

Soil Microbial Relationships in Fungal-Bacterial Bioremediation Systems: To synergy or not to be?

Micaela Buda, University of Minnesota Duluth
Written for WRIT 3150

Abstract

To address the proliferation of soil pollutants and their profound impacts on the microbiome of soil habitats, bioremediation technology has focused on using bacteria and fungi which utilize natural metabolic processes as tools for the removal of recalcitrant pollutants. Independently, fungi and bacteria have demonstrated remediation capabilities but the synergy between them offers a possibility for accelerated removal efficacies. The influence of fungal partners on bacterial communities is based on several variables, including specific fungal partners, environmental conditions, and soil pollutants, making it difficult to predict explicit microbial behaviors for entire bioremediation cases. One area of study addressing this issue is the addition of biostimulants to an inoculated substrate to aid microbial partners in their bioremediation efforts by providing an additional nutrient source that can reduce the occurrence of resource competition. In this review, an evaluation of current studies exploring fungal-bacterial responses to pollutants and bioremediation strategies is used to synthesize current insights on how this synergism may be employed in soil remediation methods. Overall, future investigations should concentrate on the long-term impacts between the indigenous microbiome within contaminated soils and the fungal-bacterial consortium employed to shed light on how these pairings will behave during comprehensive applications.

Introduction

Terrestrial environmental pollutants stem from a range of industrial anthropogenic activities that are tied to the world's energy, agricultural, and transportation sectors. These pollutants enter the biosphere through a variety of pathways and impact the health of the ecosystem and the indigenous microbial community that exists within it. One of the currently implemented waste management approaches to handle these contaminants is the use of bioremediation technology. Advancements in bioremediation technologies have obtained high-

efficiency rates at low costs, with products like carbon dioxide and water that present a relatively low ecological impact. In comparison to physical and chemical remediation methods, these factors have to use bioremediation for more comprehensive applications (Singh et al. 2020).

Historically, bioremediation methods have focused on the utilization of bacteria for their rapid growth, ease of replication, and enzymatic oxidation. However, the small, relatively simple physiology of bacteria is a limitation to what they can successfully degrade. On the other hand, fungi offer a promising solution to degrading more complex and persistent environmental compounds. Fungi use extracellular enzymes and metabolites to break down resistant materials like lignin, chitin, and microcrystalline cellulose (Atagana et al. 2006), which give them the capacity to colonize substrates ranging from wood to soil. The production of hyphae allows fungi to be effectively translocated throughout their substrate, which is unique from bacteria. Moreover, fungi have demonstrated resilience under environmentally stressed conditions. Such stressed conditions include those with reduced water accessibility, low nutrient availability, and low pH values where bacterial growth could be limited (Davis and Westlake, 1978). This same quality was illustrated as fungal strains performed better than degrader bacteria during the remediation of polycyclic aromatic hydrocarbons (PAH) in soil (Byss et al. 2008). Both bacteria and fungi have separately evolved biodegradation capabilities through differing pathways but, put together, they present an attractive opportunity to establish an impressive synergistic bioremediation instrument. One such synergistic mechanism employed by fungi and bacteria is known as the fungal highway, wherein hyphae act as transportation networks for otherwise mobility-impaired microorganisms, improving their accessibility to pollutants for subsequent degradation to take place (Ma et al. 2016).

Research has investigated a variety of soil pollutants that have been successfully degraded in different capacities by fungal and bacterial partners. Fungal and bacterial partners have been successfully used for bioremediation of diverse contaminants. These pollutants include but are not limited to, heavy metals (Ma et al. 2016; Corral-Bobadilla et al. 2019), petroleum hydrocarbons (PHC) (Mair et al. 2013; Fauzi and Suryatmana 2016; Tornberg et al. 2003; Li et al. 2020; Liu et al. 2017; Robichaud et al. 2019), and herbicides (Ellegard-Jensen et al. 2014; Wang et al. 2018; Zang et al. 2020). Undoubtedly, fungal bioremediation can be effective over a wide spectrum of pollutants, making it a cost-effective and compelling form of bioremediation technology. However, there remains a lack of research on what potential impacts bacterial-fungal remediation has on fellow degrader bacteria and indigenous soil bacteria. The soil microbial community exists in the order of billions and provides critical ecosystem services such as nutrient cycling, nitrogen fixation, and sustenance of productive soil structures for plant growth (Torsvik and Øvreås 2002). Therefore, it's valuable to know how fungi will interact with the indigenous microorganisms in the event of large-scale remediation applications that have the potential to alter the state of entire ecosystems.

