenty cabbage seeds were planted in the gel, and the growth of the cabbage was monitored. This experimental procedure aimed to assess whether water-soluble aromatic compounds isolated from various soils have a direct impact on plant growth.




**[Fig.8] Process of Collecting Water-Soluble Aromatic Compounds from Soil**

**2.1.10 Growth Changes in Soil Bacteria When Normal Soil is Treated with a Solution Containing Water-Soluble Aromatic Compounds Extracted from Healthy Soil**

① A 1 g sample of normal soil treated with a solution containing water-soluble aromatic compounds extracted from healthy soil was collected. The sample was centrifuged at 6,000 rpm to separate the supernatant from the soil. Ten microliters (10 μL) of the supernatant were inoculated onto a bacterial culture medium capable of cultivating soil bacteria. The inoculated medium was incubated at room temperature for 12 hours to observe the growth of soil bacteria.

**2.1.11 Selective Cultivation and Preservation of Actinomycetes and Growth Assessment in Wildfire-Exposed Soil**

**[Table 2] Composition of Selective Medium for Culturing Actinomycetes**

| Malt Extract Agar (MEA) | |
|---|---|
| •Malt Extract: | 10 g |
| •Yeast Extract: | 4 g |
| •Glucose: | 4 g |
| •Agarose: | 15 g |
| •Distilled Water: | 1 L |
| •pH: | 6.2 |

① A selective medium designed exclusively for the cultivation of Actinomycetes was prepared and stored at low temperatures until use.

② A 1.5 mL microtube was filled with 1 mL of sterilized triple-distilled water filtered through a 0.2 μm pore-sized filter. The tube was shaken thoroughly to homogenize the mixture.

③ The prepared microtube was centrifuged at 6,000 rpm for 10 minutes to separate the supernatant containing soil bacteria.

④ Ten microliters (10 μL) of the supernatant containing soil bacteria were inoculated onto NA (Nutrient Agar) media. The inoculum was spread evenly using a spreader for 2–3 minutes. The NA media were placed in a sealed zipper bag containing an oxygen-absorbing and carbon dioxide-releasing patch, along with the selective medium for Actinomycetes. The bag was sealed and incubated at room temperature for 24 hours. This setup facilitated the growth of anaerobic soil bacteria, including Actinomycetes, as they thrive under oxygen-deprived conditions. All procedures were conducted under a clean bench.

⑤ Similarly, 10 μL of the supernatant was inoculated into 3 mL of LB broth. The inoculated broth was placed in a sealed zipper bag containing an oxygen-absorbing and

carbon dioxide-releasing patch. The bag was sealed and incubated at room temperature for 24 hours to cultivate anaerobic soil bacteria, including Actinomycetes. All steps were conducted under a clean bench.

⑥ One milliliter (1 mL) of the culture medium containing Actinomycetes was transferred into a UV-Vis spectrophotometer. The optical density (OD) was measured at a wavelength of 600 nm to assess growth dynamics and verify changes in methanogenic bacterial populations.

⑦ The same procedures (steps 1–6) were performed using unexposed, commercially available soil as a control group.

**2.1.12 Collection and Storage of Solution Containing Water-Soluble Aromatic Compounds Produced by Actinomycetes**

① Sterilization was performed by placing normal horticultural soil in an autoclave at 121°C and 1.5 atm for 15 minutes to eliminate all microorganisms from the soil.

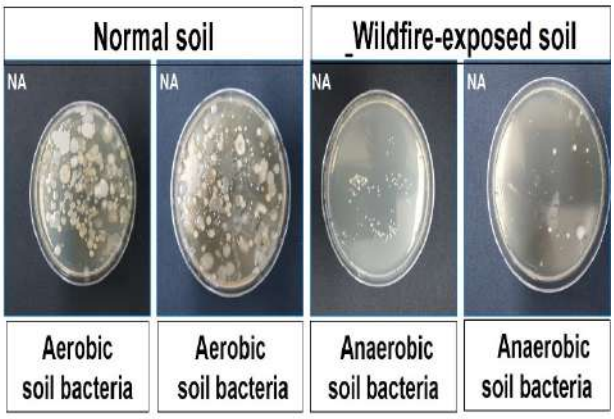
② After sterilization, 100 μL of culture medium containing Actinomycetes was inoculated into the sterilized soil. The soil was placed in the research apparatus and stored for one month. After one month, the lid of the sealed plastic container was opened, and the tertiary distilled water located between the petri dish containing the soil and the sealed container was collected. The collected water was transferred into a 15 mL conical tube and stored at 4°C under refrigerated conditions.

