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Biology 9H

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Soil Ecology Project:

How does litter affect the fungal density in the soil?

Background

Littering in the United States is a significant problem. Over 51 billion pieces of it land on US roads every year, and the annual cleanup costs nearly $11.5 billion. Of this trash, 52% comes from motorists, 22.8% from pedestrians, 16.4% from improperly covered trucks and collection vehicles, and 1.5% from improperly secured containers, dumpsters, trashcans, and recycling bins. Most of it is made up of cigarette butts (38%), but paper (22%) and plastic (19%) make up a significant amount of the litter as well (Great American Cleanup, 2006).

The problem with all this trash is that it does not decompose and disappear through natural processes as quickly as humans put it there. While foods such as bananas can decompose in a couple of weeks, items such as cigarette butts can take 1 to 5 years to decompose, and a plastic bag can take anywhere from ten to twenty years. In fact, an aluminum can can take up to 500 years before it is completely gone. Hence, the amount of trash that is littered every year can take centuries to decompose (Minnesota Department of Transportation, 2012). In the meantime, though, all this trash continues to affect the earth's surface, including the millions of living things that inhabit the soil, and this is especially true for the microbes that live there.

One such microbe, fungi, are normally actually involved in the decomposition process. They usually decompose the bodies of dead organisms and, by doing so, help recycle nutrients in the soil (Robinson, 2012). They convert dead organic matter into their own biomass, as well as releasing carbon dioxide, organic acids, and ammonium, and these converted organic matters then provide some of the necessary nutrients in the soil for other plants and organisms to survive.

Among these nutrients, the most crucial are those involved in decomposing hard, woody matter and other material that contains cellulose, proteins, lignin, and other nitrogen rich

compounds. The decomposing fungi release digestive enzymes through their cell walls which then break down the complex compounds of dead organisms into simple molecules such as ammonia, and this ammonia is then converted to ammonium and nitrate that plants can absorb to make their nucleic acids and proteins (Harrison, 2003). Plants need these critical biological molecules to start and stop their cells' chemical reactions between the five basic biological molecules that enable them to live. Hence if fungi did not transport these nutrients, plants would have a very hard time surviving.

Soil fungi are able to perform this crucial task because of the large size and surface area of their physical structure. Fungi, which are multicellular, have many threadlike filaments called hyphae that they use to develop symbiotic relationships with plants. These mutualists, known as mycorrhizae, live in or on plant roots and increase a plant's ability to take in water and nutrients from the soil (especially in less fertile soil). In return, fungi get their food from plant roots in the form of carbohydrates provided by photosynthesis. Mutualists also produce hormones and antibiotics that enhance the growth of the roots and help protect plants and themselves from diseases (Jenkins, 2005).

It is believed that most of the carbon and nutrient exchange takes place in a type of mycorrhizal fungi called arbuscular mycorrhizas (Badenko, 2004), which form vesicles with their hyphae inside the cells of the plant roots that can take many different shapes. However, the ectomycorrhizal type of fungi help plants take in phosphorus, nitrogen, and water by creating and extending root branches and increasing the root's surface area using cellular mechanisms so they can greatly help the intake of necessary nutrients for the plant (Jenkins, 2005). Either way, all these mutualist fungi can help the plants to be stronger against droughts and provide a hospitable

environment for other soil organisms to live in and get their own food from more easily (Jenkins, 2005).

It is clear, then, that without fungi in the soil, the soil ecosystem would be in shambles. However, the soil ecosystem would not be the only part of the environment that would suffer. Fungi in the soil may help plants get their nutrients, but animals get their nutrients from plants. So if the soil fungi population in an ecosystem were to drastically decrease, the number of plants could diminish as well, meaning the animals could die off due to starvation.

Yet, since soil fungi are so critical to everything in an ecosystem, any harm the trash or other litter might be causing could be harming the entire environment in which lots of different organisms live (including people). Therefore, our experiment is testing to see whether or not the common human habit of littering affects this vital microbe. Something that humans do on a day-to-day basis could cause the soil fungi population to suffer, which could cause the nutrients in the soil to not be able to get to the plants that need them. If our experiment proves this, then it is a clear sign that changes in the way society takes care of its waste need to be made.

