01 Jan 2004

Presence of Fungi on Decomposing Leaf Litter

Christina Bradstreet

Presence of Fungi on Decomposing Leaf Litter

April 1, 2005

By: Christina Bradstreet

Introduction

For many years it has been considered that leaves and other litter from riparian trees represent major energy sources in stream ecosystems. As soon as leaf litter hits the water of streams. it acquires various fungal species, which will eventually help complete the degradation of the leaf. Bacteria on the leaf litter might impair the determining factor of just how important fungi is to the decomposition of leaf litter if the bacteria was dominant and overpowered the fungi. However, it has been seen that bacteria counts of leaf litter found in streams in relatively low during the initial stages of decomposition. Various factors influence the amount and type of fungi that will be present on the leaf, including water temperature, season, types of fungi present in the particular stream being studied, and the type of leaf litter found in the vicinity. It has also been known that sole differences in the leaf litter in a particular stream also has an effect on what fungi will be dominant and the frequencies at which certain fungi might be found on the litter. Also, as leaves decompose, the chemical composition of leaves also change, which might later inhibit or stimulate various fungal species that are found on the leaf litter.

Hyphomycetes are known to be the main fungal decomposers of leaf litter. Fungal communities that might be found on the various leaf litters in streams can be characterized using either a traditional or a molecular style of approach. Using either technique the diversity of the fungi that are taking part in the leaf litter decomposition will easily become known. The traditional method of identifying fungi is microscope based and involves characterizing the fungi by counting and identifying the various conidia spores that it releases. In contrast, the molecular method is a more direct approach to identifying the various fungal communities through extraction and amplification of fungal DNA.

Traditional methods employ the art of identifying and characterizing the various conidia released by the fungi found of decomposing leaf litter. Conidia are simply asexual propagules that are typically carried downstream in order to help in the ability of the fungi to disperse and colonize in new locations. Typically, hyphomycetes will colonize available substrates, and then a few days later release conidia, which can then be used to classify the type of fungi that is found on the substrate (leaf litter). There are various ways in which conidia might be gathered from fungal decomposers: it is possible to collect the conidia out of the foam the naturally occurs on many streams, but simply taking a water sample from the stream, or by immersing leaf packs in to streams at different times of the year. Conidia are then filtered onto small filters, which are then dyed and put under the microscope. Using just visual contexts, the conidia are thus identified by comparing to other known species of fungi and their respective conidia. However, several conidia cannot be assigned to a specific fungal species with absolute certainty, which results in an underestimation of the true diversity of the fungal communities found on the leaves. Another low point to the traditional method of fungal classification is that by simply identifying conidia, the fungi that are non-reproducing or dormant are not represented since conidia are not produced. Using only the traditional

method results in an underestimated sample of the fungal decomposers found on leaf litter.

Since DNA is present in all life stages, a more direct approach to characterizing various fungal communities is through the extraction and amplification of the fungi. Therefore, all species of fungi can be detected, whether they are sporulating or not, allowing a higher diversity of decomposers to be seen. There are two major molecular techniques involved in this method. The first involves extracting and amplifying the fungal DNA by PCR using primers, and then separating the different fungal chains present using denaturing gradient gel electrophoresis (DGGE). The second method involves terminal-restriction fragment length polymorphism (T-RFPL) after the fungi have been amplified. Using DGGE, a semiquanitative estimate can be provided since fungal communities divide into bands of various widths depending on the amount of DNA per each community. However, fungi that are high in numbers might be too overpowering, which would then still obscure the less common species of fungi that are not found in large numbers. Nevertheless, this problem can be avoided through careful analysis of the data. A major problem that might be present, nonetheless, is that using the molecular method might inadvertently picks up DNA from non-targeting structures.

For our experiment we are interested in determining the types and amounts of various fungi communities found on leaf litter in streams. This will be done by placing litter bags in streams and taking them out at various intervals throughout the year. Next, the fungi will be classified according to both the molecular and traditional methods. First, conidia will be collected on filter paper and stained with a specific dye. Then the conidia will be viewed under a microscope. The conidia that are seen will be classified solely on how they look, their generalized appearance. Then how many are seen of each kind will be recorded, and then data compared between the different slides. Next, ground pieces of leaf litter will be plated, four to each plate. Fungi will be allowed to grow for a limited time until the fungi colonies are distinguishable. All the colonies will then be compared and identified solely on appearance and visualization. The amount of each type of fungi out of all the plates will then be recorded. Finally, DDGE will be used in the molecular method to classify the fungi.

Method

The experiment was conducted between October 2004 and March 2005 in the Audubon stream, which is found in Rolla, Missouri. The stream passes through an area of mixed trees and has a bed of various stones and gravel. This specific location was picked due to the relative isolation of the stream, entailing that no person or animal might come across the litter bags, and also due to the fact that the stream was easily accessible and maintained a fairly constant water level (without considering extreme weather conditions).