This review explores recent publications concerning fungal and bacterial responses to a variety of bioremediation tactics and the relationships that exist during these processes to address prospective synergistic combinations for future augmentation. This analysis of successful fungal-bacterial partnerships provides insight on how these methods can be applied in situ, while this review gives suggestions for how future studies can provide a more comprehensive understanding of these interactions across the landscape.

Fungal-Bacterial Relationships During Bioremediation

Fungi and bacteria are physiologically different, use different mechanisms to degrade contaminants, and require different levels of nutrition (Liu et al. 2017). Resource competition acts as a prominent adversary to microbial synergy in relation to bioremediation and research has suggested that such competition can hamper the efficacy of certain degrading microbes (Fauzi and Suryatmana 2016; Robichaud et al. 2019). Fungal species use their competitive ability to colonize soil, an important factor for establishing successful mycoremediation systems (Baldrian, 2008). Additionally, Byss et al. (2008) reported that the fungal species used for biodegradation can act as controls for fluctuations seen in the bacterial community existing within PAH-contaminated soil. Therefore, understanding these fungal-bacterial relationships before large-scale bioaugmentation is critical to avoid adverse effects on soil health.

Bacterial community response to fungal interactions

Microbial populations are predictably more diverse in non-stressed systems than in the stressed systems that are reflected in contaminated soils. Due to their large population size, bacteria are often distinguished through crystal violet gram staining. Gram-positive (G+) bacteria retain the purple color of the crystal violet due to their thick peptidoglycan layer. Gram-negative (G-) bacteria are unable to retain the purple color of the stain and are characterized by a thin peptidoglycan layer and cell wall. Additionally, G+ bacteria produce exotoxins only, while G- can produce both exotoxins and endotoxins. In PAH-contaminated soil, the bacterial community composition can shift toward a higher concentration of G- bacteria (Kästner et al. 1994). This shift may be explained by reports that G- bacteria populations increase when easily utilizable carbon sources become available (Marilley and Aragno 1999). This trend was supported by Tornberg et al. (2003) who found that PAH-contaminated soil showed higher concentrations of phospholipid fatty acids (PLFA) that are common in G- bacteria after separate inoculation with *H. fasciculare*, *A. vaillantii*, and *R. bicolor*. On the contrary, Byss et al. (2008) found that PAH-contaminated soil inoculated with *P. ostreatus* showed higher concentrations of G+ bacteria compared to G- bacteria after 120 days. Such varied results in bacterial composition illuminate the need for future research to pinpoint how any chosen fungal partner in a remediation system impacts both G+ and G- bacteria. Future research could predict how such a relationship between bacteria will impact the soil in the long-term context. Additional investigations into how G+ and G- bacteria impact the fate of bioremediation efficacies and fitness of the entire soil microbiome will be beneficial in advancing this technology as well.

Effects of fungal extracellular enzymes

Certain fungal species utilize extracellular enzymes such as laccase, C120, and MnP to break down the surrounding substrate. These enzymes allow fungi to degrade a wide variety of organic compounds and have been shown to be effective in the remediation of petroleum hydrocarbons (Liu et al. 2017; Yanto et al. 2017; Borràs et al. 2010). In their study of fungal-bacterial soil bioremediation, Tornberg et al. (2003) observed an enhanced level of bioavailable carbon that was used predominantly by G- bacteria. Researchers postulated that it could have been released from either fungal

