Decay of woody material: The presence of fungi and bacteria in the decomposition process of tress

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Abstract:

The complex process of wood decomposition involves a large number of decomposer microorganisms such as fungi and bacteria, which will act differently according to the chemical composition of the wood. Wood is composed of different tissues containing lignin, a complex polymer with fungicidal and antibacterial properties that protects the trees from diseases and decomposition. This study was focused on the observation of microorganism growth in association with the stages of decay of woody material. Fungal growth was found in wood samples that were placed in forest with great amount of woody decay material.The observation of microorganisms growth of wood samples placed in agar cultures, showed a greater growth of microorganisms when late decay and non decay (healthy) stage of birch tree was examined. Also, a significant result showed an association in the presence of microorganisms in decay stages of birch wood. The final results open new questions on the ecological role of bacteria and fungi in the decomposition of wood and on the decay of the fungicidal and antibacterial properties of the wood as it decomposes.

KEYWORDS: decomposition, bacteria, fungi, decay, wood

Introduction:

The study of decomposition process is important for understanding the cycle of matter involving abiotic components such nutrient cycles and trophic chains involving decomposer organisms that are fundamental in converting organic matter to inorganic. One major component of tree wood is lignin, a complex organic polymer associated with carbohydrates such as cellulose and hemicellulose in the wall of vascular plant cells.The composition and quantity of these organic substances vary widely depending on wood type and species (Li Jingjing 2001). Lignin is a complex phenolic compound known to have fungicidal and antibacterial properties, playing an important role in the protection of vascular plant tissues from invasion by infectious microorganisms (Shigo, AL 1979). Previous studies have shown inhibition of the growth of microorganisms in the presence of lignin isolated from wood (Augustin, J., & Joniak, D.1979, Wimmer, R., & Pâques, L. E. 2004).

Microorganisms such as bacteria and fungi are two of the primary agents of wood decomposition. Decomposer fungi have evolved enzymes that externally digest and absorb soluble lignin and cellulose found in the wood of trees (Zabel, R. A., & Morrell, J. J. 2012). The decomposed wood is the product of a slow, natural process that can last for decades depending on the biological and abiotic factors involved. On some occasions, this process starts with a wound that is most often caused by some species of birds or insects that extract highly energy resources from the wood or seek shelter in trees.Wounds caused in the bark of the trees leave the xylem and phloem unprotected, causing these nutrient components of trees to be vulnerable to a wide range of organisms (Shigo, A.L. 1979), including insects and microorganisms such as bacteria and fungi. Tree cells respond to the effects produced by this damage by creating toxic components that affect invaders. Organisms recognized as pioneers are better adapted to these toxins and able to break the tree's protective barrier (Shigo, A.L. 1979), beginning the processes of infection. The process of decomposition itself begins when pioneers organisms such as some insects, bacteria and decay and non decay fungi start to digest the cell walls

On the forest floor, it is possible to find woody material in different stages of decay. Thus, the objectives in this study were to 1) Observe fungal and bacterial growth from wood in various stages of decay, 2) Determine the presence or absence of bacteria and fungi in association with wood decay stages and species, and 3) Observe fungal growth in sugar maple wood disks exposed airborne fungal spores. We hypothesized that the amount of microorganisms cultivated on agar would depend on the stage of wood decay material and the wood species being examined. Additionally, we predicted that forests with more fallen trees and stages of decomposed woody material would have a greater amount of airborne fungal spores, leading to increased fungal growth on wood left overnight in these forests.

