Composting in Schools

Setting the Scene, Lab Activities, Composting Health Considerations

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Setting the Scene

- Trash Goes to School: K-12 Solid Waste Lesson Plans

Lab Activities

Observing Compost Invertebrates

by Elaina Olynciw

Background

In outdoor compost piles, a wide range of invertebrates take part in the decompostion of organic matter. Try monitoring invertebrate life in the pile over the course of the compost process. How long is it before you locate the first invertebrates? What happens to them when the pile heats up? Do you find different organisms later on, after the pile cools down?

In indoor container composting you may find fewer (or no) invertebrates, and decomposition is accomplished by microbes alone.

Materials

- light-colored trays or pans
- tweezers, spoons, or tongue depressors

Procedure

One method of collecting invertebrates is to take grab samples of compost from various locations in the heap. Some organisms such as centipedes and sowbugs will be more likely to be found near the surface. Others will be found deeper in the heap. Spread each compost sample in a large tray or pan, preferably light in color for maximum contrast. Students should use wooden tongue depressors, plastic spoons, or other instruments that will not hurt the organisms, to sort through the compost. Flashlights and magnifying lenses can be used to enhance the observation. The larger organisms, such as worms, centipedes, millipedes, sowbugs, earwigs, spiders, ants, beetles, snails, slugs, some mites, etc., can be observed with the naked eye. To get a closer look, place samples of the compost in petri dishes or watch glasses and observe them under a dissecting microscope.

An alternative method of separating small arthropods in compost is by using a "Berlese funnel". This method will provide a higher concentration of arthropods to view. Place a funnel with a 10-30 cm upper diameter in a ring stand. Attach a circle of 10mm wire mesh (hardware cloth) or window screen 8 cm below the funnel. Just below the funnel, place a vial to collect the specimens. Position a light source (25 watt) 2.0 - 2.5 cm above the funnel, or place the collecting apparatus in a sunny location. The light and heat drive the negatively phototaxic compost organisms downward through the funnel and into the collecting jar. If you use too strong a light source, the organisms will dry up and die before making it through the funnel.

Place compost in the funnel and then partially fill the vial with water if you want to observe live organisms. Observe the organisms about 2 to 4 days later. They will remain alive and float on top of the water. You can place them in a petri dish or watch glass and observe them under a dissecting microscope

or with a magnifying glass. You should find small arthropods, including many different kinds of mites, a few different insect larvae, springtails, small millipedes, ants, etc.

The organisms can be lifted out with a paint brush and maintained in small chambers of plaster of paris (mixed with powdered charcoal to aid observation). This substrate must be kept continually moist to keep the arthropods alive. Adding brewer's yeast to the substrate provides a food supply for many species.

A mixture of 90% percent ethanol and 10% glycerol can be used to collect the arthropods if preserved organisms are needed for quantitative study. A grid of 1.0cm squares can be set up on a petri dish for counting. Removal of organisms can be accomplished with an angled sewing needle that has been filed flat. The pointed end can be imbedded in a cork or wooden dowel or matchstick. Thin, drawn out pipettes can also be used and rinsed out with alcohol if organisms get stuck.

Observing Compost Microorganisms

This protocol was written by Elaina Olynciw, biology teacher at A. Philip Randolph High School, New York City, while working in the laboratory of Dr. Eric Nelson at Cornell University as part of the Teacher Institute of Environmental Sciences.

Introduction

Observe the microbial communities in your compost over the course of several weeks or months as the compost heats up and then later returns to ambient temperature. Can you identify differences in microbial communities at various stages of the composting process?

