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Independence Effects of Heat and Ash on Forest Soil Nematode-Trapping Fungi Communities

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Abstract: Heat input and ash residues are main components of vegetation fire disturbances. Understanding the distinct impacts of heat and ash on soil microorganisms is crucial to comprehend and predict the induced changes in soil ecosystem composition and dynamics following different types of fire disturbances. This study's main goal was to track the dynamic shifts in the community of soil nematode-trapping fungi (NTF) following the separate disturbances of heat and ash by means of a specifically designed experiment conducted in the field. Four simulated fire treatments, one treatment reproducing natural burning, and one control treatment were taken into account. Every ten days following the disturbance, soil samples from each treatment were collected, and soil NTF and physicochemical characteristics were measured. The results showed that: (1) Heat drastically decreased the number of strains and heavily altered the NTF community, but it also encouraged the emergence of new NTF species. Instead, no overt changes were observed in the treatment that just experienced the addition of ash on the soil. (2) When compared to treatments that received only heat input, the lower strain count of NTF recovered more quickly in the natural burning treatment which was affected by both heat and ash input. These findings suggest that the disruptive effect of fire heat on soil NTF biomass may be counterbalanced by the emergence of new species and the repairing capabilities of new nutrients introduced by ash residue. In the future, both conventional and cutting-edge techniques should be considered in research designs to better understand the ecological role of fire disturbances occurring at different intensities, as well as the mechanisms that make soil ecosystems resilient to fire, in particular the role of new species.

Keywords: fire disturbance; fire factors; nematode-trapping fungi (NTF); community structure; restoration ecology

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1. Introduction

Fire is a natural evolutionary force that has shaped the spatial distribution and composition of the Earth's ecosystems for over 450 million years [1–3]. Under the influence of rapidly accelerating global warming and intensified human activities, the frequency and intensity of wildland fires are increasing, bearing important consequences on forest ecosystems [4–6]. In an effort to protect natural assets, post-fire vegetation recovery has emerged during the 21st century as a key research area in the field of restoration ecology which aims to understand the role and impacts of fire disturbances [7]. Previous studies based on fire occurrences in several ecosystems showed different degrees of impacts and recovery patterns, mainly due to the variety of burning conditions such as burn intensity or fuel type and load. This diversity of conditions leading to divergent results makes it difficult to design comparative studies and depict general patterns. Artificial experiments may represent an alternative approach to study ecosystem impacts of various components of fire disturbances (heat intensity, amount of biomass burned, etc.) because the input variables can be controlled and manipulated [8,9].

Soil microbes connect aboveground and underground ecosystems by breaking down organic matter and minerals, fixing nitrogen, degrading organic pesticides and heavy metals, consequently regulating plant growth [10–13]. Soil microbes are very adaptable and respond fast to the stress induced by natural calamities, such as drought, temperature change (warming), and fire occurrences, having an impact on the recovery of ecosystems [14–16]. This is why understanding post-disturbance mechanisms of soil microorganisms and their relationship with above-ground life is an important foundation for forest ecosystem recovery. However, these investigations are challenging to conduct due to the extremely rich microbial biomass and the complex structure of the soil microbial communities, which may be a reason for previous divergent or contradicting findings. Choosing an ecologically well-defined microbial community that can be easily observed at the species level may represent a valuable approach to overcome these issues and provide new advancements in the study of how fire affects soil microbes.

Nematode-trapping fungi (NTF) are a class of microorganisms living parasitic or saprophytic lives in various environments. They are essential for the regulation of nematode populations and for controlling the nitrogen cycle in the ecosystem [17]. NTF are composed by a relatively small number of species (only 116 species belonging to three genera), and many research laboratories have access to sophisticated technologies for effective species-level isolation and identification. Thanks to these characteristics, NTF were successfully adopted as a target group in previous studies on fire impacts on soil microbial community structure [18], which suggested that further investigations should be encouraged.

Yunnan province of southwest China experiences frequent wildfires. Among prominent vegetation types found in the region, the Yunnan pine (*Pinus yunnanensis*) features fire-dependent traits [19] and its dead needles falling on the ground pile up to form an easily flammable fuel. Moreover, it is a local custom to ignite mountain slopes during the Spring Festival to promote the growth of mushrooms used for food and medicine, and to maintain healthier pastures [20]. Forest management and protection authorities frequently perform prescribed burns to reduce the accumulated fuel and decrease the risk of catastrophic high-intensity fires [21]. Consequently, due to the diversity of fire characteristics, in particular the different intensity and amounts of biomass burned, we carried out an experiment under a controlled environment specifically designed to evaluate the isolated effects of heat input and ash deposit on the soil surface on NTF communities found in Yunnan pine forests. We hypothesized that: (1) different components of fires (contact with flame, heat, ash residuals) have different effects on soil NTF; (2) heat, as a major variable of burn intensity affecting soils, influences soil NTF according to its magnitude; and (3) different NTF species would react differently to heat or ash inputs. The experimental findings will provide insights into soil microbial dynamics after fire that can explain the phenomena observed during real wildfire events, contributing to the theory of fire ecology and serving fire management and restoration practices in Yunnan pine forests.

