An initial study of woody-debris decomposition to reduce risk of repeated-fire incidence in tropical peatland ecosystem

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Abstract. As peatland ecosystems were formed from layered partially decomposed plant biomass, they were considered more vulnerable to fire, especially during extreme drought season. Woody debris accumulation in the field may increase the risk of peatland fire. In order to minimize the chance of repeated fire, an initial study on woody debris decomposition by employing a consortium of wood-decay microbes (consists of Scedosporium apiospermum, Pycnoporus sp., Pycnoporus sanguineus, and unidentified cellulolytic bacterial isolate) was conducted. Series of experiments of in vitro-, semi-controlled-, and field- conditions were carried out. After 12-weeks of incubation, the in vitro trial showed that all treatments on mineral-soil basal media were colonized by fungal mycelia, including the control. Meanwhile, the treatments on peat soil seem less supportive for fungal growth since only six out of ten treatments have been colonized by fungal mycelia. In semi-controlled conditions, effects of microbial inoculation showed questionable results as the trials were randomly occupied by Schizophyllum commune, which was not included in the microbial inoculants. Un-clear effects of the microbial inoculants were also observed on the field trial as no significant difference of dry-weight loss between the inoculated woody logs and the un-inoculated control. Further comprehensive studies to reduce woody debris in peatland areas are required.

1. Introduction
Tropical peatlands formed from layered partially decomposed biomass in the wetlands have unique characteristics and significant roles as the terrestrial carbon storage and water reservoir [1] and have prominent roles in conserving the biodiversity of several endangered-endemic species [2]. Peat soils are formed when the accumulation rate of organic matter exceeds its decomposition rate. The slow decomposition rate is associated with the inhibition of microbial activities due to anaerobic- and acidic-environmental conditions [3].

Recent conditions indicate that the peatland ecosystems are becoming more responsive to climate change [4]. The effects of temperature, rainfall, and other climatic variables on carbon storage, greenhouse gases (GHG) fluxes, and peat biodiversity have been widely reported [5]. In comparison to any other forest ecosystems, peatlands – especially the degraded ones – are more vulnerable to fire [6]. For example, the tropical peatland in Central Kalimantan has degraded due to agricultural expansion, land conversion into other land uses, such as the expansion of oil palm plantation, which leads to the
drained of the peatlands and makes them more susceptible to fire [7, 8]. Furthermore, during the extreme drought season, the peatlands' susceptibility to fire is increased [9].

The peat fires produce large amounts of smoke that affect human health and social activities and result in severe ecosystem damages such as increased carbon emissions, biodiversity loss, and abundant plant residues. In addition, the accumulation of agricultural waste, standing and dead left in the field, which can act as fuel for the next unintentionally fires, is more likely to increase the chance of repeated fires [10]. The phenomenon of El Nino elevates this hazardous risk [11].

Reducing fuels (the plant residues) through prescribed burning is one of the strategies that may reduce the risks of wildfire incidence [12]. However, applying it in the peatland areas is perilous as the fire may develop uncontrolled. There is an idea to decompose woody biomass as an alternative way to reduce flammable materials as well as re-cycle the organic carbon to plant nutrients. As a part of a biogeochemical cycle, the decomposition of plant residues consisting of lignocellulosic compounds naturally occurs in every type of ecosystem, including in the peatlands. However, because of the variation of lignocellulose proportion and composition, the degradation rates of each plant part are various. Moreover, the decomposition of woody debris is not as easy as other plant parts, e.g., leaf litters, due to its lignin content and the degree of cellulose crystallinity [13].