Initially, one gram of leaf litter was placed into 20 leaf litter bags and closed. These bags were then placed in one of four locations in the stream. Each location was relatively close to the next. However, to avoid losing all liter bags in the event of a storm, high water, or animal contact the bags were placed in four different groups or locations. Three to four bags were then collected about once a month. However, during the last month various oak leaves were also collected out of the stream and brought back to the lab for the same analysis in order to make sure that the litter bags did not facilitate any differences that might not normally occur in the streams.

At the field site, each litter bag that was collected was immersed in water collected from the stream that day in their own individual plastic bags. The same was also done with the leaves collected during the last month. Water temperature and pH were also measured, and a water sample taken to take back to the laboratory. Once in the laboratory, a small amount of water from one plastic bag was placed in a shallow tray. The litter bag from that particular plastic bag was then opened and the leaf litter put into the tray. Using a cork borer, ten cores of approximately 10mm in diameter were taken. Five of these cores were placed in recently made aeration tubes. The tubes consisted of 50mL plastic tubes that were filled with 40mL of distilled water. 10mL pipettes were then connected to aeration tubing, and the pipettes were then placed into the water. The cores were allowed to aerate for approximately two to three days. The last five cores were wrapped well in aluminum foil and placed in small petri dishes. These were then put into the freezer for further analysis at a later date. This process was repeated for all leaf litter bags and the oak leaves collected during the last month of the experiment.

After the cores were allowed to aerate in the tubes for two to three days, a 10mL sample of the water was taken out of each tube. This water was then filtered, and the filter paper collected. The filter paper was then placed onto a microscopic slide, dyed with methyline blue, and covered with a clean cover sip. After allowing time for the conidia on the filter paper to take up the dye, the slide was then observed under the microscope. The various types of conidia present and the differing amounts of each were then counted and recorded. The various leaf litter bags collected at each individual time were then compared in order to obtain an overall picture of the fungal communities found on the decomposing leaf litter. After conidia had been collected from the aeration tubes, three of the cores were taken out of each tube individually, placed under running distilled water for approximately five to ten seconds in order to remove any unwanted bacteria or specimens, and then placed in a cleaned blender filled with distilled water. The leaf cores were then chopped up to small pieces and the solution then poured into a shallow tray. Agar plates that had previously been made, each of which contained nutrients and various antibiotics (to prevent unwanted bacterial growth) was then labeled with each designated litter bag. Each plate was then divided into four regions. Using aseptic technique, an approximate 1mm piece of leaf from the litter bags was placed into each of the four regions of the plate. This was done three times for each litter bag collected, except for the last month were each litter bag and each individual oak leaf had five plates. The fungi were allowed to grow and amplify until the various colonies were distinguishable to the naked eye on the agar plates. The various colonies discernable were then noted and

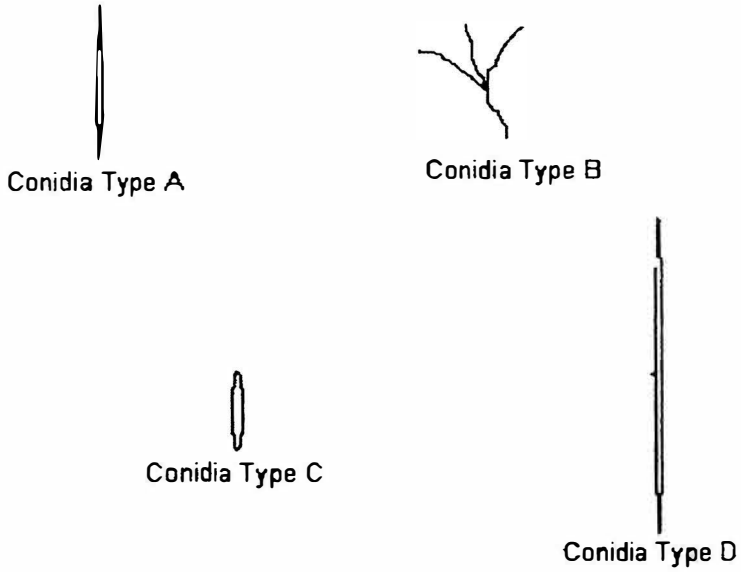
compared. This completed the traditional method of identifying the various types of fungi located on the leaves.

Next, DNA was extracted from the frozen leaf cores using PCR and various fungal primers. Primer pair ITS3GC will be used to amplify the ITS2 region of the fungi. This primer contains a GC clamp, which will allow accurate separation of the various DNA strands while running the DGGE. The rest of the ITS region was amplified using the primer ITS5, that was labeled with fluorescent dye. Polymerase chain reaction (PCR) was performed with PCR beads. After amplification the DNA was then placed on a gel that was electrically charged. The various strands of DNA would separate out on the gel due to differing lengths of each strand after running the DGGE. Each strand would then represent a single fungal colony.