2. Materials and Methods

2.1. Study Site and Experimental Design

The surface soil (0–10 cm) and litter used in our study was collected in June 2019 in a forest area dominated by Yunnan pines (*Pinus yunnanensis*), located in Dali, Yunnan Province, China (100°9′ E, 25°38′ N, elevation: 2214 m a.s.l.) (Figure S1A). To assure comparability among burn experiments, the soil was mixed and blended to create a homogeneous soil environment (Figure S1C). Then, the soil was placed in ad-hoc experimental plot, which was built at the outdoor experimental lab of Dali University (100°17′ E, 25°68′ N, elevation: 2004 m a.s.l.) (Figures S1D and S2). The experimental plot consisted of six 20-cm deep rows, and each one was further divided into five 10 × 10 cm units, as shown in Figures S1F and S2. In total, there were 30 individual units well-separated from one another by heat-resistant bricks.

The experimental burns took place in June 2019. The six plot lines corresponded to five different treatment groups, hereafter defined as short-term flaming (FS), long-term flaming (FL), heating without flames (charcoal), cool ash (ash), and natural burning, as well as one control group, hereafter referred to as 'Control' (Figure S1G–I). Each group was replicated 5 times (5 units). These treatments are trying to simulate the isolated input of different fire components occurring during a wildfire, namely the contact with flame for different durations, heat, and ash deposition. They are summarized in Figure S1, and described as follows:

- Short-term flaming group (FS): representing a short time burn of 20 min. We used a flame gun to replicate the chemical reaction induced by the flames of a fire and the associated heat directly on the soil but without the residual ashes resulting from the biomass combustion.
- Long-term flaming group (FL): representing a long time burn of 60 min. In this treatment, the flame gun was used as in FS group, but for a duration of 1 h.
- Heating without flames group (Charcoal): In this treatment we placed 200 g of smoldering charcoal in a thin iron box and left on the soil units for 1 h, to expose the soil to heat without being directly affected by the contact with the flames and preventing ash inputs.
- Cool ash group (ash): We spread ash, after cooling (removing heat or flames' effects), over the soil units, corresponding to burning (in a different location) the same amount of litter layer accumulated on the pine forest stand (approximately 260 g for a 10 × 10 cm soil unit).
- Natural burning group: 260 g of the previously collected litter layer of the pine forest was placed on each soil sample and burned as in a natural surface forest fire.
- Control group: no treatment was performed on the soil units.

2.2. Soil Temperature Measurement and Soil Sampling

Prior to the treatment, two-channel thermocouples were put into the soil at a depth of 2 cm, 5 cm, and 10 cm to monitor the temperature variation during the experiments. Soil samples were then collected one day after the treatment and every ten days for a total of 100 days using a handmade, scaled, stainless-steel tube (Figure S3) along the whole soil depth of 0–10 cm. With 6 treatments and 5 replicates sampled 11 times, we obtained 330 samples for analysis.

2.3. Measurement of Soil Physicochemical Properties

Because changes in soil physicochemical properties require time after a fire disturbance, measurements were performed at the 1st, 50th, and 100th day after the treatments.

The soil moisture content (MC) was measured on fresh samples dried at 105 °C until the weight was stable (~8 h). Soil pH, Soil organic matter (OM), total nitrogen (TN), and total phosphorus (TP) were measured from naturally dried samples. Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5 *w/v*. Soil organic matter was determined using the $K_2Cr_2O_7$ oxidation method. TN was determined using the Kjeldahl method. TP was determined with $NaHCO_3$ extraction and H_2SO_4–$HClO_4$ digestion and analyzed using the molybdenum blue method.

2.4. Isolation, Identification and Count of NTF

The soil-sprinkling method was used to isolate NTF. Hence, 5 × 1 g of soil was spread on a Corn Meal Agar (extract of 20 g cornmeal, agar 18 g, adding water to the final volume of 1000 mL, CMA) plate, separately. Each sample was plated on 3 replica petri dishes which resulted in 15 replicates for each sample. To encourage the creation of the traps, nematodes (*Panagrellus redivivus*) were employed as bait. This nematode species is commonly used as food source for fish larvae and can be conveniently purchased online (http://www.atcc.org/ (accessed on 8 September 2018)), making it an optimal choice for studies on nematophagous fungi [22,23]. Then, pure cultures were obtained by single spore isolation. Finally, the purified NTF strains were identified using biological and morphological standard procedures described in the literature [18,22,24].

Because NTF could not form discernable colonies on CMA medium, the occurrence of a given species in the 15 replicates was used to represent its abundance [25].

2.5. Data Analysis

To quantify the effect of the various treatments on soil physicochemical characteristics, we plotted their deviation range from the reference Control group at the three assessment times (0, 50, 100 days after treatment). Differences in chemical characteristics as well as total species occurrences among treatments were evaluated for each sample taken over time using ANOVA with Fisher's LSD and Duncan test or nonparametric independent-sample Kruskal–Wallis test according to homogeneity of variance and distribution normality assumptions. The same tests were performed on every widely distributed species whose occurrence count was more than 10 within each treatment through the whole experiment.

Because during the treatments the temperature in the soil at 2 cm depth had the largest variation among the three depths, it was selected to analyze the relationship between the maximum temperature and NTF total occurrence count, which was conducted in GraphPad 7.0.