Material and Methods:

Study Sites:

The area of study was located in the Upper Peninsula of Michigan along the border of Wisconsin and Michigan at the University of Notre Dame Environmental Research Center (UNDERC). Three areas of forests differing in tree species composition and amounts of decaying wood material were chosen to study fungi that may be important in the decomposition of coarse woody debris. A forest dominated by sugar maples (*A. saccharum)* and white birch (*B. papyrifera*) (46°.22380 N, 089.51958°W) was chosen as the first sample plot due to its large amount of wood decaying wood material. The second sample plot was chosen at a forest dominated by eastern hemlock trees (*T. canadensis)*, located in the area by Plum Lake (46°.21719 N, 089.49039°W). The final sample plot mostly dominated by sugar maple trees (46°.22150 N, 089.52187°W)

Data collection:

All study sites started from a placed 15 m from the road. The starting point was chosen as the zero point of an XY axis, from which a random number was chosen on the X-axis and on the Y-axis. This point was chosen as the center of a circle that was formed by measuring a diameter of 20 m. The circumference was divided into four equal parts. At each end,10 m from the center, petri dishes moistened with water and containing a fresh disk of wood from a sugar maple tree (of 7 cm in diameter) were placed on the forest floor. A petri dish was also placed in the center of the circle. The petri dishes remained in the forest for 24 hours, after which they were collected and sealed with parafilm paper to observe the growth of fungi in each.This same procedure was repeated for all sample sites. The wood samples were observed every day for a total of 14 days. In order to measure the amount of decomposing matter present in each forest plot, a 20 m transect was measured from the center of the circular plot to obtain a greater representation of the decomposed woody material present in the forest and to make counting work easier. Within these circular plots at all three sites, all fallen trees, branches and dead standing trees that were at least 1 m long and 5cm in diameter were counted.

In addition, to observe the growth of microorganisms such as bacteria and fungi in woody material in different stages of decomposition, a transect of 100 m was established, located 15 m from the road in the sugar maple and birch forest stand. Six random numbers along the 100 m were selected from which 70 m perpendicular transects were drawn. In each line, maple and white birch trees in three different stages of decomposition;totally decomposed (decay), moderately decomposed (medium) and without any signs of decomposition (healthy) were sampled. A total of 36 wood samples were removed from under the bark of each tree. Exactly 0.40 grams of each sample were placed in petri dishes filled with LB AMP agar. All plates were kept in a box at approximately 25 ° C. The samples were observed for 7 days and graph paper of 1 mm^2 squares was used to measure the area of the petri dish covered by microorganisms at the end of the week

Statistical analysis was carried out in the SYSTAT and R programs. A 2-Way ANOVA was used to analyze the proportion of the petri dish area covered by microorganisms across tree species and decay stages. Chi squared analyses were used to analyze the presence or absence of microorganisms such as bacteria and fungi in association with the decay stages of maple and birch wood.

Results

Fungi growth in wood disks

Fungi appeared on most disks and the presence of fungi was not associated with wood disks left at a particular site (X^2 (3, N = 20) = 2.8571, p =0.4932; Table 1).

Microorganisms growth in decay wood stages

A 2-Way ANOVA showed a significant interaction (2, N = 36) = 7,0 p =0.003 in the study of fungi area proportion covered in different stages of decaying wood material. A Tukey HSD test showed a significant difference (p=0.047) between proportion covered of decaying and healthy stages of birch wood . However, the association between area covered by microorganisms and different stages of wood decay material showed non-significant results (2, N = 36) = 2,552, p =0.094) , but with a trend towards significance. Analysis of tree species and microorganisms area covered also showed non significant results (p=0.915).

A chi-squared test used to analyze whether the presence of fungi and bacteria was associated with certain decay stages of wood showed a nonsignificant result (X^2 (4, N = 18) =3,4, p =0,493) for the sugar maple trees.However, the presence of fungi and bacteria or both were differently associated with the different stages of decomposition for the birch trees (X^2 (4, N = 18) =9,6545 p = 0,04567).

There was no difference in distribution of types of microorganisms present (either fungi or bacteria) between the two tree species (X^2 (2, N = 18) =0,1385 p = 0.9331).Observational study of microorganism growth showed the presence of at least 3 different colonies of bacteria, as well as the presence of filamentous fungi.