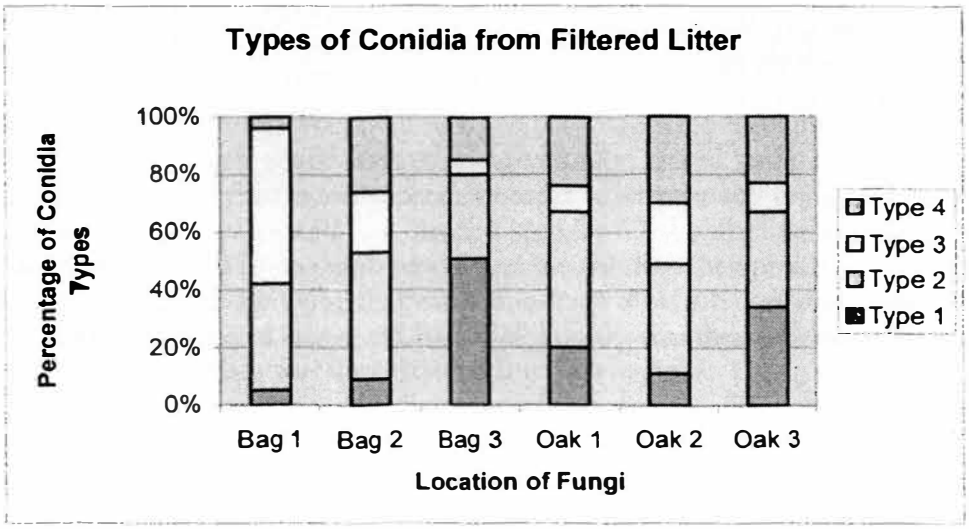
Results

Due to the different seasons that encompassed this experiment, water temperatures and amounts of conidia seen followed the expected trends. More conidia were seen during the winter season, and then fewer and fewer amounts were noticed as the season progressed. At the end of the experiment, conidia counts were at a low when compared to other numbers gathered throughout the previous portions of the experiment.

The amount of conidia collected on the filter paper after filtration varied depending on the time of the year. More conidia were seen in the middle of the experiment, during the early winter season, than at the beginning (autumn season) and the end (late winter season). After the filter papers were allowed to sit and take up the dye, the slides containing the filter paper was viewed under the microscope. Types of conidia seen were noted. Only four major conidia types were seen, though they were only identified by shape and visualization, not correct names and identities. Below are the four types of conidia seen:

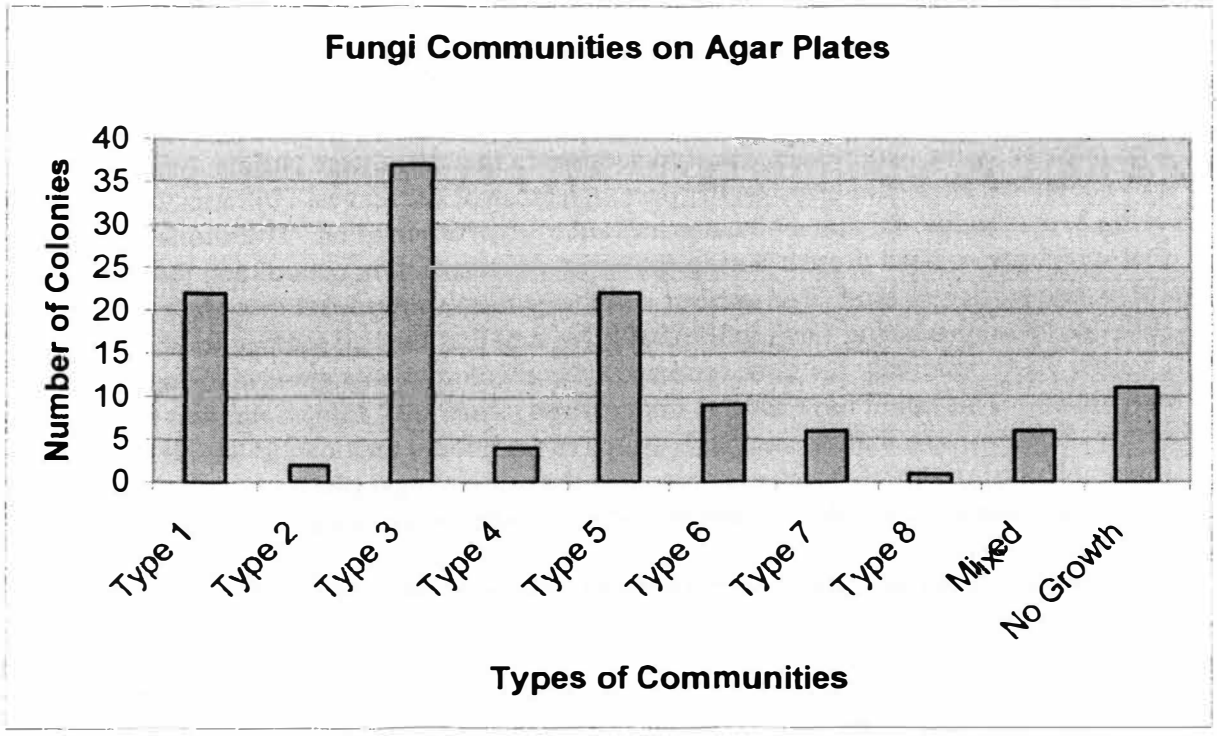


After counting all six slides (three leaf litter bags and three oak leaves), the differing amounts of each conidium were noted. It was seen that the numbers correlating to each individual conidia type varied, and the variation had no definite pattern. Below is a graph that compares the percentages of each conidia type per slide (taken from the last month).



As can be seen, the most prominent conidia spore found on the leaf litter happened to be Type 2. This denotes the fact that approximately the same fungi are inhabiting and decomposing the varies leaves found in the various locations, since the same types of conidia are being released.

Next, we observed the various fungi colonies growing on nutrient agar plates once small pieces of ground up leaf litter was placed on the plates. The result showed approximately eight different types of colonies, some of the specks showed no growth, and few of them showed several colonies, meaning that the leaf speck was not small enough to isolate only one fungal colony. The fungi colonies were classified according to appearance. Type 1 was a white colony with a yellow circle in the center. Type 2 was a white colony was a small brown circle located in the center. Type 3 was once again a white colony, but contained a pink circle in the center of the colony. The fourth type was dark yellow with a very dark brown center. The fifth was once again white, but this time containing a dark gray to black center. The sixth colony was yellow, and the center was the gray/black. The seventh was simply a small yellow dot, and the eighth was a dark yellow colony with a faint, yet large green circular center. The main colony that was noted seemed to be the third type, with the first coming in a close second. The following chart depicts the number of colonies that were found belonging to each individual type of fungi:



Results from the molecular method of classifying fungi that help in the decomposition of leaf litter found in streams were inconclusive. The experiment is still underway, and will continue to be so for the next two months. Therefore, there are no results to report as of yet.

Discussion:

The number of conidia that were in the water varied depending on the time of year. The end results were obtained from the filtrate that we took during our last outing during the last month of the experiment, when conidia levels were low. The results showed that approximately four types of conidia spores were released at that time, spanning all three leaf litter bags and all three oak leaves that were subjected to the experiment. However, the number of each individual conidium found varied depending on the leaf litter bag or the leaf. The number of fungi colonies present, however, remained relatively stable. Towards the beginning of the experiment, many bacteria species were seen, on both the nutrient agar plates as well as the filter paper microscopic slides. The bacteria inhibited our ability to see and note the different fungal and conidia types. This was corrected by simply washing all the leaf cores off under running tap water for approximately five seconds. Also, antibiotics were added to the petri dishes to help control the bacteria growth, which should not and had no apparent effects of the fungi.

The purpose of the experiment was to demonstrate that there were various types of fungi present on leaf litter that helps on the decomposition of the leaves. This was to be done using both a traditional and molecular method of experimentation. Traditional showed that fungi were indeed present during the entire decomposition period, and that it was not solely one particular kind. The various conidia present showed that there had to be more than one spore producing fungi in the leaf litter, and that they all seemed to be in each sample. Also, relatively the same amount of fungi colonies was present throughout the experiment. This could have several implications: there are a limited amount of fungal species in the stream that we sampled, using the traditional method did not allow for all fungi colonies present to be seen, or also that the nutrient agar plates only allowed limited growth of certain fungi colonies (not all fungi are adapt to growing on agar plates).

Unfortunately, as of this time, our results using the molecular method are inconclusive.

Our study showed that fungi do play a part in decomposition and are present throughout the entire process. Exactly what kind of fungi was present was inconclusive, however a visualization of several fungi colonies and conidia were seen. With more extensive testing, it is highly probable that we will soon know the exact fungi that help in the decomposition of leaf litter in Missouri streams.

References:

Nikolcheva, L, Bourque, T & Barlocher, F. *Fungal diversity during initial stages of leaf decomposition in a stream.* New Brunswick, Canada. Pages 246-253.

Nikolcheva, L & Barlocher, F. *Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence.* "Environmental Microbiology." New Brunswick, Canada.

Nikolcheva, L & Barlocher, F. *Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream.* "Mycological Progress." New Brunswick, Canada.

Unknown. *Diversity of conidia of aquatic hyphomycetes assessed by microscopy and by DGGE.*