Community composition in the different treatments was investigated using cluster analysis, while dissimilarity of communities at different assessment times (every 10 days after treatment) was illustrated with a PCoA plot based on Bray–Curtis distance. Finally, a redundancy analysis was performed to evaluate the interrelationships between soil physiochemical characteristics and the NTF communities at the three post-treatment time points (0, 50, 100 days).

3. Results

3.1. Soil Physicochemical Properties

Significant differences ($p < 0.01$) among treatments were found for all five soil physicochemical characteristics at each of the three temporal samples (0, 50, 100 days after fire), with pH, OM, and TN showing the most pronounced differences (Figure 1). Pairwise comparisons revealed that heat input decreased soil moisture at all three temporal samples in the two flaming groups and the charcoal group, but soil pH, moisture, OM, TN, and TP increased in the treatments including ash input (Table S1).

3.2. Differences in the Counts of All NTF Strains

The counts of all NTF strains in the flaming, natural burning, and charcoal treatments were lower than that in the Control and the counts in the FL and charcoal treatments were significantly lower ($p < 0.05$). There was no discernible difference in the count of the ash treatment when compared to Control. When compared to the ash treatment, the count decreased significantly in FL treatments ($p < 0.05$), but not in the other experimental treatments (FS, charcoal, or natural burning treatments) ($p > 0.05$). There was also no discernible difference between the flaming and natural burning treatments or between the flaming or natural burning and charcoal treatments (Figure S4).

3.3. Relationship between NTF Community and Heat Input

After one hour of continuous flaming operation, FL treatment reached the highest temperature of 420 °C among the treatments at a soil depth of 2 cm. It is important to note that although the peak temperature in the charcoal treatment was lower than that in FS treatment, its duration was an hour as opposed to FS treatment's 20-min (Table S2).

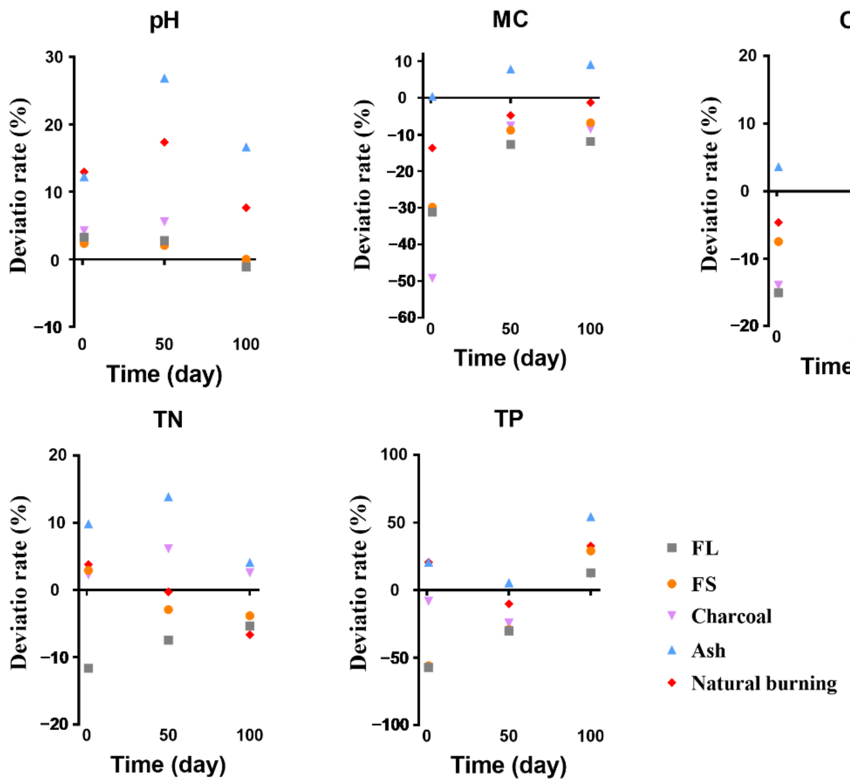

Figure 1. The deviation rate of soil chemical properties in the treatments when compared with Control. MC: moisture content; OM: organic matter; TN: Total nitrogen; TP: Total phosphorus. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with Charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any disturbance.

Peak temperature and the count of all NTF strains in each treatment were inversely correlated ($R^2 = 0.96$, $p < 0.01$). The higher the peak temperature, the lower the count. It is important to note that while the peak temperature in the charcoal treatment was lower than that in the FS treatment, the count of NTF in the charcoal treatment had a longer treatment time (1 h) than the FS treatment (20 min) (Figure 2).

3.4. Time-Varying Changes in the Counts of All NTF Strains

Through the whole experiment, the counts of all NTF strains were significantly lower in flaming, charcoal, and natural burning treatments than in the Control and ash treatments ($p < 0.05$) and the only exception is the natural burning treatments at the 80th day when the count was higher than that in Control. However, from the 90th day, it dropped sharply without recovering to the initial level by the end of the experiment. There was no significant difference between flaming, natural burning, and charcoal treatments ($p > 0.05$). In the ash treatment, the counts were constantly maintained at high levels, similar to those of the Control, showing no significant difference ($p > 0.05$). Viewed from the overall fluctuation, the counts in the flaming and charcoal treatments varied less in the first half of the experiment, especially at higher temperatures. However, in the second half of the experiment, the volatility was higher. In the natural burning treatment, the fluctuation was more obvious in the early stage than in the late stage. In the ash treatment, the fluctuation was less pronounced in the early stage of the experiment, but later it increased gradually to converge with the level of the Control (Figure 3 and Figure S5).