Discussion:

In this study, significant results obtained from a 2-Way ANOVA suggest an association with the area covered by microorganisms growing in different stages of decaying wood material. Microorganisms covered a greater area in decomposed birch material than healthy birch tree material, this suggests that decomposition may affect different species in different ways. This result supports the hypothesis that the growth of microorganisms is greater in those samples that are more decomposed, since the chemical composition of these wood samples is different than that of the original healthy sample.This observation is likely a consequence of decomposer microorganisms allowing them to grow faster in habitats with more cover by decomposing trees. Soil nutrient supply is also important in the decomposition process of woody debris material in contact with the forest floor, as the chemical composition of litter affects the decomposition rates and the microorganisms present in the soil (Bradley, R., & Mailly, D. 2004). Decomposition rates of woody material could also be explained by the amount of lignin present in tree species, sugar maple and birch are both hardwood trees, which, according to previous study, have a slightly lower lignin content in their wood than softwood trees (Sjostrom 1993). However, this study was not focused on this comparison, though this would be an interesting study that could be carried out in the future

In order to observe and compare the presence of microorganism types (fungi or bacteria) in each stage of decomposition between different species, results obtained by Chi-squared analysis showed a nonsignificant difference between organisms present in the maple samples and a significant difference between organisms present in birch. No fungi was found in decomposed samples, while bacteria was found in these samples. These results suggest that different organisms play different roles in different stages of decomposition (Moorhead, D. L., & Sinsabaugh, R. L. 2006)

The agar used in this experiment was an LB agar with antibiotic penicillin. The emergence of bacteria in wood cultures at different stages of decomposition suggests that these bacteria are resistant to antibiotic ampicillin and were able to grow without problems in this medium. The agar conditions were also optimal for the growth of filamentous fungi. It is important to note that some cultures may have been contaminated by external bacteria present in the environment and not from the study material. Some bacterial colonies began at the border of the petri dish rather than in the center by the wood sample, suggesting an outside source of contamination. However, contamination by bacteria did not exclude the presence of bacteria from the study subject, so that these polluting colonies did not influence the final results of this study.

Final results of this study may suggest important information for future researchers interested in the study of decomposition stages of wood in association with tree species, the importance of soil nutrients in the decomposition cycle, bactericide and fungicide properties of wood, and the specific ecological roles of microorganisms in the process of decaying wood. Allowing the expansion of a deeper knowledge regarding the importance of the cycle of matter

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References

Gierlinger, N., Jacques, D., Schwanninger, M., Wimmer, R., & Pâques, L. E. (2004). Heartwood extractives and lignin content of different larch species (Larix sp.) and relationships to brown-rot decay-resistance. *Trees*, *18*(2), 230-236.

Johnson, C. E., Siccama, T. G., Denny, E. G., Koppers, M. M., & Vogt, D. J. (2014). In situ decomposition of northern hardwood tree boles: decay rates and nutrient dynamics in wood and bark. *Canadian journal of forest research*,*44*(12), 1515-1524.

Li, J. (2011). Isolation of lignin from wood. p.6

Moorhead, D. L., & Sinsabaugh, R. L. (2006). A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, *76*(2), 151-174.

Saunders, M. R., Fraver, S., & Wagner, R. G. (2011). Nutrient concentration of down woody debris in mixedwood forests in central Maine, USA.

Shigo, A.L.; Larson, E.vH. 1969. A photo guide to the patterns of discoloration and decay in living northern hardwood trees. Res. Pap. NE-127. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station p.100.

Shigo, A.L. 1979. Tree decay: an expanded concept. Agric. Info. Bull. No. 419. Washington,

Sjöström, E. (1993). Wood Chemistry Fundamentals and Applications (2nd edition). San Diego, CA: Academic Press, Inc. DC: U.S. Department of Agriculture, Forest Service p.73.

Sommerville, D., Bradley, R., & Mailly, D. (2004). Leaf litter quality and decomposition rates of yellow birch and sugar maple seedlings grown in monoculture and mixed-culture pots at three soil fertility levels. *Trees*, *18*(5), 608-613.