Materials

- compound microscope
- .85% NaCl (physiological saline)
- methylene blue stain (Prepare stain by adding 1.6 g methylene blue chloride to 100 ml of 95% ethanol, then mixing 30 ml of this solution with 100 ml of 0.01% aqueous solution of KOH)

Procedure

1. Make a wet mount by putting a drop of water or physiological saline on a microscope slide and transfering a small amount of compost to the drop. Make sure not to add too much compost or you will not have enough light to observe the organisms.
2. Stir the compost into the water or saline (the preparation should be watery) and apply a cover slip.
3. Observe under low and high power. You should be able to find many nematodes (they should be very wiggly), flatworms, rotifers (notice the rotary motion of cilia at the anterior end of the rotifer and the contracting motion of the body), mites, springtails and fast-moving protozoans. Pieces of fungi mycelia can be seen, but might be difficult to recognize. Bacteria can be seen as very tiny, roundish particles, which seem to be vibrating in the background.
4. If you want to observe the bacteria directly, you can prepare a stained slide and observe the slide using a 100X oil immersion lens. To prepare a stained slide, mix a small amount of compost with a drop of physiological saline on a slide. Spread with a toothpick. Let the mixture air dry until you see a white dried film on the slide. Next fix the bacteria to the slide by passing the slide through a hot flame a few times. Stain the slide using methylene blue stain. Flood the slide with the methylene blue stain for one minute and then rinse with distilled water and gently blot dry using blotting or filter paper.

5. Fungi and actinomycetes may be difficult to recognize with the above technique because the entire organism (including the mycelium, reproductive bodies and cells) will probably not remain together. Fungi and actinomycetes will be observed best if you can find fungal growth on the surface of the compost heap. The growth looks fuzzy, powdery, or like a spiderweb. Lift some compost with the sample on top, and and prepare a slide with cover slip to view under the microscope. You should be able to see the fungi well under 100X and 400X. The actinomycetes can be heat-fixed and gram-stained to view under oil immersion at 1000X.
6. To separate nematodes, rotifers, and protozoans, a continuous column of water leading from the compost to the collection vial is necessary, and the following adaptation of the above method should be used: The compost is put into a beaker with the screen stretched across the top and taped in place. The beaker is then turned over into the funnel. Plastic tubing is placed at the end of the funnel stem and a screw clamp is placed a few inches below the end of the funnel stem on the pastic tubing.The plastic tubing should lead into a collection vial or small beaker. The clamp is closed and water is poured into the funnel until the beaker is about 1/2 filled. After a few days the clamp is slightly and slowly opened and organisms which have concentrated at the end of the tubing should fall into the vial.

Techniques for Detailed Study of Compost Microorganisms

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Collecting Samples

Microorganisms are not distributed uniformly throughout compost; they commonly occur in clumps or colonies ranging from few to thousands of individual cells. The populations vary greatly depending upon the amount of undecomposed organic matter and the micro-environment in a specific location. How wet the sample is, and whether it contains anaerobic or aerobic regions, also will affect the types of microbial life that are found. Multiple samples therefore should be taken to determine microorganism numbers or activity in compost.

Calculating Dry Weight

Water content of different composts may vary greatly. When comparing how much microbial activity there is in a gram of compost, you must allow for the difference in water content so that you can accurately compare what is happening in equal amounts of two different composts while discounting the weight of the water.

To determine the ratio of wet to dry weight of a compost, a sample of wet compost is weighed and then dried for 24 hours in a 105-110C oven. It is then reweighed, and the ratio between wet and dry weight is calculated.

When using the actual (wet) compost in a study, the moisture ratio is used to calculate how much compost to use. For example:

Amount of vegetable waste compost needed = 5 g

Predetermined wet weight of a sample = 4.3 g

Measured dry weight of the same sample = 2.8 g

Ratio of wet/dry = 1.54
Actual amount of compost needed for the experiment would be:
5 g x 1.54 or 7.7 g (wet weight)

Culturing Microorganisms

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The procedures to use in culturing micooganisms depend on which types of organisms you wish to study.

Culturing Bacteria
To culture bacteria, the following media should be used to prepare agar plates:
Growth Media: 1/10-strength Trypticase Soy Agar (TSA) Media:

Ingredients:
2 g Trypticase Soy Agar
7.5 g Bacto Agar
500 ml distilled water

Mix the ingredients, autoclave for 20 minutes, and pour into sterile petri dishes.

Plate out the bacteria using a 10^{-7} dilution starting with 5 g dry weight of the compost in 45 ml of an autoclaved .06M $NaHPO_4/NaH_2PO_4$ buffer of 7.6 pH. (approximately 4:1 dibasic:monobasic). Put this first dilution in a blender for 40 sec. at high speed.