Experiment

I. Problem: How will the presence of litter change the fungi population density in the soil?

II. Hypothesis: The presence of litter will decrease the soil fungi population density.

III. Procedure:

   a. Independent variable: presence of litter on the surface of the soil

   b. Dependent Variable: population density of fungi in the soil

c. Negative control: plot of soil with no litter added to the surface.

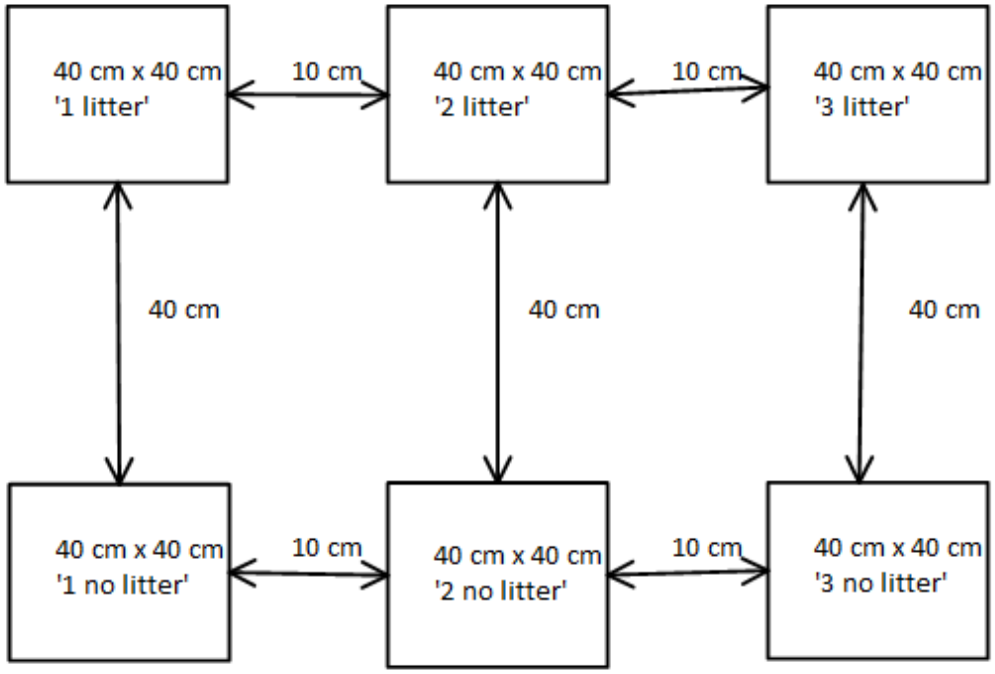
d. Positive control: density of all fungi populations before litter is added

e. Controlled Variables: coordinates of plots, type of litter including brand and size, amount of litter, size of plots, plant life, amount of time litter is present, spacing of litter, spacing of stakes, spacing between plots, type of tools used when extracting soil, amount of soil extracted, type of water and amount used to dilute the soil, new micro pipette tips for each use, labeled petri plates for each dilution and plot number and type, same food given to fungi, amount put onto petri plates, amount of soil in the 10^0 culture tubes, same dilutions plated in each experiment, amount of time for fungi colonies to grow, equation used to find density of fungi population, degree diluted kept the same in all of the trials,

f. Step by step instructions

    1. Go to the lawn on the edge of Roland Park Country School near the street, find a patch of grass at the coordinates N-39.357 W-76.637 and measure a 40x 40cm square on a patch of soil and mark it with flags with ‘1 litter’ on them on the corners. Do the same with the rest of the plots labeling them according to the picture in step 2.

2. Measure and label the plots as shown in picture:



3. Make sure all soil samples taken in steps 4-6 are taken on the same day at the same time to control for weather.
4. Use the Soil corer which is 2 cm in diameter and place it in the center of the plot labeled “1 litter”, stick the soil corer into the ground until it reaches 15 cm into the ground.
5. Rotate the soil corer clockwise to gather a soil sample.
6. Slowly take out the column from the ‘1 litter’ plot and put the soil sample into a plastic bag (immediately after rotating the soil corer)and mark it ‘trial 1 litter before’ Do steps 4 and 5 for the rest of the six plots and label them accordingly to their trial and plot and place each soil sample into its own separate respective new plastic bag.
7. **Do steps 8-15 on the same day at the same time.**