**2.1.13 Changes in Soil Bacterial Composition, Plant Growth, Soil Moisture Retention, Porosity, Organic Matter, Earthworm Growth, and Methanogenic Bacteria in Wildfire-Exposed Soil Treated with a Solution Containing Water-Soluble Aromatic Compounds Produced by Actinomycetes**

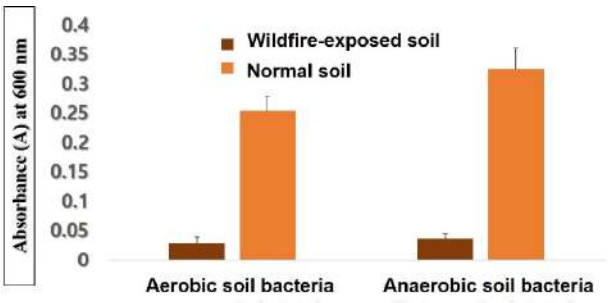
① Based on the previously described methodology, a solution containing water-soluble aromatic compounds produced by Actinomycetes was applied to wildfire-exposed soil. Changes were monitored in the following parameters: soil bacterial composition, plant growth, soil moisture retention capacity, porosity, organic matter content, earthworm growth rate, and methanogenic bacterial populations.

## 2.2 Research Results

### 2.2.1 Alterations in the Composition of Soil Bacterial Communities in Wildfire-Exposed Soil




**[Fig.9] Changes in Aerobic and Anaerobic Soil Bacteria in Wildfire-Exposed Soil**



**[Fig.10] Changes in Aerobic and Anaerobic Soil Bacteria in Wildfire-Exposed Soil Measured by Optical Density**

Wildfire-exposed soil showed a significant reduction in both the diversity and quantity of aerobic and anaerobic soil bacteria.

### 2.2.2 Changes in Plant Growth in Wildfire-Exposed Soil


**[Fig.11] Plant Growth Changes in Wildfire-Exposed Soil**

Significant inhibition of plant (cabbage) growth was observed in wildfire-exposed soil. This suppression is believed to be associated with the substantial reduction in both the quantity and diversity of anaerobic and aerobic soil bacteria, which are critical for regulating the cycling of organic matter. The lack of sufficient organic matter supply, resulting from this bacterial decline, likely impeded plant (cabbage) growth.

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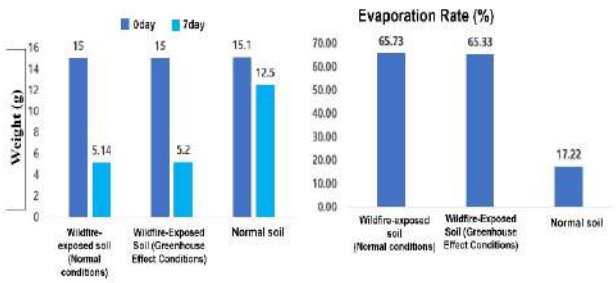
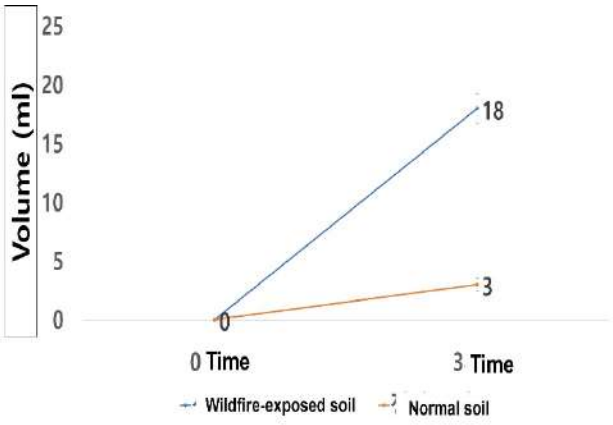
### 2.2.3 Changes in Moisture Retention and Porosity of Wildfire-Exposed Soil



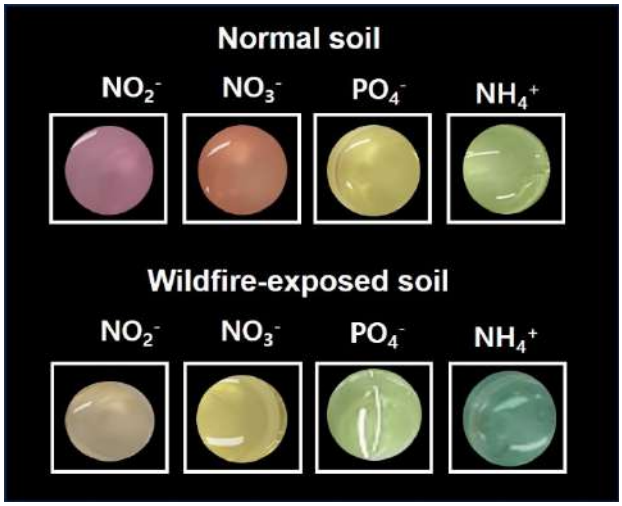

**[Fig.12] Changes in Moisture Retention Capacity of Wildfire-Exposed Soil**




**[Fig.13] Changes in Porosity of Wildfire-Exposed Soil**

In wildfire-exposed soil, moisture evaporation was found to be significantly accelerated, and the porosity between soil particles increased, preventing the soil from retaining moisture effectively. This phenomenon is likely directly related to the significant reduction in the diversity and quantity of anaerobic and aerobic soil bacteria, as observed in previous studies. These bacteria play a crucial role in regulating soil porosity, suggesting that their depletion in wildfire-exposed soil contributes to the loss of its moisture retention capacity.