Decomposition which is carried out by microorganisms and resulting CO2 efflux into the atmosphere, seems contradictory to the primary role of peatlands as the global carbon stores. However, decomposition always occurs in nature, even in un-disturbed natural peatland ecosystems, as a part of the carbon dynamic on the earth [14]. Regarding the main role of peatland ecosystems as global carbon stocks, the efforts to prevent repeated fires by accelerating woody debris decomposition is a challenging process and requires deep considerations when applied in large areas. The process should be well-designed in order to address the uniqueness and complexity of peat ecosystems as well as ensure that woody-debris decomposition can be well-managed. Thus, in order to accelerate the woody-debris decomposition process in the peatland ecosystem, this initial study tested the feasibility of employing microbial consortium to decompose woody materials through series of experiments in in-vitro-, semi-controlled-, and field-conditions.

2. Materials and Methods

2.1. Microbial isolates and preparation of woody-debris specimens

Several microbial isolates and their combination were used for the in-vitro test as listed in Table 1, while for the semi-controlled condition test, only the mixed isolates (S9) was applied and compared to the control.

In these trials, the microbial inocula were applied in the form of either spores- or cells- suspensions for fungal or bacterial isolates, respectively. Before the application, the fungal isolates were cultured on sterilized, moistened wood chips in flasks, incubated for 1 – 2 weeks at 30°C in a shaker incubator to induce fungal sporulation. Spores were extracted by pouring 50 ml sterile water into the flask and soaking it, and then the suspended spores were poured into other sterile flasks. This process was repeated three times to ensure that the spores were suspended as much as possible. The bacterial cells suspension was prepared by culturing a 10µl-loop of the bacterial colony into 50 ml NB media and incubated for two days at 30°C in a shaker incubator.

Various wood samples were used at the three different stages of this study. It corresponded to the availability of woody debris at the time and place the study was conducted. The wood samples for the in-vitro study were random (the plant origin was unknown) because they were collected from branches scattered around the arboretum of the Forest Research and Development Center in Bogor. In the semi-controlled trials, woody rods of Agathis sp. and Araucaria sp. were used, while in the field trials, the log segments of Cratoxylon arborescens and Shorea balangeran were used for the experiments. All woody samples were sterilized twice at 121°C, 1 atm, 30 minutes prior to the application of the treatments.
Table 1. Variation of spore suspension applied for in vitro wood-decomposition test.

| No. | Code | Description |
|-----|------|-------------|
| 1   | S0   | Control : sterilized water |
| 2   | S1   | Spore suspension of *Scedosporium apiospermum* st.1 |
| 3   | S2   | Spore suspension of *Scedosporium apiospermum* st.2 |
| 4   | S3   | Spore suspension of *K.C* (fungal isolate) |
| 5   | S4   | *Mixture 1*: S-1, S-2, and S-3 (1:1:1) |
| 6   | S5   | Spore suspension of *Pycnoporus* sp. |
| 7   | S6   | Spore suspension of *Pycnoporus sanguineus* |
| 8   | S7   | *Mixture 2*: S-5 and S-6 (1:1) |
| 9   | S8   | Cells suspension of *K.G* (bacterial isolate) |
| 10  | S9   | *Mixture 3*: Mixture of all microbial isolates |

2.2. Wood decomposition in controlled condition (in vitro test)
The in-vitro test was carried out in the INTROF-CC laboratory by inoculating spores suspension of the tested isolates onto sterile wood rods (diameter & length ± 2cm), which were placed on two different basal media (sterilized mineral- or peat- soils) in 300ml-jars (Figure 1). Variation of treatment was listed in Table 3. The experiment was conducted for 12 weeks. Fungal colonization on the wood surface was observed, and dry-weight reduction of the wood samples was measured.

![Figure 1](image)

Figure 1. Wood-rods on basal media (mineral soil or peat) for in vitro wood decomposition test.