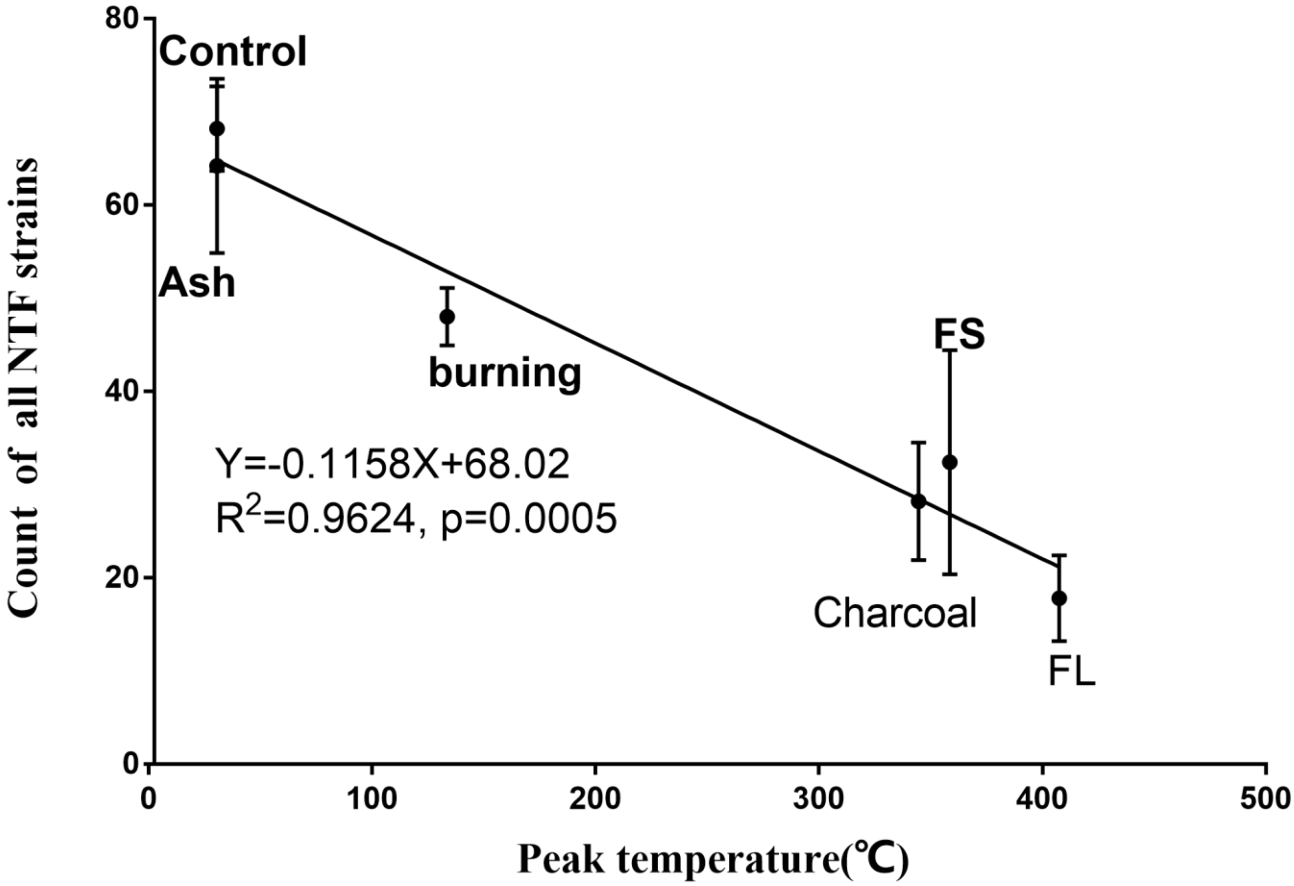

Figure 2. Relationship between the average count of all nematode-trapping fungi (NTF) strains in each treatment and peak temperature.

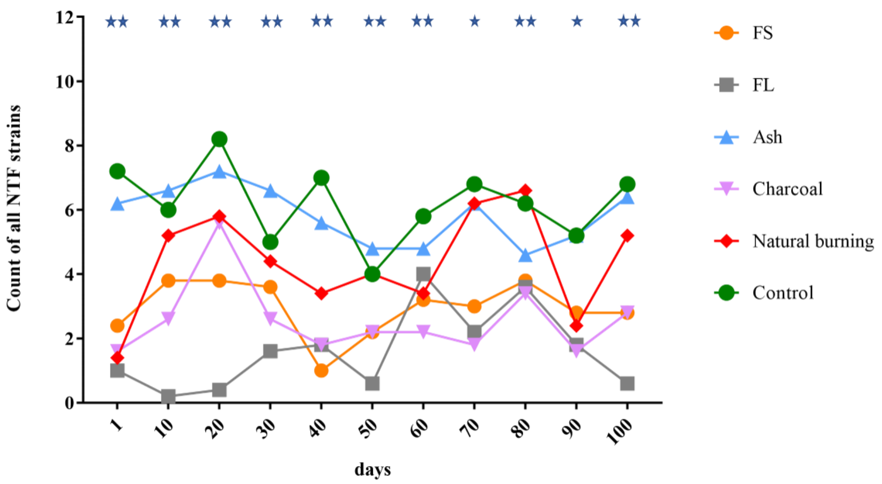

Figure 3. Dynamic of the counts of all nematode-trapping fungi (NTF) strains in each treatment and difference among treatments. ★: $p < 0.05$; ★★: $p < 0.01$. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with Charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any treatment.