### 2.2.4 Changes in Organic Matter Content of Wildfire-Exposed Soil

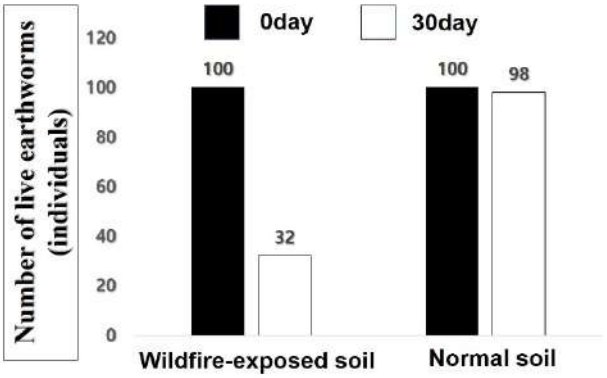



**[Fig.14] Changes in Organic Matter Content in**

**Wildfire-Exposed Soil**

The study revealed that the organic matter content in wildfire-exposed soil significantly decreases. This reduction is likely directly related to the substantial decline in the diversity and quantity of anaerobic and aerobic soil bacteria, which are responsible for the cycling of organic matter, as observed in previous research

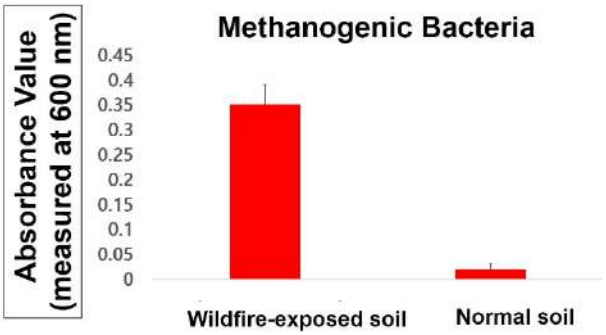
### 2.2.5 Changes in Earthworm Growth Rate in Wildfire-Exposed Soil




**[Fig.15] Changes in Earthworm Growth in Wildfire-Exposed Soil**

It was observed that earthworms could not survive normally in wildfire-exposed soil. Earthworms typically feed on soil and excrete nutrient-rich castings; however, wildfire-exposed soil is severely deficient in nutrients, which likely impedes their survival. This conclusion aligns with previous findings indicating a significant reduction in the organic matter content of wildfire-exposed soil, further supporting the connection between soil nutrient depletion and the inability of earthworms to thrive.

### 2.2.6 Growth Changes of Methanogenic Bacteria in Wildfire-Exposed Soil

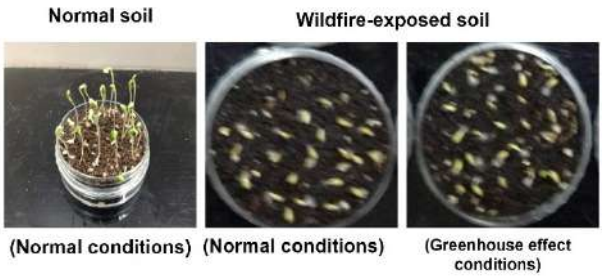



**[Fig.16] Growth Changes of Methanogenic Bacteria in Wildfire-Exposed Soil**

The study revealed that, while the overall number and diversity of soil bacteria significantly decreased in wildfire-exposed soil, methanogenic bacteria, which produce methane, showed a substantial increase in their population. This finding suggests that wildfires may exacerbate greenhouse gas emissions by promoting the growth of methane-producing bacteria, potentially leading to further environmental challenges.

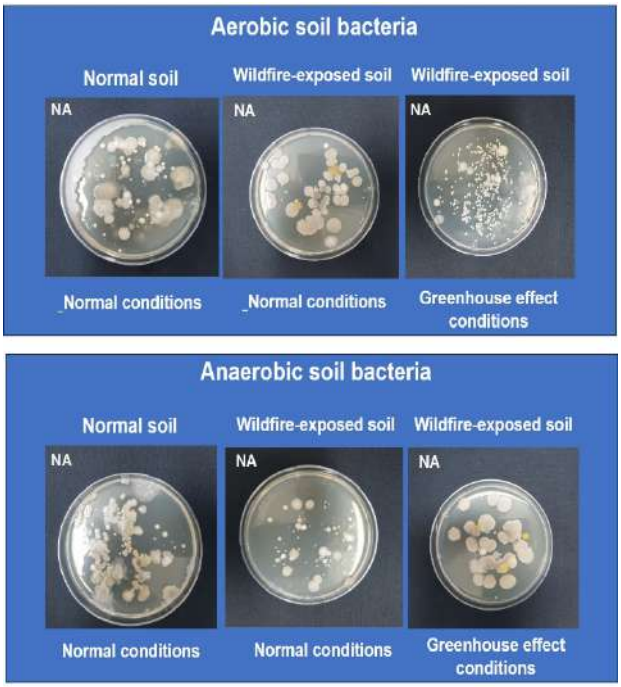
### 2.1.7 Assessment of Soil Recovery After One Month of Storage Under Greenhouse Effect Conditions Induced by Global Warming and Normal Conditions