mycelia or through fungal decomposition processes, or a byproduct of G⁺ bacterial decomposition. Liu et al. (2017) found that G⁻ bacteria *B. licheniformis* Y-1 was able to use laccase produced by *P. ostreatus* as a carbon source when present in low concentrations. These findings suggest that fungal enzymes could be creating an optimal environment for G⁻ bacterial growth, which can accelerate biodegradation processes. Conversely, Liu et al. (2017) also reported that when present at high concentrations, laccase had an inhibitory effect on *B. licheniformis* Y-1 productivity in PHC-contaminated soil. Liu et al.'s report suggests that the concentration of laccase within the bioremediation system plays a critical role in fungal-bacterial synergism. At the same time, Lladó et al. (2013) reported that concentrations of MnP and laccase in *I. lacteus* cultures demonstrated no changes in the presence of bacteria. Also, this study found that native soil bacteria reduced *T. versicolor* laccase levels, though Borràs et al. (2010) showed that *T. versicolor* lacked advanced degradation capabilities when active native PAH-degrading microbiota was present. This result indicates that *T. versicolor* laccase did not have an adverse effect on the bacteria during the remediation process, but rather the bacterial community may have in turn impeded the fungal laccase activity. Since fungal remediation is principally attributed to such degrading enzymes, it's imperative that interactions between autochthonous soil bacteria and fungal degrader partners are understood before in situ application takes place.

Bacterial and Fungal Responses to Bioremediation Mechanisms

Bioremediation can be approached with a variety of strategies depending on the preliminary soil conditions, contaminant species, and status of the autochthonous microbiome. The two prevailing mechanisms for soil bioremediation are biostimulation and bioaugmentation. Biostimulants are characterized as any supplementary component that acts as a catalyst for the biodegradation of soil pollutants. Bioaugmentation consists of the addition of a microorganism or microbial consortium to the contaminated environment (Yanto et al. 2017). Both techniques have demonstrated varying levels of effectiveness individually, and even less is known about their capabilities when paired together.

Addition of biostimulants

Biostimulants can function as a nutrient source for fungi and bacteria species to accelerate their remediation capabilities. Based on their findings that both fungal strains *Trametes versicolor* and *Irpex lacteus* reveal bacterial inhibition during the bioremediation of high molecular weight polycyclic aromatic hydrocarbons (HMW-PAH), Borràs et al. (2010) suggested that there may be resource competition taking place in the soil. This inhibition was exhibited as bacterial degradation reduced consistently after fungal partners were introduced to the contaminated substrate. Additional research has indicated that a periodic reduction in available nutrients can decrease enzymatic activities within the soil (Margesin and Schinner 2001). Accordingly, the addition of nutrients that can be used by both bacterial and fungal degraders may help to reduce the probability of resource competition while facilitating bioremediation efforts. At the same time, it has been reported that hydrocarbon remediation efficacy of soil bacteria

can be reduced within a carbon-rich environment. This efficacy can cause bacteria to preferentially use carbon additives rather than carbon pollutants during metabolic processes (Lladó et al. 2013). While some studies have focused on the injection of nutrients into the system post-inoculation, others take the approach of enrichment before inoculation through using bulking materials like straw (Byss et al. 2008; Liu et al. 2017) and barley wheat (Atagana et al. 2006). Currently, there remains a high degree of variability among tested biostimulation amendments within the literature. A plethora of fungal-bacterial-pollutant combinations exist, yet little research has been focused on identifying feasible biostimulants that cater to both degrader partners simultaneously. Mair et al. (2013) found that the addition of an inorganic NPK (nitrogen-phosphorous-potassium) fertilizer to PHC-contaminated soil led to an increase of 60-75% in total soil respiration compared to that of natural attenuation processes. This increase suggests that NPK fertilizer could be a viable stimulant for both fungi and bacteria since respiration was measured for the entire soil microbial population. Although this study reported that NPK fertilizer had stimulating effects on total soil bacteria specifically, it remains unclear what relationships existed between soil fungi and fertilizer. In a separate study, Yanto et al. (2017) employed periodic biostimulation and bioaugmentation (PBB) using malt extract as the stimulant. They found that the addition of PBB increased the activity of fungal degrading enzymes (C12O, laccase, and MnP) within the soil, in conjunction with a higher overall removal efficiency of crude oil hydrocarbons. On the other hand, Borràs et al. (2010) found that the population of degrader bacteria increased within the mineral medium BMTM during HMW-PAH bioremediation processes. Ultimately, research has demonstrated a spectrum of results concerning biostimulant amendments, including responses from individual fungal and bacterial populations to whole microbial communities. For those studies that lacked insight on both fungal and bacterial responses relating to biostimulants, it's worth investigating whether stimulated effects in one species would transcend to both degrader partners.