8. Use a clean transfer pipette to add 10 ml of the sterile water to each of the six 15 ml culture tubes, label the tubes "10^0 :before 1 litter", "10^0 : before 2 litter", "10^0 : before 3 litter", "10^0: before 1 no litter" , "10^0 : before 2 no litter", and "10^0 : before 3 no litter".
9. Use the same pipette to add 9 ml to each of the second set of six 15 ml culture tubes, label the tube "10^{-1} : before 1 litter", "10^{-1}: before 2 litter", "10^{-1}: before 3 litter", "10^{-1} : before 1 no litter" , "10^{-1} : before 2no litter", and "10^{-1}: before 3 no litter".
10. Use the same pipette to add 9 ml to each of the second set of six 15 ml culture tubes, label the tube "10^{-2} : before 1 litter", "10^{-2}: before 2 litter", "10^{-1}: before 3 litter", "10^{-2} : before 1 no litter" , "10^{-2} : before 2no litter", and "10^{-2}: before 3 no litter ".
11. Place 1cc of your soil sample from the '1 litter' bag into the "10^0: before 1 litter" culture tube; Cap the tube and shake vigorously.
12. Use a new clean pipette labeled '1 litter', remove 1 ml of the soil/water mixture from the "10^0: before 1 litter" tube and place into the "10^{-1}: before 1 litter" tube and shake the tube. Then using the same pipette take 1 ml of the soil/water mixture and place it in the culture tube marked "10^{-2}: before1 litter".
13. Take three 3M Petrifilm ™: yeast and mold count plates and label one with "10^0: before1 litter", one with "10^{-1}: before1 litter", and one with "10^{-2}: before 1 litter".
14. Next, on the plate marked "10^0 : 1 litter", place 100 µl from the culture tube labeled "10^0 : 1 litter". On the plate marked"10^{-1}: 1 litter", place 100 µl from the

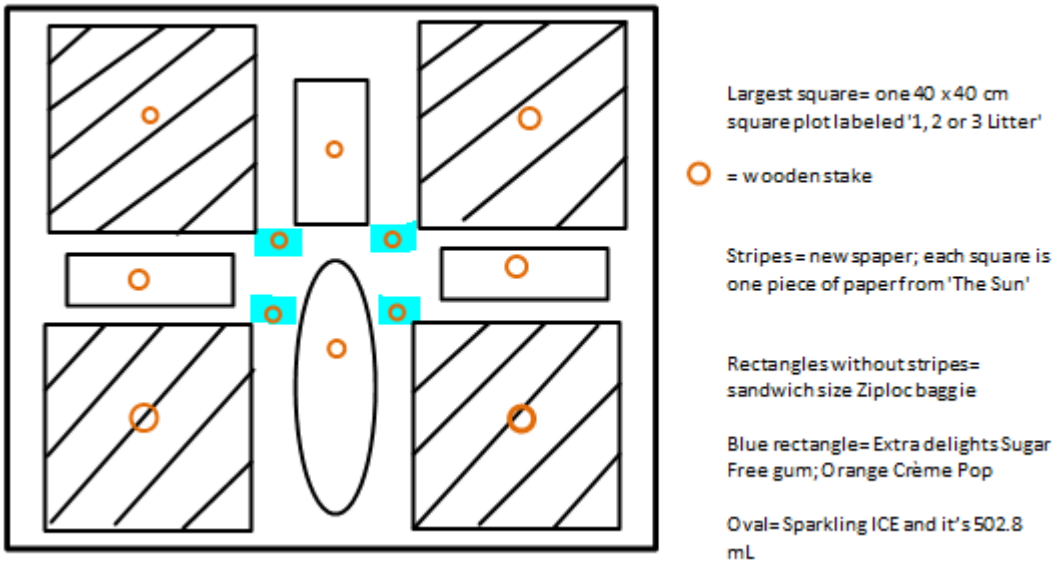
culture tube labeled "10^{-1}: 1 litter". On the plate labeled "10^{-2}: 1 litter", place 100 µl from the culture tube labeled "10^{-2}: 1 litter".