2.3. Wood decomposition in semi-controlled condition
The semi-controlled trial was carried out in an outdoor environment around the INTROF-CC laboratory in Bogor. Sterile wood rods were put on the sterilized basal media (mineral soil or peat) in clean perforated plastic boxes, as described in Figure 2. Spore suspension of microbial consortium (S-9) was sprayed along the wood surfaces, then incubated outdoor for 12 weeks. Treatments combination of four variables conditions, *i.e.*, (1) basal media: mineral soil (pH ± 7) and peat (pH ± 4); (2) wood species: *Agathis* sp. and *Araucaria* sp.; (3) aerobic conditions: fully- and less- aerobic; (4) microbial inoculations: un-inoculated controls and inoculated ones, were applied. Reduced dry-weight of wood samples pre- and post-treatments were recorded. Any visual signs of microbial growth were observed weekly.
Figure 2. Semi-controlled conditions of wood decomposition trials. The less-aerob boxes were covered by the porous plastic lid; the fully-aerob boxes were covered by gauze materials.

2.4. Wood decomposition in field condition
The field trials were conducted in two different areas, i.e., in Banjarbaru, South Kalimantan (for unflooded condition) and Pulang Pisau, Central Kalimantan (for flooded/inundated condition). The consortium of microbes applied in these trials consist of Pycnoporus sanguineus, Pycnoporus sp., Nodulisporium sp., Earliella scabrosa, and Schizophylum commune spores. The addition of S. commune spores in this trial refers to the trial results in semi-controlled conditions, which showed its high adaptability in colonizing wood logs. The trials were taken into account three variables, i.e. (1) wood types: logs of Cratoxylon arborescens and Shorea balangeran; (2) peat conditions: un-flooded and flooded/inundated peats (Figure 3); (3) inoculation: un-inoculated controls and inoculated ones. The trials were observed for six months. Dry-weight of wood samples pre-and post-treatment, as well as any visual signs of fungal growth, were recorded. At the end of observation, fungal diversity in the wood logs was assessed by re-isolating fungal culture from the wood samples.

Figure 3. Wood decomposition trials in field condition (unflooded- and inundated- peats).

3. Results and Discussion
3.1. In vitro trial
The use of mineral soil and peat as basal media in the in-vitro trial was aimed to evaluate the influence of these two different media, which have different pH levels, on microbial colonization of the wood samples. Results showed that both mineral soil and peat were sufficient for the growth of fungal spores (Figure 4), but the mineral soil showed better influence than the basal peat media. After 12 weeks of incubation, all wood samples that were put on the mineral soil have been successfully colonized by fungi, even for the control (S0), in which 67% of the wood samples were colonized by fungal mycelia.
Meanwhile, the basal peat media appears to be less conducive for the growth of fungal decomposers. 6 of 10 and 9 of 10 of the treatments on the peat- and mineral- soils, respectively, showed 100% mycelial colonization (Figure 5, indicated by the blue line in the graph).

![Figure 4. Invitro wood decomposit ion test: Fungal growth on the wood samples.](image)

*Figure 4. Invitro wood decomposit ion test: Fungal growth on the wood samples.*

![Figure 5. Percentage of weight loss of woody-rod samples and mycelial colonization after 12-weeks in vitro decomposition.](image)

*Figure 5. Percentage of weight loss of woody-rod samples and mycelial colonization after 12-weeks in vitro decomposition.*

This study revealed that the percentage of colonization did not linearly correlate to decomposition rate. A variation in the dry-weight loss of the wood samples was observed even for those with 100% mycelia colonization (Figure 5). In the basal soil media, a consortium of all microbial isolates (S9) seems relatively effective in decomposing the wood (dry weight was reduced 38%), while in basal peat media, the same microbial consortium decomposed only 7% of the total biomass. Treatment S6 (spore suspension of Pycnoporus sanguineus) showed good decomposing activities both in peat- and soil-conditions (Figure 5). During 12-weeks observation, the fungus decomposed 33.5% and 20.9% wood biomass in soil (pH 6.5) and peat (pH 4), respectively.