Utilization of Spent Mushroom Substrate

A novel advance in biotechnology is the utilization of spent mushroom substrate (SMS) due to its demonstrated ability to bind to environmental pollutants and improve nutrient concentrations in the soil (Meaky and Okechukwu 2019). Spent mushroom substrate is the pasteurized organic product from mushroom harvesting (Corral-Bobadilla et al. 2019). Manure is used as a substrate in SMS which presents additional environmental problems including the release of methane, a known greenhouse gas, into the atmosphere. There is also a lack of storage and disposal solutions for SMS residue making it a linear system that has historically lacked an apparatus to cycle the resources back into production. Therefore, using SMS as a bioremediation technology presents an opportunity for economical and ecologically considerate use of resources. Nevertheless, no technique is flawless, as shown by the finding that sterilized SMS applied with atrazine can lead to an initial decline in soil pH (Meaky and Okechukwu 2019). Studies have shown that a neutral pH is optimum for bacterial growth, suggesting that a lowered pH would have inhibitory effects on bacteria degraders (Cho et al. 2016). Despite this, studies have reported successful biodegradation of chlorimuron-ethyl, atrazine, and heavy metal species using SMS derived from *Pleurotus eryngi* (Zang et al. 2020), *Pleurotus ostreatus* (Meaky and Okechukwu 2019), and *Agaricus bisporus* (Corral-Bobadilla et

al. 2019) respectively. Interestingly, Meaky and Okechukwu (2019) found that after an initial decline in the bacterial population the soil bacteria population was significantly higher ($P < 0.05$) in week six of the bioremediation treatments compared to week two. This increase in population suggests that certain bacterial populations can recover from an initial impairment in the presence of atrazine using SMS.

Synergistic Efficacy During Bioremediation

Certain bioremediation mechanisms have focused on using a consortium of fungi and bacteria to take advantage of the potential for complimenting biodegradation capacities. However, these consortiums have had inconsistent results, particularly for the bioremediation of hydrocarbon pollution from crude oil and petroleum waste. Fauzi and Suryatmana (2016) reported that the petrophilic fungal consortium paired with azobacter bacterial species successfully degraded 0.22% ppm/day of crude oil waste. Likewise, Liu et al. (2017) found that, when *P. ostreatus* and *Bacillus licheniformis* Y-1 are used in conjunction with each other, they had the highest degradation rates for TPH ($57.72 \pm 5.55\%$) and PAH ($49.05 \pm 6.49\%$). Despite these results, Borràs et al. (2010) reported weak synergistic effects between indigenous microbes and *I. lacteus* during PAH bioremediation efforts. Similar results were observed between indigenous soil bacteria and *T. versicolor* by Borràs et al. (2010) and Lladó et al. (2013). In addition to complex hydrocarbon pollutants, the bacterial-fungal consortium *P. eryngiu*-SMS-CB was successful at chlorimuron-ethyl degradation (herbicide) (Zang et al. 2020). Although hydrocarbons and crude oil waste in general certainly don't represent the entire spectrum of terrestrial environmental pollutants, a substantial amount of research has been carried out to understand fungal-bacterial synergy during its remediation. The opposing results established for these interactions elucidate a challenging framework for identifying how fungal-bacterial synergy will behave in future remediation endeavors.