**[Fig.17] Storage of Normal Soil and Wildfire-Exposed Soil for 30 Days**

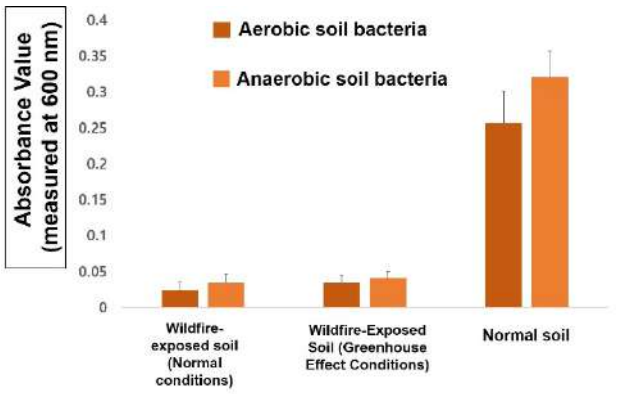



**[Fig.18] Plant Growth Results After 30 Days of Storage in Normal and Wildfire-Exposed Soils**

When wildfire-exposed soil was stored for 30 days and used to grow plants (cabbage), the plants were unable to grow normally. Additionally, it was observed that plants grew even less when wildfire-exposed soil was subjected to greenhouse effect conditions.

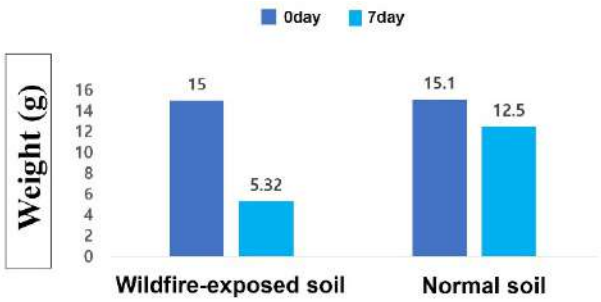


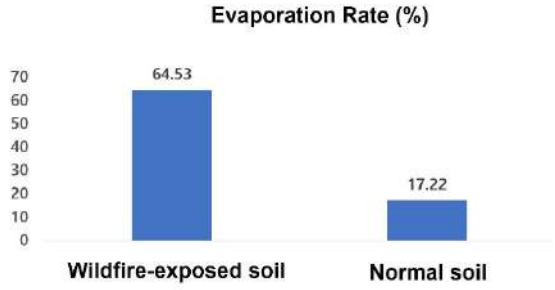

**[Fig.19] Changes in Aerobic and Anaerobic Soil Bacteria in Normal and Wildfire-Exposed Soils After 30 Days of Storage**




**[Fig.20] Changes in Aerobic and Anaerobic Soil Bacteria in Normal and Wildfire-Exposed Soils After 30 Days of Storage Using Optical Density Measurements**

The results showed that even after 30 days of storage, aerobic and anaerobic soil bacteria in wildfire-exposed soil were unable to grow normally. Furthermore, when wildfire-exposed soil was subjected to greenhouse effect conditions, the quantity and diversity of aerobic and anaerobic soil bacteria decreased even further.




**[Fig.21] Changes in Moisture Retention Capacity of Normal and Wildfire-Exposed Soils After 30 Days of Storage**

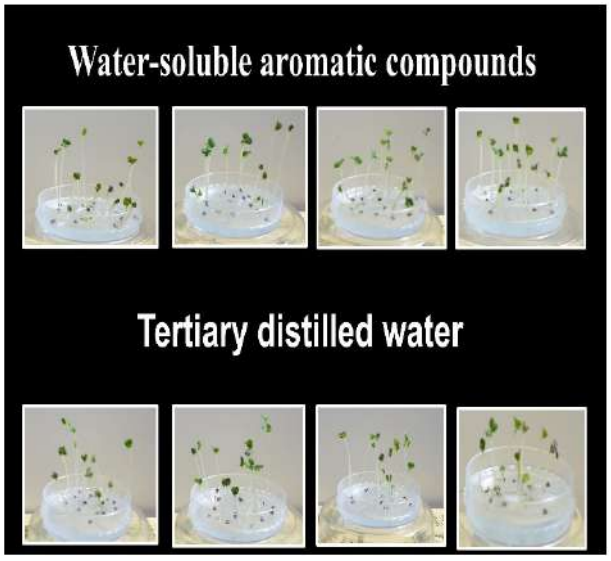
After 30 days of storage, wildfire-exposed soil still failed to recover its moisture retention capacity.