Spores of white-rot fungi showed better survival and growth in peat conditions compared to other inoculum. They colonized 100% woody samples and decomposed wood biomass 9.5% (*Pycnoporus* sp.), 20.9% (*Pycnoporus sanguineus*), 15.8% (combination of *Pycnoporus* sp. and *P. sanguineus*). It seems that *Pycnoporus sanguineus* is a potential fungal isolate for developing inoculum-starter to decompose wood debris in peatland conditions. Application of *Scedosporium apiospermum* spores (S1, S2 and S4) was not recommended. Despite less colonization and low wood-decomposing activities, the *S. apiospermum* has been reported as an opportunistic pathogen to humans [13]. In patients with cystic
fibrosis, infection with *S. apiospermum* may cause airway obstruction [15, 16]. In order to prevent undesired effects, it is better to avoid further application of this isolate as a part of the wood-degrader microbial consortium.

3.2. Semi-controlled trial

In general, mineral soil- and peat soil- basal media under the fully aerobic conditions showed a greater dry-weight loss than less-aerobic conditions. However, in comparison to the control of each treatment combination, the effects of the microbial consortium on improving wood decomposition seem to work consistently on the less-aerobic conditions. Inoculated rods of *Agathis* sp. and *Araucaria* sp. were decomposed greater than the un-inoculated ones. Decomposition of wood samples in basal soil media showed significant differences between the control (un-inoculated) and the inoculated of both *Agathis* sp. and *Araucaria* sp. wood samples. However, in the basal peat media, the significant difference was only observed on the *Araucaria* samples. In aerobic conditions, either in soil- or basal peat media, the controls showed greater decomposition rates rather than the inoculated ones (Figure 6).

![Figure 6](image_url)

**Figure 6.** Percentage of decomposed wood samples after 12-weeks trial. Wood A is *Agathis* sp., wood B is *Araucaria* sp.

Wood decomposition by filamentous fungi is generally known as an aerobic process. Oxygen availability is required to generate ATP for cellular metabolism of the fungi to activate CAZy (carbohydrate-activate enzymes) genes which are necessary for the decomposition of cellulose, hemicellulose, pectin, and lignin [17]. However, a recent transcriptomic study conducted by Mattila and co-workers [18] reported that oxygen depletion might lead to an alternative wood decomposition strategy which resulted in a higher cellulolytic activity under the anaerobic condition compared to the aerobic one. The oxygen depletion activates more than 200 genes of the fungi and induces a wide array of secreted enzymes required for the catabolism of the released pentose and hexose sugars and galacturonic acids [18].

The obvious and direct effects of the application of microbial consortium on wood decomposition under the semi-controlled conditions seem difficult to be evaluated. In this trial, spores of *Pycnoporus* sp., *P. sanguineus* (Polyporales), and *Scedosporium apiospermum* (Sardiomycetes) were applied, but the signs of these fungi were only observed on a few wood samples in the first three weeks of the observation period. On the other hand, most of the wood samples, either placed in soil- or basal peat media, either in the boxes with less- or fully- aeration, were randomly overgrown by the sporocarp of *Schizophyllum commune* – an Agaricales fungus (Figure 7). Thus, it seems that the spores of this Agaric fungi were already present in the environment, then occupied and dominated the decomposition of wood samples during the experiment took place.
3.3 Field trial

The result showed that decomposition rates of the trial systems which were placed on the un-flooded peat soil were lower than those in inundated peat soil (Figure 8). At first glance, this data seems contradictory to the common phenomenon where drainage (un-flooded) soil with its aerobic surface usually results in an increased decomposition rate of organic matter [3]. However, if the data was correlated to the facts, the inundated site's experience fluctuated cycles of flooded-and-dry, ambient temperature, and sunlight exposure depending on seasonal rainfall changes. Therefore, a higher decomposition rate of trial systems located in the inundated peat soil compared to those in un-flooded peat soil can be understood. Changes in the soil environment have a strong influence on microbial structure and abundance, as well as its activities as bio-decomposer agents [3].