3.5. Time Variation in the Counts of the Widely Distributed NTF Species

Arthrobotrys musiformis, *A. thaumasia*, *A. oligospora*, and *Dactylellina parvicolla* all had counts above 10. Hence, they were classified as widely distributed NTF species.

Based on how the same NTF species responded to different treatments: (1) The counts of *A. musiformis* in the flame, natural burning, and charcoal treatments considerably dropped at the beginning, followed by a slight recovery as time went on, but remained lower than the Control at the end of the treatment. In general, the ash treatment matched the Control. Statistical analysis revealed that, with the exception of the 60th day, there was a significant difference ($p < 0.05$) among the treatments at every time point. (2) The variance in the counts of *A. thaumasia* was quite comparable to that of *A. musiformis* except from the 50th day when the counts were at their lowest. Statistical analysis revealed a significant difference ($p < 0.05$) among treatments at each time point, with the exception of the 50th and 60th day. (3) The counts of *A. oligospora* did not change significantly ($p > 0.05$) during the majority of time intervals, with the exception of the 1st, 30th, and 70th days and these counts remained at extremely low levels across all treatments. However, on the 30th day, the natural burning and ash treatments experienced a considerable increase, but then another sharp decrease followed. (4) The counts of *Dac. parvicolla* remained extremely low and did not differ significantly ($p > 0.05$) from the Control at any time points except for the 40th, 60th, and 80th days under any treatment (Figure 4).

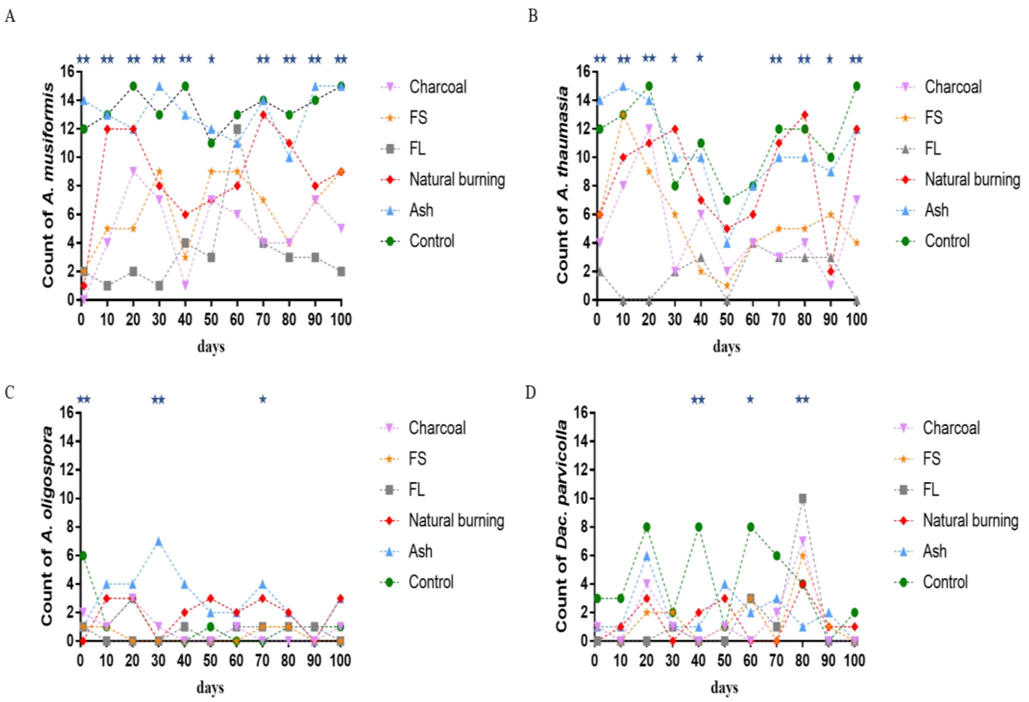

Figure 4. Dynamic of the counts of the widely distributed species in each treatment and difference among treatments. A: *A. musiformis*; B: *A. thaumasia*; C: *A. oligospora*; D: *Dac. parvicolla*. ★: $p < 0.05$; ★★: $p < 0.01$. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any treatment.

When comparing the responses of the different species to the same treatment, it can be observed that the variation range of the species with high content in their natural habitat (*A. musiformis* and *A. thaumasia*) was greater than that of the species with low content (*A. oligospora* and *Dac. parvicolla*) when heat input was present during fire disturbance (Figure S6).

3.6. Differences within the Composition and Structure of the NTF Community

According to cluster analysis, the communities of the flaming and charcoal treatments were more similar to one another than those of the natural burning and ash treatments, which were more similar to the Control (Figure 5). The order of the polygon areas formed by the NTF communities at each time point in the different treatments was FL > Charcoal > FS > Natural Burning > Ash > Control. The larger the polygon, the higher the variation it represented. Additionally, polygon placement of ash treatment was near Control, whereas the others were farther away (Figure 6).