**2.2.8 Design and Development of Research Equipment for Collecting Water-Soluble Aromatic Compounds Produced by Soil, and the Collection of Such Compounds**

**[Fig.22] Collection of Water-Soluble Aromatic Compounds Produced by Healthy Soil**

Water-soluble aromatic compounds produced by healthy soil were collected and stored in a low-temperature refrigerator to prevent deterioration and contamination until they were used for research.

**2.1.9 Evaluation of Growth Changes in Plants (Cabbage) Exposed to Water-Soluble Aromatic Compounds Collected from Soil**




**[Fig.23] Effects of Water-Soluble Aromatic Compounds Produced by Healthy Soil on Plant (Cabbage) Growth**

Water-soluble aromatic compounds produced by healthy soil were found to have no direct impact on plant growth

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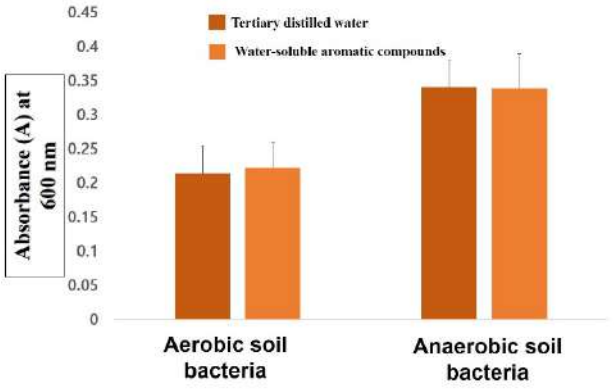
**2.2.10 Growth Changes in Soil Bacteria When Normal Soil is Treated with a Solution Containing Water-Soluble Aromatic Compounds Extracted from Healthy Soil**

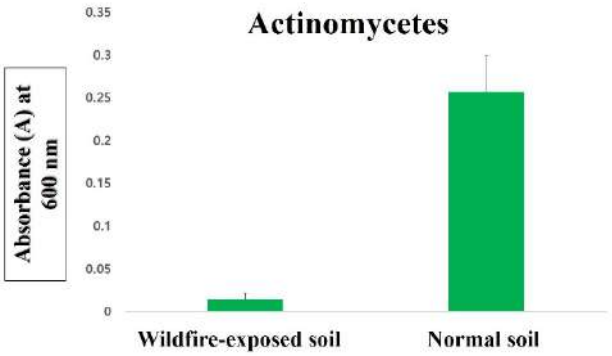



**[Fig.24] Effects of Water-Soluble Aromatic Compounds Produced by Healthy Soil on Soil Bacterial Growth**

Water-soluble aromatic compounds produced by healthy soil were found to have no significant impact on the growth of soil bacteria.

**2.1.11 Selective Cultivation and Preservation of Actinomycetes and Growth Assessment in Wildfire-Exposed Soil**

**[Fig.25] Actinomycetes Cultivated from Healthy Soil**




**[Fig.26] Distribution Changes of Actinomycetes in Wildfire-Exposed Soil**

In wildfire-exposed soil, the quantity and diversity of Actinomycetes were significantly reduced. This observation aligns with previous findings and suggests that Actinomycetes, which produce water-soluble aromatic compounds, are particularly vulnerable to heat compared to other bacteria in soil [8]. Consequently, their population decreases drastically when soil is exposed to wildfire conditions.

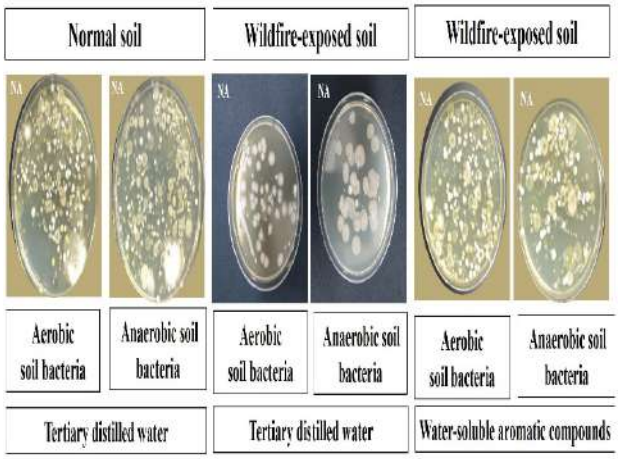
Based on these findings, a hypothesis was formulated: if Actinomycetes are cultivated and introduced into wildfire-affected soil, it may be possible to address the soil degradation issues caused by wildfires.

### 2.2.12 Collection and Storage of Solution Containing Water-Soluble Aromatic Compounds Produced by Actinomycetes

**[Fig.27] Collection of Water-Soluble Aromatic Compounds Produced by Actinomycetes in Soil**

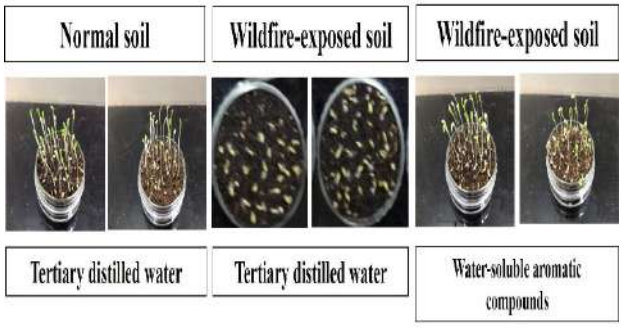
Water-soluble aromatic compounds were collected after selectively cultivating only Actinomycetes in sterilized soil.