Zabel, R. A., & Morrell, J. J. (2012). *Wood microbiology: decay and its prevention*. Academic press p.44.

Zemek, J., Košíková, B., Augustin, J., & Joniak, D. (1979). Antibiotic properties of lignin components. *Folia microbiologica*, *24*(6), 483-486.

	Site 1	Site 2	Site 3	Control
Has fungi	4	2	4	4
No fungi	1	3	1	1

Table 1. Presence of fungi at the three study sites and in the control. The distribution of fungi did not vary among study sites or the control (X^2 (3, N = 20) = 2.8571, p =0.4932)

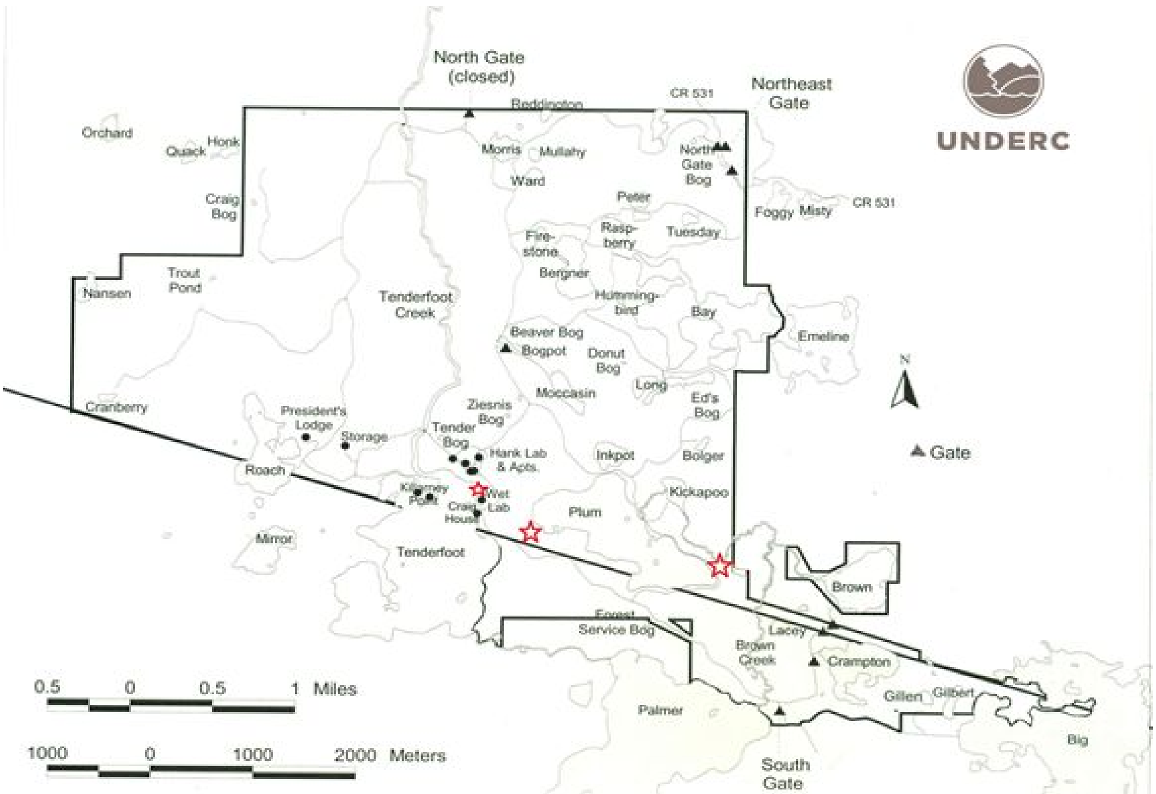

Figure.1 Location of study sites at UNDERC property. Sample plots are marked with stars. Start top left represents study site one, star above right represents study site two and star between both stars represents study site three.