15. Repeat steps 11-14 for "2 litter", "3 litter", "1 no litter", "2 no litter", and "3 no litter" on the same day at the same time labeling them with "before" following the dilution and plot number.
16. Allow all of the plates to grow for 120 hours at room temperature.
17. Examine each of the plates for individual fungi colonies on the same day at the same time, count the yeast and mold separately. Molds are indicated by larger 'fuzzier' circles and yeasts are defined dots that are solid colored. Choose the plate with the fewest colonies but the lowest dilution for both mold and yeast to make your estimates of the number of fungi in the original 1 cc soil sample (you may use a different dilution level for the yeast and mold) using the following formula:

    # Microbes in 1 cc of soil= # Colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

18. On the plots labeled '1 litter', '2 litter', and '3 litter' 40 x 40 cm squares place 4 gum wrappers, two plastic sandwich sized Ziploc baggies and one sparkling ICE bottle on each plot and secure them down with wooden stakes (see diagram in step 20).
19. On the three plots labeled '1 no litter', '2 no litter', and '3 no litter', place wooden stakes into the ground where you would have placed the litter (see diagram in step 20).

20. Space them like this:



21. Wait 5 days then take the gum wrappers, bags of chips and aluminum cans off of the plots.
22. Repeat steps 3-17 this time label the baggies, and culture tubes, and nutrient agar plates with 'after' following the dilution and plot number.
23. Be sure to have recorded all data into your table.

Data and Analysis

IV. Data and Analysis

A. Data Table

Fungal Densities Before Putting Litter on the Plots (# /1 cc of soil)

Trial	Yeast # in 1 cc of soil	Mold # in 1 cc of soil	Total fungi in 1 cc of soil
Litter 1	40000	100000	140000
Litter 2	6000	90000	96000
Litter 3	60000	120000	180000
No Litter 1	40000	50000	90000
No Litter 2	11000	40000	51000
No Litter3	5000	6000	11000

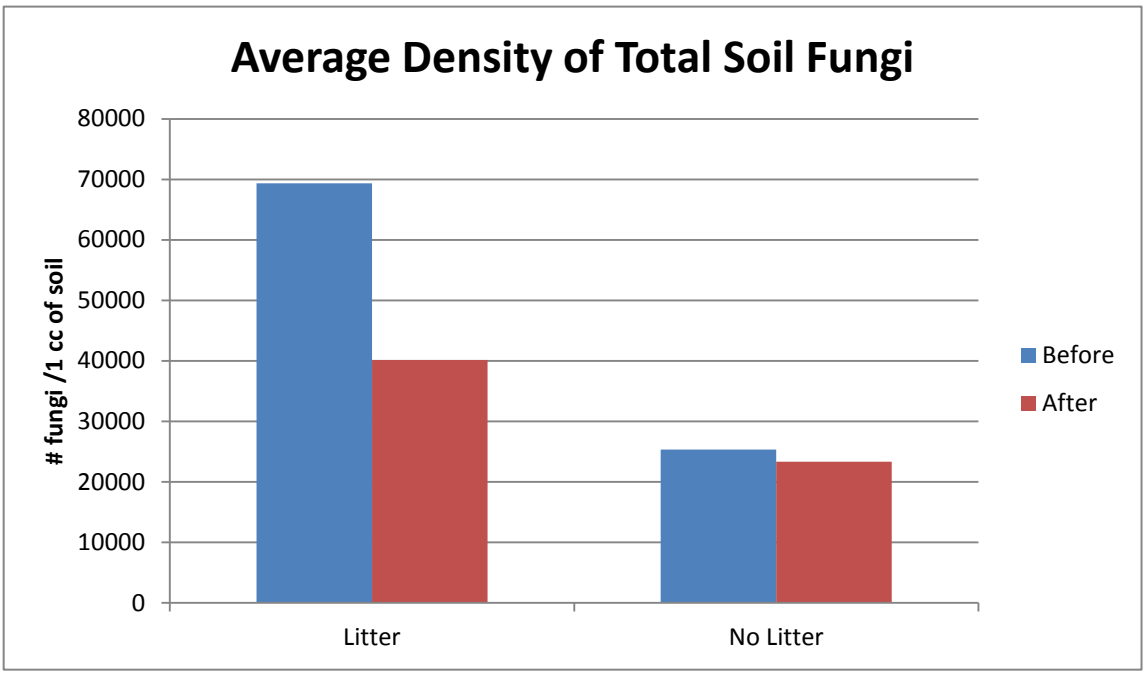
Fungal Densities After Putting Litter on the Plots (# /1 cc of soil)