### 2.1.13 Changes in Soil Bacterial Composition, Plant Growth, Soil Moisture Retention, Porosity, Organic Matter, Earthworm Growth, and Methanogenic Bacteria in Wildfire-Exposed Soil Treated with a Solution Containing Water-Soluble Aromatic Compounds Produced by Actinomycetes



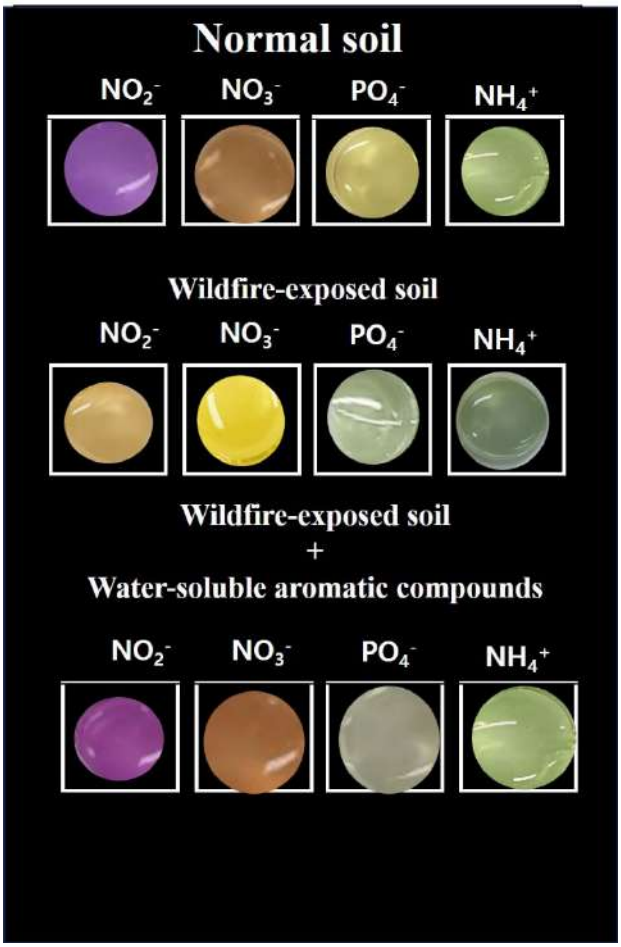

**[Fig.28] Changes in Growth of Anaerobic and Aerobic Soil Bacteria After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

The study confirmed that the growth of anaerobic and aerobic soil bacteria recovered in wildfire-exposed soil after the application of water-soluble aromatic compounds produced by Actinomycetes.



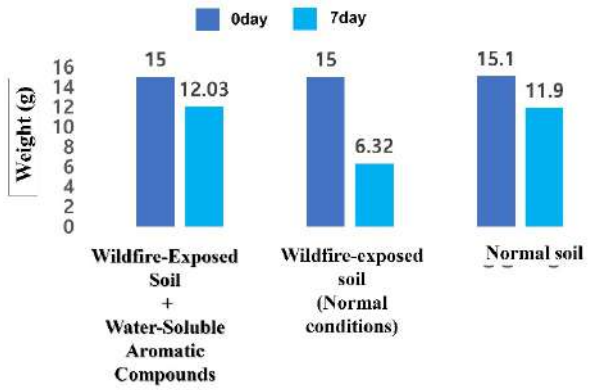

**[Fig.29] Changes in Plant Growth After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

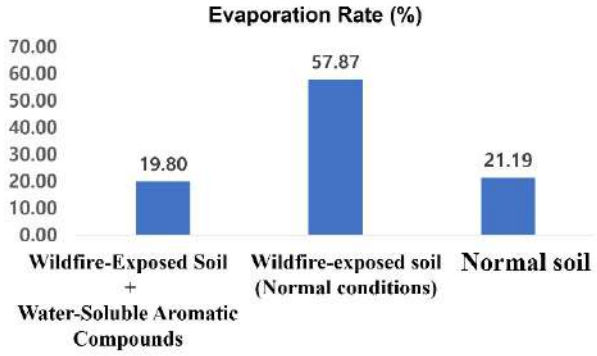
It was observed that plant (cabbage) growth returned to normal in wildfire-exposed soil following the application of water-soluble aromatic compounds produced by Actinomycetes.