Implications for Future Research

While there is a strong case for employing fungal-bacterial partnerships to degrade terrestrial environmental pollutants, there remains a degree of ambiguity for how a consortium will impact the local soil microbial community and pollutant concentration in the long run. The mixed results within the literature suggest that remediation efficacies are dependent on a wide variety of variables, including indigenous soil microbial community, specific fungal partners, the form of bioremediation technology employed, and local environmental parameters. Although researchers have examined a range of bioremediation techniques, resource competition between bacteria and fungi along with the competitive ability of fungi remain barriers to the successful synergy between the two partners and may be further amplified by biostimulation supplements. Mixed results have been reported as well for the use of SMS, which has demonstrated remediation success in some capacities. However, SMS's ability to reduce the pH of its surrounding environment when added to contaminated soil is an impairment to overall synergistic processes. Although research has shown that certain consortiums successfully work together to degrade hydrocarbon waste products, the efficiency rates are inconsistent, and the consortiums' proficiencies have yet to be explored for

other soil pollutants. Most of the published research regarding these processes had an inoculation period of 120 days or less. Future studies should focus on the long-term effects of fungal-bacterial bioremediation on the landscape and assess the overall sustainability of these technologies. It's important to know how long treatment will take, how much maintenance it will need, and how much it will cost to execute a large-scale bioremediation effort. Moving forward, research should address what ramifications nutrient biostimulation could have in the long term as excess nutrients may pose a threat to microbiomes in the soil. Moreover, nutrient runoff from agricultural fields is already a troublesome threat to the ecosystem. For the same reasons, there needs to be a focus on the interactions between the consortium and indigenous microorganisms present in the contaminated soil. This focus would help to determine a more accurate degradation efficiency that could be applied in a polluted environment. Ultimately, the use of fungi and bacteria paired together as a bioremediation and waste management mechanism for recalcitrant environmental pollutants provides a promising advancement in remediation technologies. Further research should be performed so that low-impact remediation efforts can replace other environmentally costly methods and large-scale augmentation can be achieved.

References

- Atagana HI, Haynes RJ, Wallis FM. 2006. Fungal Bioremediation of Creosote-Contaminated Soil: A Laboratory Scale Bioremediation Study Using Indigenous Soil Fungi. *Water Air Soil Pollution*. 172(1):201–219. doi:10.1007/s11270-005-9074-x.
- Baldrian P. 2008. Wood-inhabiting ligninolytic basidiomycetes in soils: Ecology and constraints for applicability in bioremediation. *Fungal Ecology*. 1(1):4–12. doi:10.1016/j.funeco.2008.02.001.
- Borràs E, Caminal G, Sarrà M, Novotný Č. 2010. Effect of soil bacteria on the ability of polycyclic aromatic hydrocarbons (PAHs) removal by *Trametes versicolor* and *Irpex lacteus* from contaminated soil. *Soil Biology and Biochemistry*. 42(12):2087–2093. doi:10.1016/j.soilbio.2010.08.003.
- Byss M, Elhottová D, Tříška J, Baldrian P. 2008. Fungal bioremediation of the creosote-contaminated soil: Influence of *Pleurotus ostreatus* and *Irpex lacteus* on polycyclic aromatic hydrocarbons removal and soil microbial community composition in the laboratory-scale study. *Chemosphere*. 73(9):1518–1523. doi:10.1016/j.chemosphere.2008.07.030.
- Cho S-J, Kim M-H, Lee Y-O. 2016. Effect of pH on soil bacterial diversity. *J ecology environ*. 40(1):10. doi:10.1186/s41610-016-0004-1.
- Corral-Bobadilla M, González-Marcos A, Vergara-González EP, Alba-Elías F. 2019. Bioremediation of Waste Water to Remove Heavy Metals Using the Spent Mushroom Substrate of *Agaricus bisporus*. *Water*. 11(3):454. doi:10.3390/w11030454.