Fluctuation in temperature can affect the decomposition rate of recalcitrant- and complex structure-organic carbons, which require high activation energy. A minor increase in the ambient temperature can substantially increase the decomposition rate [19]. Hydrological changes can also enhance extracellular enzyme activities – that have substantial roles in organic matter decomposition, increase microbial biomass, as well as release a large amount of phosphorus- and nitrous- oxide [20]. This indicates that changes in the micro-environment will alter microbial structure and function, leading to modification of carbon-, phosphorous- and nitrogen- cycles, resulting in a higher decomposition rate as described in Figure 8.

![Figure 7. Fruit bodies of Schizophyllum commune (Agaricales) grown on the wood samples randomly.](image1)

![Figure 8. Percentage of dry-weight loss of the treated wood logs.](image2)

Effects of fungal consortium inoculation in wood decomposition remain unclear. Both the uninoculated control and the inoculated woody logs showed insignificant differences in dry weight loss, except for the Cratoxylon arborescens placed in the inundated peatland area (Figure 8). All woody log
samples were colonized by fungal structures such as fruiting bodies, mycelia, and rhizomorphs (Figure 9). Nonetheless, the inoculated fungal consortium's viability consists of *Pycnoporus* sp., *P. sanguineus*, *Nodulisporum* sp., *Schizophyllum commune*, and *Earliella scabrosa*, is doubtful. Although some fruit bodies of basidiomycota (such as *Pycnoporus* and *Trametes*) were visually observed as grown in some woody logs (Figure 9), the results of the ITS1 and ITS4 sequences showed that Ascomycotina (which comprising of *Aspergillus* spp., *Trichoderma* spp., *Curvularia eragrostidis*, *Lasiodiplodia pseudotheobromae*, *Neurospora calospora*, *Paecilomyces* sp., and *Daldinia eschscholtzii*) were the most abundant fungal phylum isolated across the woody log samples (Table 2).

**Figure 9.** Fungal signs (fruit bodies, mycelia, rhizomorphs etc.) grown on the wood logs samples.

**Table 2.** Identification of fungal cultures re-isolated from the decay wood logs in field condition.

| No. | Fungal Genera/Taxa          | Numbers of Isolates | GenBank Accession No.          | Isolates Abundance (%) |
|-----|----------------------------|---------------------|--------------------------------|-------------------------|
| 1.  | *Aspergillus aculeatus*     | 1                   | MK811100                       | 2.13                    |
| 2.  | *Aspergillus niger*         | 3                   | MK886749, MK828713             | 6.38                    |
| 3.  | *Aspergillus* sp.           | 8                   | MF094213, MK775953, MH935986  | 17.02                   |
| 4.  | *Trichoderma koniopsis*     | 1                   | MH153621                       | 2.13                    |
| 5.  | *Trichoderma reesei*        | 4                   | MH398538                       | 8.51                    |
| 6.  | *Trichoderma peltatum*      | 1                   | MH863656                       | 2.13                    |
| 7.  | *Trichoderma* sp.           | 9                   | MK871297, MK871102, MK870998, LC109300 | 19.15 |
| 8.  | *Curvularia eragrostidis*   | 1                   | MF038158                       | 2.13                    |
| 9.  | *Daldinia eschscholtzii*    | 1                   | MK849923                       | 2.13                    |
| 10. | *Lasiodiplodia pseudotheobromae* | 1     | KM006441                       | 2.13                    |
| 11. | *Neurospora calospora*      | 1                   | MH861163                       | 2.13                    |
| 12. | *Paecilomyces* sp.          | 1                   | MG827159                       | 2.13                    |
| 13. | *Schizophyllum* sp.         | 1                   | MK732120                       | 2.13                    |
| 14. | *Syncephalastrum racemosum* | 1                   | KF305750                       | 2.13                    |
| 15. | Un-identified               | 9                   | ---                            | 19.15                   |