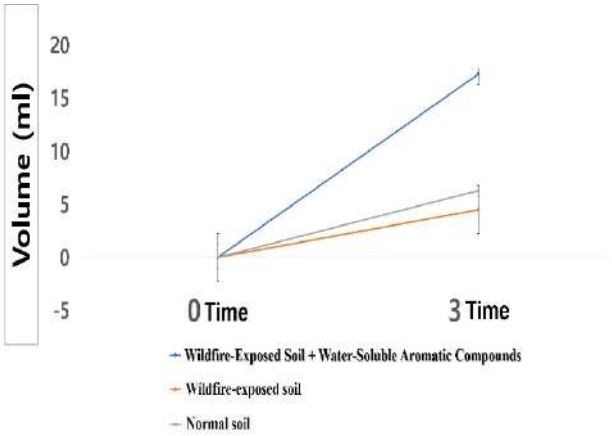

**[Fig.30] Changes in Soil Organic Matter After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

After applying water-soluble aromatic compounds produced by Actinomycetes to wildfire-exposed soil, the organic matter content recovered to levels similar to those of normal soil.



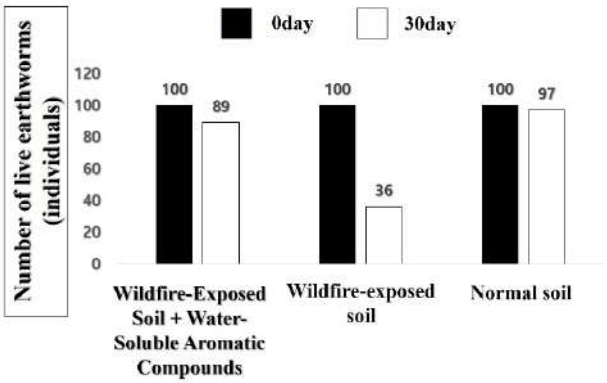




**[Fig.31] Changes in Soil Moisture Retention After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**



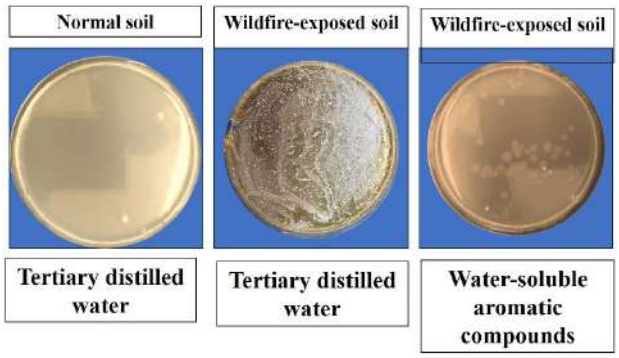

**[Fig.32] Changes in Soil Porosity After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

The study demonstrated that the application of water-soluble aromatic compounds produced by Actinomycetes to wildfire-exposed soil restored its moisture retention capacity and improved porosity to levels capable of holding water effectively.



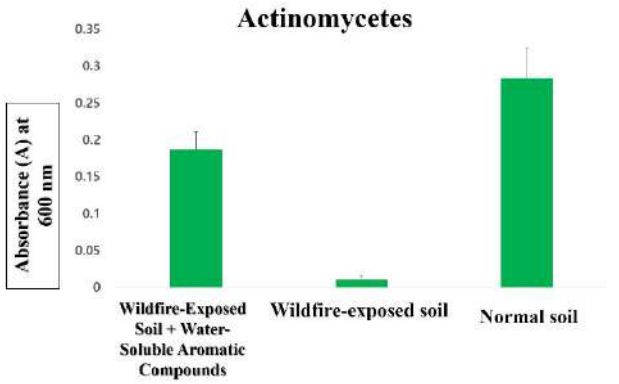
**[Fig.33] Changes in Earthworm Growth After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

The research confirmed that applying water-soluble aromatic compounds produced by Actinomycetes to wildfire-exposed soil created an environment where earthworms could grow and thrive normally.




**[Fig.34] Changes in Methanogenic Bacteria After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

It was observed that the application of water-soluble aromatic compounds produced by Actinomycetes to wildfire-exposed soil significantly reduced the population of methanogenic bacteria.




**[Fig.35] Changes in Actinomycetes Growth After the Application of Water-Soluble Aromatic Compounds Produced by Actinomycetes to Wildfire-Exposed Soil**

The study ultimately confirmed that the application of water-soluble aromatic compounds produced by Actinomycetes to wildfire-exposed soil restored the population of Actinomycetes.

## 3. Conclusion

The impacts of wildfires on soil were identified as follows: First, there was a significant reduction in the populations of anaerobic and aerobic soil bacteria, which are essential for the supply and cycling of organic matter. This decline resulted in a reduction in soil moisture retention and porosity, impairing the soil's ability to retain water and leading to increased evaporation compared to normal soil. Furthermore, the organic matter content in wildfire-exposed soil was significantly depleted, which inhibited plant growth. Additionally, the population of methane-producing bacteria, a major contributor to the greenhouse effect, increased substantially. It was also observed that wildfire-exposed soil showed no signs of natural recovery even after prolonged storage.