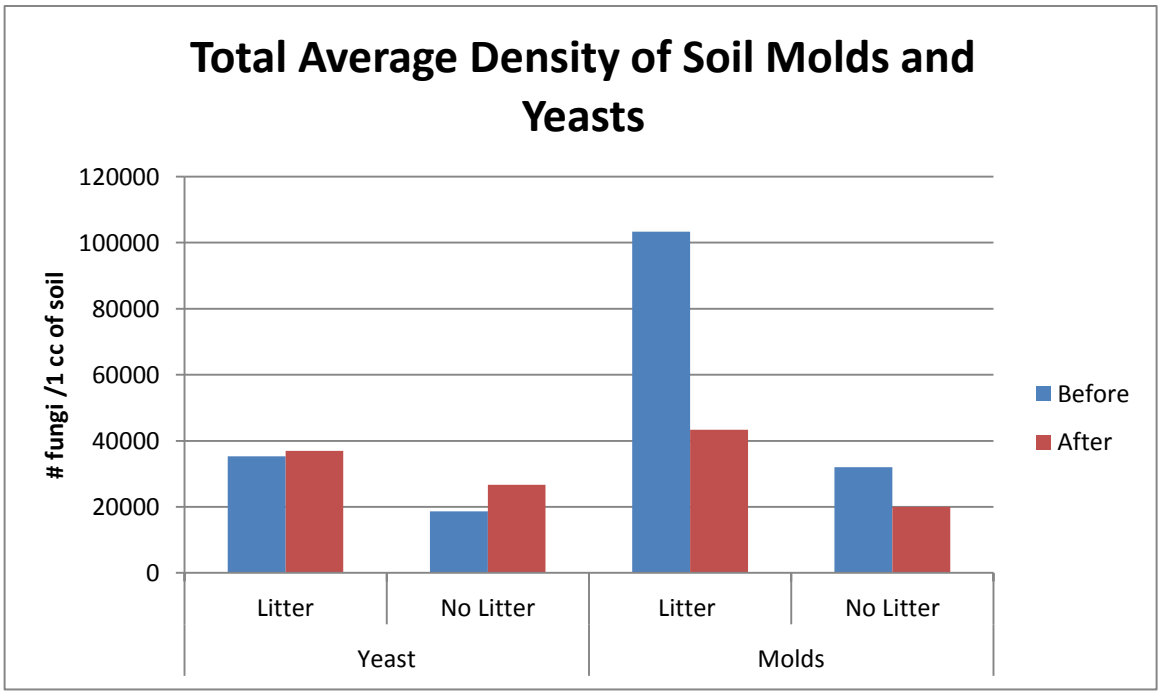
Trial	Yeast # in 1 cc of soil	Mold # in 1 cc of soil	Total fungi in 1 cc of soil
Litter 1	11000	30000	41000
Litter 2	70000	50000	120000
Litter 3	30000	50000	80000
No Litter 1	10000	10000	20000
No Litter 2	30000	30000	60000
No Litter3	40000	30000	70000