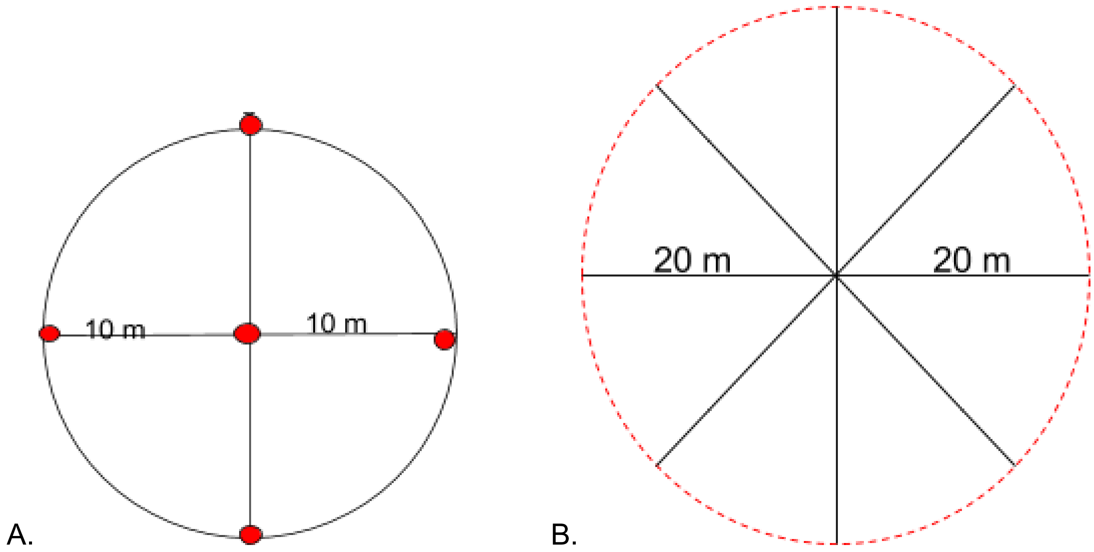

Figure 2: A: Sample plot used to placed petri dishes with disk of wood in the center and in each cardinal direction. B: Sample plot used to count the amount of wood decay material present in each forest.

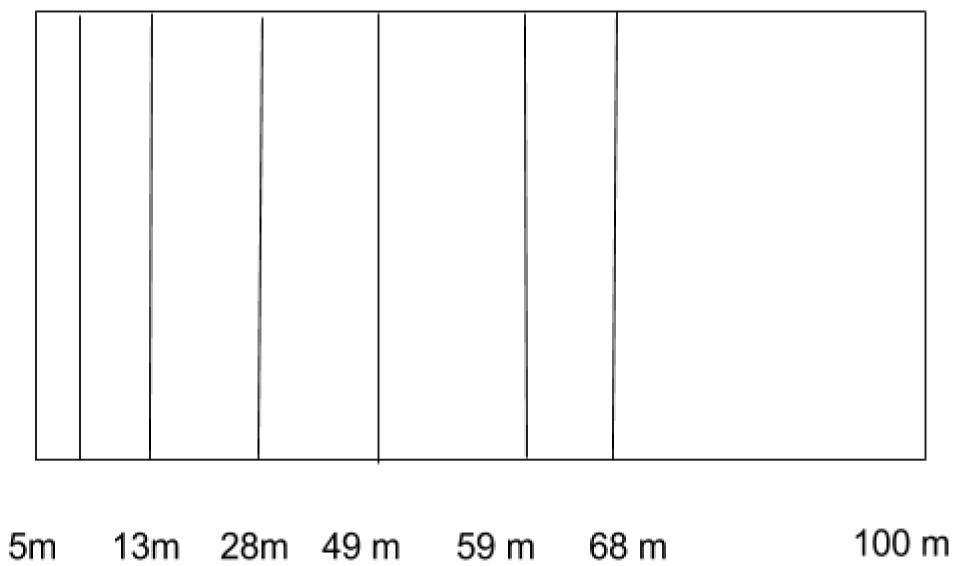

Figure 3. Transects of forest located in site one used to collect pieces of decay stages of woody material.