Domination of Ascomycotina in fungal community inhabited in peatland areas have been reported by many studies. A study on microbial communities of peat swamp forests in Brunei Darussalam using
Illumina sequence analysis reported that the abundance of Ascomycotina in that site was 54.1% of all fungal sequence, followed by Basidiomycotina (15.4%) and 30.1% of the detected sequence were unclassified [21]. Data compilation on fungal species richness in peatlands across Europe, North- and South- America stated that anamorphic ascomycetes were the largest group of fungi isolated from the explored peatlands (63% of all species cultured), which predominantly genera of *Penicillium* and *Acremonium* [14]. Domination of these micro-fungi groups is associated with their characteristics that have prolific sporulation and fast growth rates, as well as are easily cultured on most standard media (such as PDA, MEA) under a wide range of growing conditions.

Microbial community (fungi, bacteria, archaea) structures, abundance, and activities may shift significantly in response to environmental changes, such as water regime, soil environment, ambient temperature, etc. [3]. Microbial community structure and its decomposition activities in peatland ecosystems are influenced by many factors, such as variation in the water table, land-use patterns, age of drainage, and peat thickness [22]. Effects of a water-table variation on microbial communities vary depending on the peat's depth profile and oxygen availability [23]. In acrotelm (oxic zone), a low-water-table site is likely to encounter a more frequent drying and wetting cycle than a high-water-table site. The drying and wetting cycle leads to alternate aerobic and anaerobic physiological responses of the microbes. This process selectively enriches resilient microbes that can tolerate changes in a physical environment and physiological functions [22]. Physiologically, dry-wet cycling can affect both carbon and nitrogen metabolisms [24]. Denitrifying assemblages are preferred under wetter and low-oxygen conditions, while carbon metabolisms involving enzyme systems such as phenoloxidase, β-glucosidase, and many hydrolases enzymes are active in high-oxygen conditions [23, 25].

On the contrary, microbial communities in the anoxic zone are shifted through different possible mechanisms. Firstly, exposing microbial communities in prolonged stable anoxic conditions will affect the diversity and abundance of anaerobic-methanogens bacteria [26, 27]. Secondly, the availability of dissolved organic matters (root exudates from plant communities and degradation products of lignocellulosic materials) in the water-saturated anoxic zone will affect the quantity and composition of the microbial communities [28].

Fire incidence may affect microbial community structures. Nutrient supply for microbial communities is influenced by root exudates and lignocellulosic degradation products and the aromatic and aliphatic carbon products resulting from fire incidence [28]. As such carbon joins the carbon pool after a fire event, the abundance and activity of microbial communities decreased immediately. However, after 5 – 10 years, the state of microbial assemblages reflects the recovered and adapted communities [22]. It shows that microorganisms are the most resilient communities in response to environmental changes, including fire incidences. The resilience of microbes as communities in their native environment explains why attempts to introduce certain microbes either as single isolates or a consortium becomes ineffective. These discourses showed that dealing with wood decomposition in the peatland ecosystem requires a comprehensive research design and more holistic approaches for developing strategies on woody-waste management in this unique ecosystem.

4. Conclusion

The study revealed that both trials of fungal inoculation on woody logs conducted in semi-controlled- and field- conditions did not significantly enhance the wood decomposition. Thus, even though fungi have crucial roles in an ecosystem as decomposers that can decay organic material, including woody debris, introducing certain fungal consortiums into the woods in environmental conditions seems not necessarily conducted due to ineffectiveness. Since wood decomposition is a complex process and involves various biotic- and abiotic- factors, more holistic perspectives (not only considering fungi–wood interactions) to improve strategies to reduce woody debris are required. In addition, other factors which also have influenced the woody-debris decomposition, such as weather, soil condition, decomposer agents (microbes, nematodes, insects, and other animals), as well as the involvement of social communities, should be taken into account for future strategies on repeated fire prevention.
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**Authors' contribution**

LA, SH designed & conducted the experimental trials, prepared the first draft of the manuscript; SAF conducted molecular identification of the microbes; all authors contributed to the manuscript development and revision.