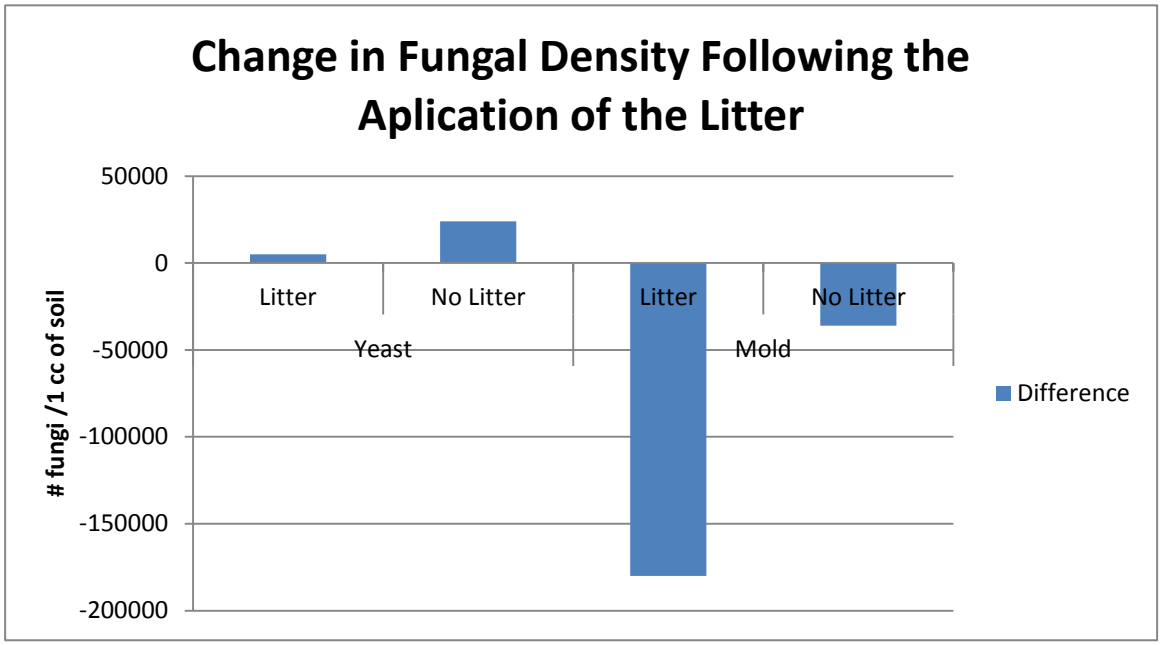
Average Fungal Densities (# /1 cc of soil) in Litter and No Litter Plots

	Yeast /1 cc of soil		Molds / 1 cc of soil		Total fungi /1 cc of soil	
	Litter	No Litter	Litter	No Litter	Litter	No Litter
Before	35333	18667	103333	32000	69333	25333.5
After	37000	26667	43333	20000	40166.5	23333.5

B. Graph


Average Density of Total Soil Fungi
fungi /1 cc of soil
80000
70000
60000
50000
40000
30000
20000
10000
0
Litter
No Litter
Before
After


Total Average Density of Soil Molds and Yeasts
fungi /1 cc of soil
120000
100000
80000
60000
40000
20000
0
Litter
No Litter
Litter
No Litter
Yeast
Molds
Before
After



Conclusion

Our hypothesis states that adding litter to the plots of soil will decrease the fungi population in the soil. We chose this hypothesis because litter can release toxins into the ground such as methane and leachate (Nathanson, 2012). These toxins have the ability to destroy cells and kill living organisms such as fungi. After testing our hypothesis, we found that it was correct. Our first graph shows the Average Density of Total Soil Fungi. It shows us that for the plots without litter, the average population of fungi in the soil began with 25333.5 fungi per 1 cc of soil to 23333.5 fungi per 1 cc of soil. There was only a slight decrease of 2,000 total fungi per 1 cc of soil between the first and second time the soil was tested. This shows us that the fungi population was hardly effected by anything in the environment. The soil that was tested with litter had a much greater decrease in the fungi population. The density decreased from 69,333 fungi per 1 cc of soil to 40,166 fungi per 1 cc of soil, making a total decrease of 29,166.5 total

fungi per 1 cc of soil. However, in our second graph that shows the Total Average Density of Soil Molds and Yeasts, we see that there was a greater increase of yeasts in the No Litter plots than in the Litter plots. We also see that the amount of molds in the Litter plot decreased much more than the amount of molds in the No Litter plots. When a fungus is in its yeast form, it means that it is in protection mode. Because there was a greater yeast increase in the No Litter plot than in the Litter plot, we can see that there was some other environmental factor that could have affected the fungal density. This led us to make a third graph, that shows the Change in Fungal Density Following the Application of the Litter. In this graph, we can see that even if the decrease in mold in the No Litter plots was subtracted from the decrease in mold from the Litter plots, the drop in the fungi density is still considerably larger than the drop in the No Litter plots. This shows that even with the other mysterious environmental factor, there was a greater decrease in the fungi density with litter than without. Because our soil plots were near a road, and it had rained prior to our testing, we believe that the mysterious environmental factor that increased the density of yeast in the no litter plots could be from the run off of the road. The data that we have collected leads us to ask further questions to research such as: "What is the effect of road pollutions on the fungal density in soil?" and "Which type of litter is the most harmful to the fungi population?"

References

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