The investigation into the underlying causes of these issues revealed that **Actinomycetes**, the primary microorganisms responsible for producing water-soluble aromatic compounds in soil, are particularly sensitive to heat compared to other soil bacteria. Consequently, the number and diversity of Actinomycetes were drastically reduced in wildfire-exposed soil. To mitigate these issues, water-soluble aromatic compounds produced by Actinomycetes were extracted and applied to wildfire-exposed soil. This treatment restored the normal growth of Actinomycetes in the soil, effectively addressing various soil problems caused by wildfires.

Moreover, the study demonstrated that soil DNA content is closely linked to soil health. Soil DNA serves as a critical indicator of microbial diversity and activity, reflecting the richness and functionality of microbial communities that are vital for soil health. These communities play key roles in organic matter decomposition, nutrient cycling, and supporting plant growth. Higher soil DNA content signifies an abundant

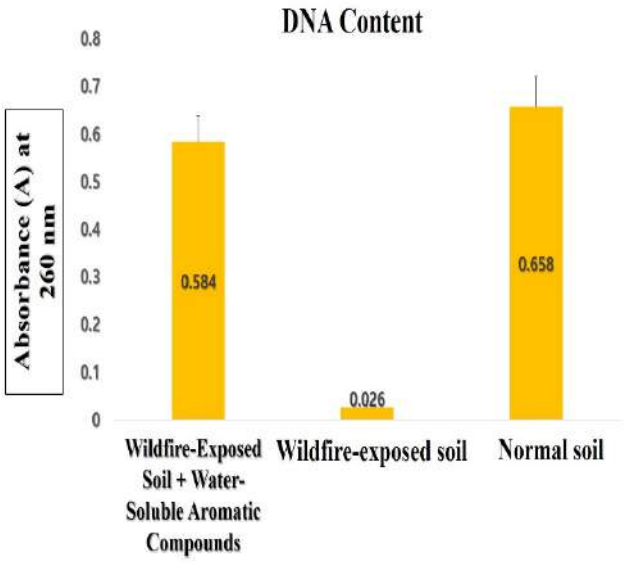
and active microbial community, which is a crucial characteristic of healthy and fertile soil.

The research compared the DNA content of wildfire-exposed soil, normal soil, and wildfire-exposed soil treated with water-soluble aromatic compounds produced by Actinomycetes.


**[Fig.36] Kit Used for Soil DNA Extraction**

DNA was extracted from wildfire-exposed soil, normal soil, and wildfire-exposed soil treated with water-soluble aromatic compounds produced by Actinomycetes using the **FastDNA® Spin Kit for Soil** (Product No. 116560200, 50 preps).

- Up to 500 mg of soil was placed into a Lysing Matrix E tube.
- Sodium Phosphate Buffer (978 µL) and MT Buffer (122 µL) were added to the tube.
- The sample was homogenized at a speed of 6.0 for 40 seconds using the **FastPrep® Instrument**.
- Centrifugation was performed at 14,000 x g for 5–10 minutes to separate the supernatant.
- The supernatant was transferred to a clean 1.5 mL microcentrifuge tube, and 250 µL of PPS was added. The mixture was manually shaken 10 times.
- After centrifugation at 14,000 x g for 5 minutes, the supernatant was transferred to a clean 15 mL tube.
- Binding Matrix (1.0 mL) was added to the supernatant, mixed thoroughly, and allowed to bind for 2 minutes using a rotator. The tube was then left for 3 minutes to allow the silica matrix to settle.
- Excess liquid was carefully removed without disturbing the binding matrix.
- The remaining mixture was loaded into a **SPIN™ Filter** and centrifuged at 14,000 x g for 1 minute. This process was repeated until all liquid was filtered.
- The filter was washed with 500 µL of SEWS-M buffer containing ethanol, followed by centrifugation at 14,000 x g for 1 minute.
- The **SPIN™ Filter** was air-dried for 5 minutes.
- DES solution (50–100 µL) was added to the filter, and the tube was incubated at 55°C for 5 minutes to enhance DNA yield.
- DNA was eluted by centrifugation at 14,000 x g for 1 minute and used for PCR or other downstream applications.
- DNA content was quantified using a UV-SPECTROPHOTOMETER at a wavelength of 260 nm.




**[Fig.37] Comparison of DNA Content in Wildfire-Exposed Soil, Normal Soil, and Treated Soil**

The results revealed that the DNA content of wildfire-

exposed soil treated with water-soluble aromatic compounds produced by Actinomycetes was restored to levels comparable to those of normal soil. This finding confirms that soil DNA content is a vital indicator of microbial diversity and activity, reflecting the richness and functionality of microbial communities that are essential for soil health. The restoration of DNA content demonstrates the recovery of microbial balance within the soil, indicating that the applied treatment effectively mitigated the impacts of wildfires on soil health.

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## 【評語】180026

Wildfire is one of the most important nature disturbances in the environment , driving restructuring microbiological system. This research were identified the populations of anaerobic and aerobic soil bacteria, which play a critical role in the decomposition and cycling of organic matter. The restoration of actinomycetes populations, enabling their normal growth in the affected soil. The number and diversity of Actinomycetes present a key role int the wildfire-exposed soil.