Perform serial dilutions to 10^{-7} and add 0.1 ml of the dilution per plate. Incubate at 28C for 4 days. Count the colonies as colonies per unit after 4 days. Prepare slides of specific colonies the same day.

Culturing Actinomycetes
Growth Media: 1/50-strength TSAPoly B

Ingredients:
0.4 g Typticase Soy Agar
10.0 g Bacto Agar
500 ml distilled water
10 mg Polymixin B in 10 ml 70% Ethanol

Mix the first 3 ingredients, autoclave for 20 minutes, and cool to room temperature. Add the antibiotic and pour into sterile petri dishes.

Plate out the actinomycetes using a 10^{-7} dilution starting with 5 g dry weight of compost in 45 ml of the autoclaved buffer. Put this first dilution in a blender at high speed for 40 sec.

Perform serial dilutions to 10^{-7} and add 0.l ml of the final dilution to each plate.

Incubate the plates at 28C for 14 days.

Take counts and samples of actinomycetes colonies after 14 days. Many of the colonies will look powdery white. However, some may take on a rough appearance and produce a variety of pigments.

Note: If you are comparing mesophilic compost to thermophilic compost, you should prepare double the usual number of plates so that you can incubate plates at both 28C and 50C.

Culturing Fungi
Growth Media: 1/3-strength PDARP

Ingredients:
6.5 g Potato Dextrose Agar
5.0 g Bacto Agar
500 ml distilled water
15 mg Rifampicin in 10 ml Methanol
15 mg Penicillin G in 10 ml 70% Ethanol

Mix the first 3 ingredients, autoclave for 20 min. and cool to room temperature. Add the antibiotics and pour into sterile petri dishes.

Plate out the fungi using a 10^{-4} dilution starting with 5 g dry weight of compost in 45 ml of the autoclaved phosphate buffer. Put this first dilution in a blender at high speed for 40 sec.

Perform serials dilutions to 10^{-4} and add 0.l ml of the final dilution to each plate.

Incubate the plates at 28C for 3 days.

Take counts and samples of fungal colonies at 3 days.

Preparing Slides of Microorganisms

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Bacteria
Use sterile techniques to prepare your slide! Use an inoculating needle to add a drop of saline to a clean slide. Take a sample of a single bacterial colony and mix it into the saline. Let air dry until a white film appears. Heat fix by passing the slide through a flame a few times.

Actinomycetes
Follow the above procedure but try to get a portion of the colony on the slide intact. You can try lifting it with a sterile scalpel. It will be too crowded to observe on most of the slide, but at the edges of the colony you will be able to see the pattern that the filaments form.

Fungi
Lift a portion of the colony intact onto a clean slide (it will still be attached to the agar), add a cover slip and observe without staining. Look at the edges of the colony where the sample will be thinner and there will be enough light to observe.

Staining Slides
Gram staining can be used to make slides of bacteria and actinomycetes:

Preparation of Gram Stains

Crystal Violet:

Dissolve 2 g of crystal violet in 20 ml of 95% ethanol. Add this solution to 80 ml of a 1% Ammonium Oxalate solution. Let stand for 24 hours and filter.

Gram Iodine:

Add 1 g Iodine and 3 g Potassium Iodide to 300 ml distilled water. Store in an amber bottle.

Decolorizer: 95% Ethyl Alcohol

Safranin:

Add 2.5 g safranin to 10 ml 95% ethanol. Add this solution to 100 ml distilled water

Procedure for Gram Staining

1. Flood slide with crystal violet - 20 sec.
2. Wash with distilled water - 2 sec.
3. Flood slide with Gram iodine - 1 min.
4. Decolorize by tilting slide and drop by drop rinsing with 95% ethanol until ethanol runs clear - about 10 to 20 sec.
5. Wash with distilled water - 2 sec.
6. Flood with safranin - 20 sec.
7. Wash with distilled water - 2 sec.
8. Blot dry.

Monitoring the Composting Process

As composting proceeds, a number of changes occur in its physical, chemical, and biological characteristics. Monitoring some of these variables will help you to assess the status of your compost and to compare the progress of systems with different initial conditions or ingredients.