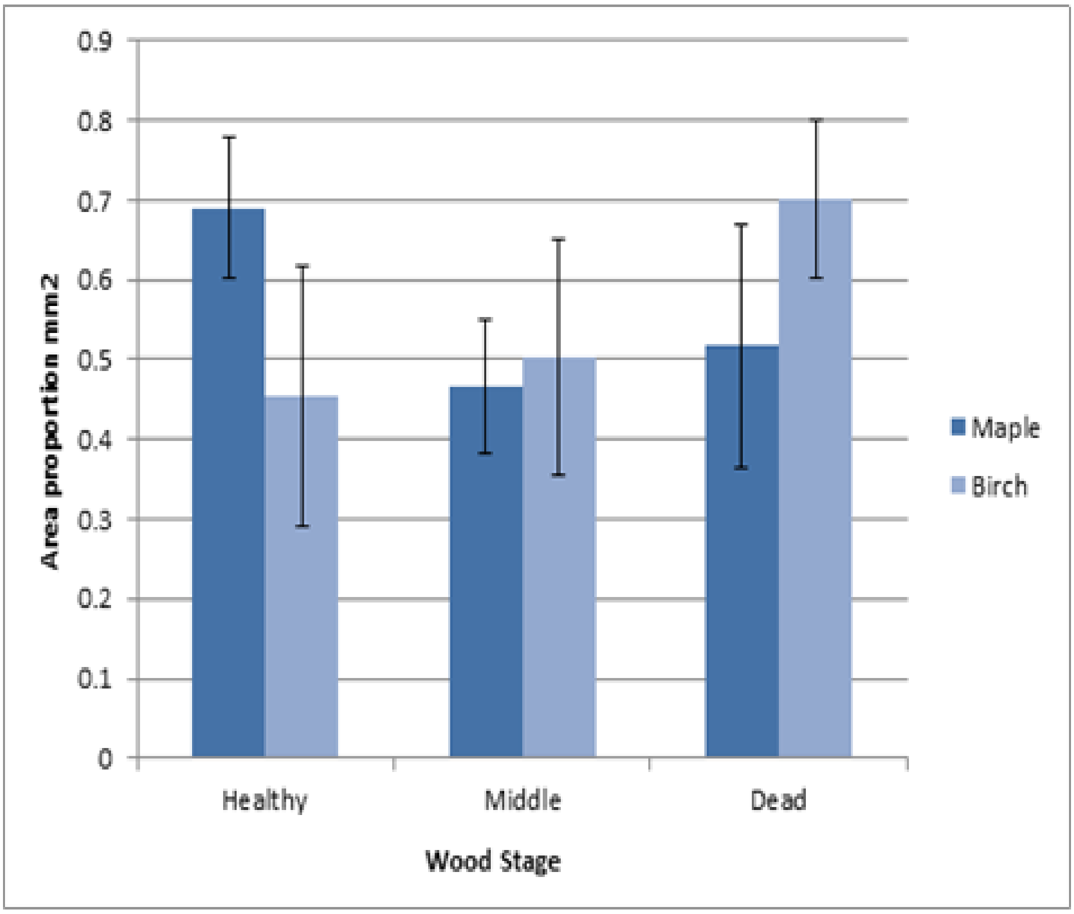

Figure 4. Association of area covered by microorganism with decay stage and tree specie. A 2-Way ANOVA showed a significant interaction between wood stage and species on the proportion of the area in the petri dish covered by microorganisms (2, N=36)=7.00, p=0.031). Error bars represent standard deviation.