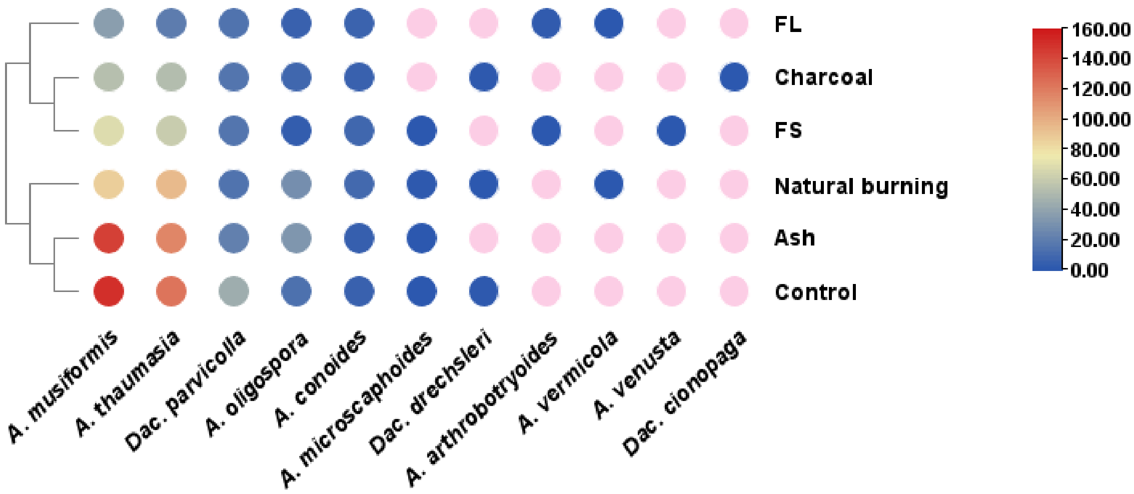

Figure 5. The nematode-trapping fungi (NTF) community composition of each treatment. Pink plots indicate no detection. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with Charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any treatment.

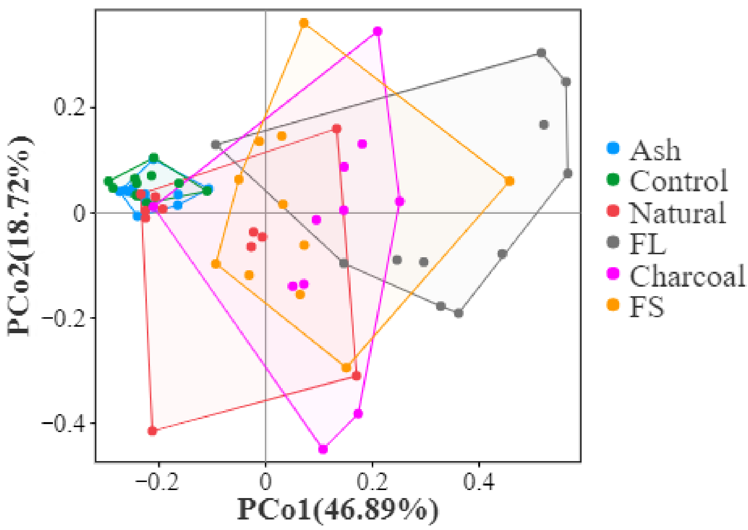

Figure 6. Principal coordinates analysis (PCoA) ordination plot showing the changes of nematode-trapping fungi (NTF) communities in each treatment at different time points. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with Charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any treatment.

Additionally, new species, that were not isolated in the Control appear following the flame, natural burning, and charcoal treatments. In particular, two species (*Arthrobotrys arthrobotryoides* and *Arthrobotrys vermicola* in FL, and *A. arthrobotryoides* and *Arthrobotrys venusta* in FS) appeared in the flaming treatments, while one species appeared in the charcoal treatment (*Dactylella cionopaga*) and one in natural burning treatment (*A. vermicola*) (Figure 5).

3.7. Interrelationships between Soil Physicochemical Properties and NTF Community

Redundancy analysis revealed that Axis1 explained 54.61% of the variation of the NTF community while Axis2 explained 8.07%. The physicochemical characteristics of the soil, specifically OM and TN, were significantly associated with the NTF community ($p < 0.05$) and accounted for 43.6% and 22.9%, respectively, of the variation of NTF community (Figure 7 and Table S2).