- J. S. Davies, D. W. S. Westlake. 1979. Crude oil utilization by fungi. *Canadian Journal of Microbiology*. 25(2): 146-156. <https://doi.org/10.1139/m79-023>
- Ellegaard-Jensen L, Knudsen BE, Johansen A, Albers CN, Aamand J, Rosendahl S. 2014. Fungal–bacterial consortia increase diuron degradation in water-unsaturated systems. *Science of The Total Environment*. 466–467:699–705. doi:10.1016/j.scitotenv.2013.07.095.
- Fauzi M, Suryatmana P. 2016. Bioremediation of crude oil waste contaminated soil using petrophilic consortium and *Azotobacter* sp. *Journal of Degraded and Mining Lands Management; Malang*. 3(2):521–526. doi:http://dx.doi.org.libpdb.d.umn.edu:2048/10.15243/jdmlm.2016.032.521.
- Kästner M, Breuer-Jammali M, Mahro B. 1994. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). *Appl Microbiol Biotechnology*. 41(2):267–273. doi:10.1007/BF00186971.
- Liu B, Liu J, Ju M, Li X, Wang P. 2017. Bacteria-white-rot fungi joint remediation of petroleum-contaminated soil based on sustained-release of laccase. *RSC Adv*. 7(62):39075–39081. doi:10.1039/C7RA06962F.
- Lladó S, Gràcia E, Solanas AM, Viñas M. 2013. Fungal and bacterial microbial community assessment during bioremediation assays in an aged creosote-polluted soil. *Soil Biology and Biochemistry*. 67:114–123. doi:10.1016/j.soilbio.2013.08.010.
- Ma X, Ding N, Peterson EC, Daugulis AJ. 2016. Heavy metals species affect fungal-bacterial synergism during the bioremediation of fluoranthene. *Appl Microbiol Biotechnology*. 100(17):7741–7750. doi:10.1007/s00253-016-7595-4.
- Mair J, Schinner F, Margesin R. 2013. A feasibility study on the bioremediation of hydrocarbon-contaminated soil from an Alpine former military site: Effects of temperature and biostimulation. *Cold Regions Science and Technology*. 96:122–128. doi:10.1016/j.coldregions.2013.07.006.
- Margesin R, Schinner F. 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. *Applied Microbiology and Biotechnology*. 56(5–6):650–663. doi:10.1007/s002530100701.
- Marilley L, Aragno M. 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. *Applied Soil Ecology*. 13(2):127–136. doi:10.1016/S0929-1393(99)00028-1.

Meaky E, Okechukwu H. 2019. Spent Mushroom Substrate: A Potential in situ Biostimulant in a Herbicide-Polluted Soil. *American Journal of Research*.:15.

Robichaud K, Girard C, Dagher D, Stewart K, Labrecque M, Hijri M, Amyot M. 2019. Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North. *Chemosphere*. 220:47–55. doi:10.1016/j.chemosphere.2018.12.108.

Singh RK, Tripathi R, Ranjan A, Srivastava AK. 2020. Chapter 9 - Fungi as potential candidates for bioremediation. In: Singh P, Kumar A, Borthakur A, editors. *Abatement of Environmental Pollutants*. Elsevier. p. 177–191. [accessed 2020 Sep 29].
<http://www.sciencedirect.com/science/article/pii/B9780128180952000096>.

Tornberg K, Bååth E, Olsson S. 2003. Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. *Biol Fertil Soils*. 37(3):190–197. doi:10.1007/s00374-002-0574-1.

Torsvik V, Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*. 5(3):240–245. doi:10.1016/S1369-5274(02)00324-7.

Wang J, Li Xinyu, Li Xu, Wang H, Su Z, Wang X, Zhang H. 2018. Dynamic changes in microbial communities during the bioremediation of herbicide (chlorimuron-ethyl and atrazine) contaminated soils by combined degrading bacteria. *PLOS ONE*. 13(4):e0194753. doi:10.1371/journal.pone.0194753.

Yanto DHY, Hidayat A, Tachibana S. 2017. Periodical biostimulation with nutrient addition and bioaugmentation using mixed fungal cultures to maintain enzymatic oxidation during extended bioremediation of oily soil microcosms. *International Biodeterioration & Biodegradation*. 116:112–123. doi:10.1016/j.ibiod.2016.10.023.

Zang H, Liu W, Cheng Y, Wang H, An X, Sun S, Wang Y, Hou N, Cui C, Li C. 2020. Bioremediation of Historically Chlorimuron-Ethyl-Contaminated Soil by Co-Culture Chlorimuron-Ethyl-Degrading Bacteria Combined with the Spent Mushroom Substrate. *Microorganisms*. 8(3):369. doi:10.3390/microorganisms8030369.