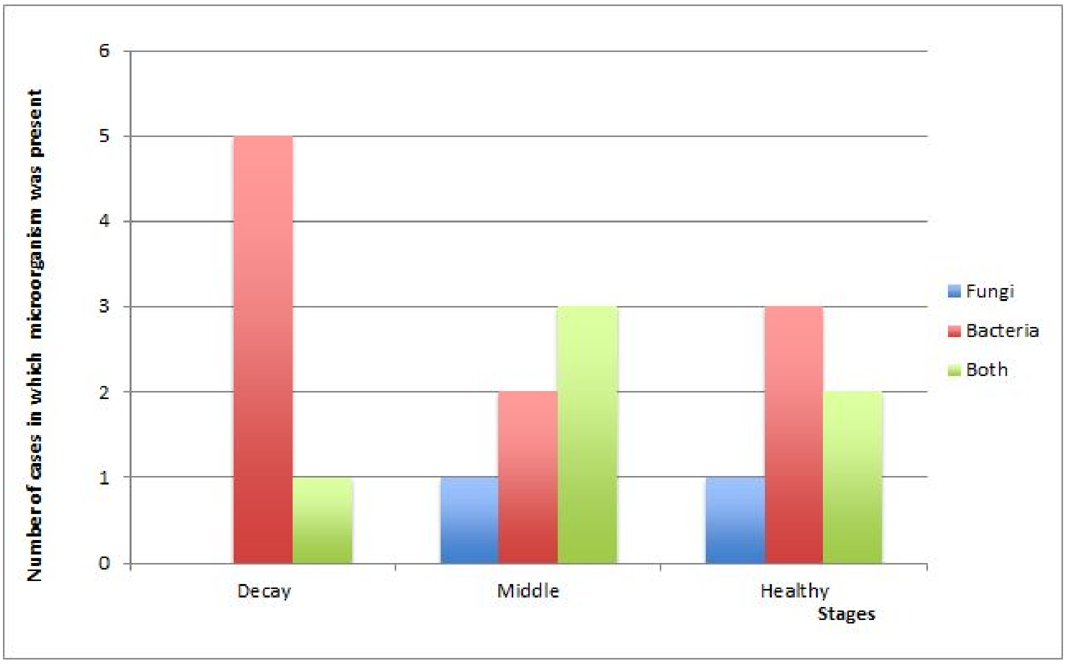

Figure 5. Number of cases in which microorganisms was present in decay stages of sugar maple tree wood.(X^2 (4, N = 18) =3.4, p =0.4932)