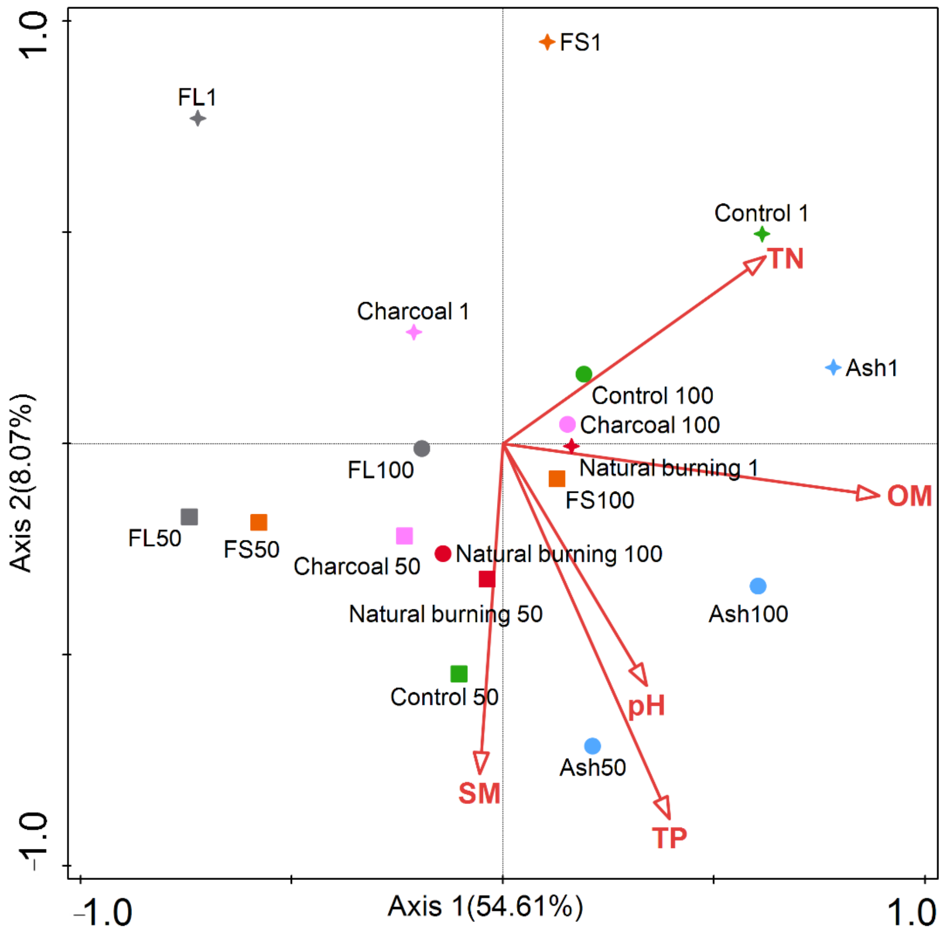

Figure 7. Constrained ordination diagram for samples in the first two redundancy analysis axes based on the soil physicochemical characteristics of the different treatments and their interrelationships with the structure of the soil nematode-trapping fungi (NTF) communities. FS: treated with flame gun and the burn duration was 20 min; FL: treated with flame gun and the burn duration was 60 min; Charcoal: treated with Charcoal; Ash: treated with cooled ash; Natural burning: treated with dry pine needles placed directly on the soil and let burn naturally and ashes were left on the soil after burn; Control: without any treatment. Numbers behind the group name shown the detection time point. 1: The 1th day; 50: The 50th day; 100: The 100th day.

4. Discussion

4.1. Effects of Heat Input on Soil NTF Communities

According to the changes in soil NTF communities observed in the experiment, there was a substantial linear relationship between the counts of NTF strains and peak temperature: the higher the peak temperature, the lower the counts, leading to more extended recovery time. The number of NTF strains also varied depending on the duration of the high temperature period. The number of NTF strains decreased with increasing duration at constant peak temperature. These findings are in line with the findings of the microbiological alterations in soil caused by fire disturbance [26,27]. So, it is clear that key elements in determining the effects of fire disturbances on soil NTF are the peak temperature and the duration of the high temperature period. However, their effects on how microbial communities are organized have not been addressed in studies based on simulation trials and wildland fires, including this one. Given that different species have varied limits on their ability to tolerate high temperatures, the peak temperature may be more significant for species diversity while peak temperature duration primarily affects overall biomass. In order to investigate the impacts of peak temperature and duration in the future, it is important to strengthen the intended experimental investigations.

Since heat is more easily transferred into deep soil at higher temperatures and for longer periods of time [28,29], the effects on the microorganisms dwelling in the deep soil layer would be larger. Recent studies revealed that deep soil-dwelling microorganisms played a decisive role in postfire recovery [30], especially as a germplasm resource for the recovery of microbes in shallow soil [18]. The upward migration of microorganisms from the deep soil to rebuild the microbial communities in the shallow soil will therefore be weaker as a result of the negative effects created by prolonged high temperatures, which will affect the recovery of the entire ecosystem. Furthermore, the higher the temperature, the greater the impact on the soil quality, particularly physical properties, such as water-holding capacity, which will be greatly reduced [31,32].

It should be highlighted that although heat has a negative impact on the entire biomass, new species appeared in all experimental treatments that included heat input (Figure 5), which is consistent with earlier comparable findings [33–35]. Unfortunately, there has not been much research conducted on the mechanisms underlying the appearance of new species in burned areas. Our earlier research indicated that following fire disturbance, the dominant NTF distributed in shallow soil drastically declined, and their vacant niches were subsequently filled by the dominant NTF that were initially distributed in deeper soil [18]. This could be a contributing factor in the appearance of new species. Second, heat input encouraged the resurgence of dormant species [36] and the rapid reproduction of species whose biomass was low prior to fire disturbance, making them detectable following fire disturbance [26,37,38]. In conclusion, while the heat from fire would have a negative impact on soil microbial biomass during a short period of time, it would have a favorable impact on the ecosystem's species diversity [39–41]. Therefore, research on the emergence of new species, particularly their role in ecosystem restoration following fire disturbance, should be of concern.