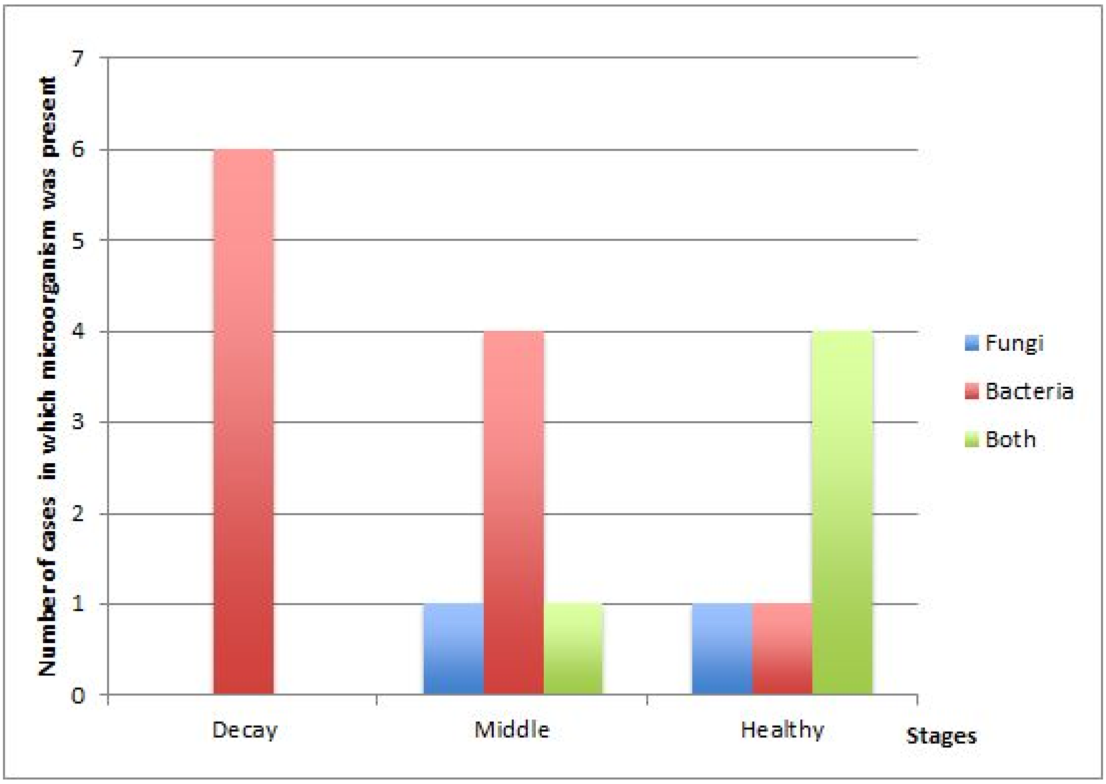

Figure 6.Number of cases in which microorganisms was present in decay stages of birch tree wood (X^2 (4, N = 18) =9.6545, p =0.046)

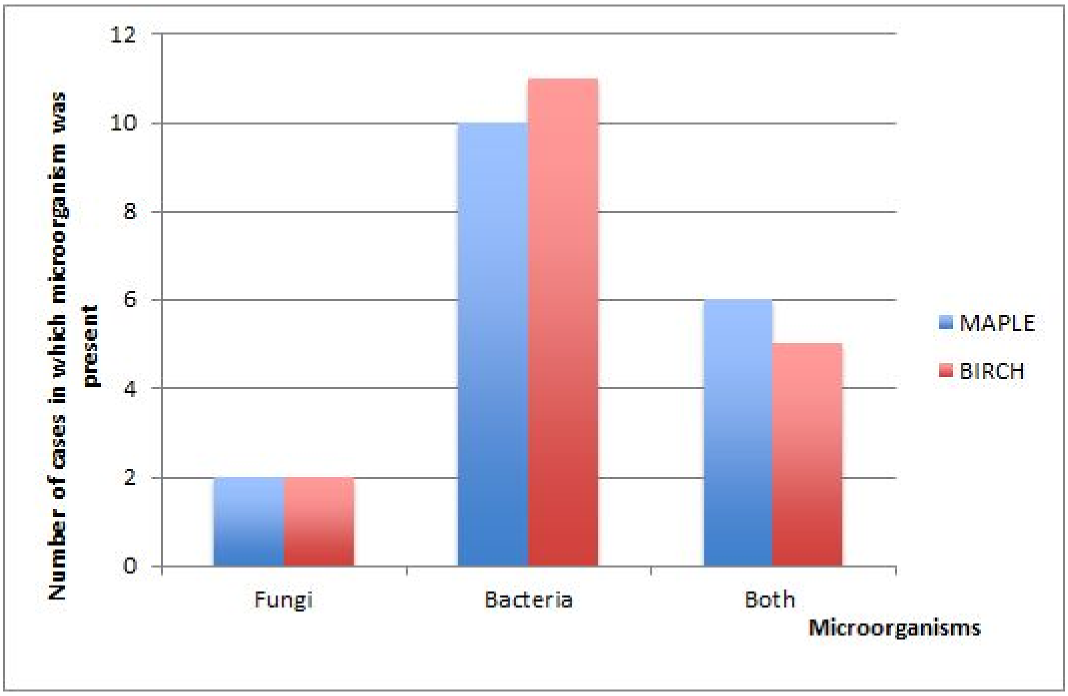

Figure 7. Number of cases in which microorganisms was present in two species of trees (X^2 (2, N = 18)= 0.13853, p =0.9331)