4.2. Effects of Ash on Soil NTF Communities

For a long time, researchers were concerned about the influence of ash created by fire on soil microorganisms and research findings pointed to changing soil physical and chemical properties indirectly affecting soil microorganisms [42]. Our findings support this evidence in part: on the 50th day following the addition of the ash, the pH, OM, and TN did change noticeably (Ash treatment). However, the independent impact of ash on the overall biomass (the total number of strains identified) and the community structure of NTF was not immediately apparent. Furthermore, it is important to note that even while the overall biomass in the natural burning treatment dramatically decreased, it recovered quicker than in other heat input treatments. This demonstrates once again that heat has a detrimental effect on soil NTF, and it also suggests that ash may help the soil NTF recover after fire interference, indicating that more consideration needs to be given to the role of ash in post-fire recovery. Of course, changes in the soil's physicochemical characteristics, such as soil moisture, pH, organic matter, amount of nitrogen, phosphorous, and potassium, only occur when the ash is incorporated into the soil through rain, freeze-thaw cycles, or the activity of earthworms and other creatures [43]. Each of these processes requires time to complete. Overall, ash had a weak independent impact on soil NTF, but it could help the community recover after heat disturbance. More study on the role of ash in ecosystem recovery as well as hysteresis effects should be performed.

4.3. Interference of Fire on Different NTF Species

Different microbial species were shown to be differently susceptible to heat [44–47]. However, these findings came from studies on the combined effects of heat and ash. In this study, we found that the counts of the four widely distributed NTF species significantly fell in the charcoal and the two burning treatments (FS & FL), demonstrating that the NTF reaction to heat was nearly universal. When the impact of ash was taken into account, the patterns of change diverged: the number of *A. oligospora* grew while the numbers of the other three species decreased, with *Dac. parvicolla* suffering the greatest fall (>50%). *A. oligospora* belongs to the NTF genus *Arthrobotrys*, which has a strong saprophytic capacity [48,49] and can effectively utilize organic substances. When ash was added, the organic matter in the soil was enhanced and the growth of *A. oligospora* was encouraged significantly. In contrast, the genus *Dactylellina* member *Dac. parvicolla* preferred to enhance predation following fire [50]. Hence, ash had little impact on the biomass recovery of *Dac. parvicolla* after the fire. These observations suggest that NTF species differ in how they react to fire disturbance not only due to differences in how sensitive they are to heat, but also other factors, such as organic debris [42]. Therefore, relying just on the differences in susceptibility to heat to explain the impacts of fire on soil microbes is insufficient.

On the other hand, a variety of responses to fire disturbance also offer the chance for soil microbes and even the soil ecosystem to recover quickly following the fire. Future research on soil microbial community structures following fire should concentrate on the processes, growth characteristics, ecological roles, and environmental adaptability [51]. Technology at the gene level will not be sufficient. Instead, conventional cultural methods, sophisticated function genomics, and metabonomics will be necessary to accomplish these tasks. NTF generate several natural compounds, and knowledge on these substances allowed for its usage as a model organism for studies on gene function [22]. Therefore, NTF can be used as a model species in the future to research the ecological implications of soil microbial community changes following a fire, to investigate how organisms adapt to their new environments after a fire and how they shape those environments, and to provide more insightful data for ecological restoration following fire.

4.4. Limitations of Designed Fire Experiment

To our knowledge, this is the first attempt to disentangle the various factors interacting during a fire disturbance and affecting soil microorganisms using a new methodological approach. Fire is a dynamic process where several factors, such as fire intensity, fuel load, site conditions, and wind, determine the resulting ecological effects. As such, it is very difficult to understand the complex mechanisms regulating soil microorganisms after fire, and our method inevitably has some limitations. For example, the fireline intensity is dependent on the fuel load, and the fire severity will determine the amount and quality of ash that will be deposited on the soil surface. In this sense, it is difficult to robustly infer the relative contribution of fire types/behavior and combustion products to the soil dynamics in our experiment. Even so, our results provide new and interesting insights. More experiments are planned to tackle these problems.

5. Conclusions

The primary variables implied in a fire disturbance, heat and ash, have divergent effects on soil NTF and different effect processes. Heat has a negative impact on the biomass of soil NTF and obviously changes the NTF community structure. The impact was influenced by the duration of the high temperature and was positively correlated with the temperature peak. Ash has little impact on the community or biomass of the NTF but supports the recovery of NTF from the negative effects of heat. For post-fire restoration purposes, it is recommended to carefully consider the peak temperature and duration of the fire disturbance, as well as the contribution of ash residues providing nutrients influencing the composition and succession of soil microbial communities after fire.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fire6010027/s1, Figure S1: Study site and treatment; Figure S2. Drawing of the experimental plot illustrating how the various treatments are arranged; Figure S3. Handmade soil sampler (left: sketch, right: picture); Figure S4. Counts of all nematode-trapping fungi (NTF) strains in each group. Error bars show the SD of the absolute values; Figure S5. Difference in the counts of all nematode-trapping fungi (NTF) strains among groups at each time point with t test; Figure S6. Variation over time in the counts of widely distributed species; Table S1 Soil physicochemical properties of each group at the three time points; Table S2 The peak temperature at a depth of 5 cm in different groups; Table S3 Simple effects of soil Physicochemical properties on nematode-trapping fungi (NTF) communities.

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