Monitoring Compost Moisture

Composting proceeds best at a moisture content of 40-60% by weight. At lower moisture levels, microbial activity is limited. At higher levels, the process is likely to become anaerobic and foul-smelling.

When you are choosing and mixing your compost ingredients, you may wish to measure the moisture content. After the composting is underway, you probably don't need to repeat this measurement because you can observe whether appropriate moisture levels are being maintained.

If your compost starts to smell bad, chances are it's too wet. Excess water fills the pore spaces, impeding diffusion of oxygen through the compost materials and leading to anaerobic conditions. Mixing in additional bulking agent such as dry wood chips, cardboard pieces, or newspaper strips is likely to

alleviate the problem. If you are composting in a bioreactor with drainage holes, you may notice leachate draining out. This liquid is often rich in nutrients and can be diluted for use on plants. You may find it useful to record the amount of leachate produced by each system, for comparison with initial moisture content, temperature curves, or other variables.

If you are blowing air through your compost system, you will need to be careful not to dry it out. If the temperature drops sooner than expected and the compost looks dry, moisture may have become the limiting factor. In this case try mixing in some water and see if the temperature rises again.

Monitoring Compost Temperature

Temperature is one of the key indicators in composting. Is the system heating up? How hot does it get? How long does it remain hot? How does mixing affect the temperature profile?

Heat is generated as a byproduct of microbial breakdown of organic material, and you can use the temperature of your compost to gauge how well the system is working and how far along the decomposition has progressed. For example, if your compost heats up to 40 or 50C, you can deduce that the ingredients contained adequate nitrogen and moisture for rapid microbial growth.

To take your temperature readings, make sure to use a probe that reaches deep into the compost. Leave the probe in place long enough for the reading to stabilize, then move it to a new location. Take readings in several locations, including at various depths from the top and sides. Compost may have hotter and colder pockets depending on the moisture content and chemical composition of ingredients. Can you find temperature gradients with depth? Where do you find your hottest readings? For systems in which air enters from the bottom, the hottest locations tend to be two-thirds or more of the way up. Is this true for your system?

By graphing compost temperature over time, you can tell how far along the decomposition has progressed. A well constructed compost system will heat up to 40 or 50C within two to three days. As readily decomposable organic matter becomes depleted, the temperature begins to drop and the process slows considerably.

The temperature at any point depends primarily on how much heat is being produced by microorganisms and how much is lost through aeration and surface cooling. How long the system remains hot therefore depends on the chemical composition of the ingredients as well as the size and shape of the system. Moisture content also affects temperature change; since water has a higher specific heat than most other materials, drier compost mixtures tend to heat up and cool off more quickly than wetter mixtures, providing adequate moisture levels for microbial growth are maintained.

Monitoring Compost pH

Why is compost pH worth measuring? Primarily because you can use it to follow the process of decomposition. Compost microorganisms operate best under neutral to acidic conditions, with pH's in the range of 5.5 to 8. During the initial stages of decomposition, organic acids are formed. The acidic conditions are favorable for growth of fungi and breakdown of lignin and cellulose. As composting proceeds, the organic acids become neutralized, and mature compost generally has a pH between 6 and 8.

If anaerobic conditions develop during composting, organic acids may accumulate rather than break down. Aerating or mixing the system should reduce this acidity. Adding lime (calcium carbonate) generally is not recommended because it causes ammonium nitrogen to be lost to the atmosphere as ammonia gas. Not only does this cause odors, it also depletes nitrogen that is better kept in the compost for future use by plants.

At any point during composting, you can measure the pH of the mixture. In doing this, keep in mind that your compost is unlikely to be homogeneous. You may have found that the temperature varied from location to location within your compost, and the pH is likely to vary as well. You therefore should plan to take samples from a variety of spots. You can mix these together and do a combined pH test, or test each of the samples individually. In either case, make sure to make several replicate tests and to report all of your answers. (Since pH is measured on a logarithmic scale, it doesn't make sense mathematically to take a simple average of your replicates.)

pH can be measured using any of the following methods. Whichever method you choose, make sure to measure the pH as soon as possible after sampling so that continuing chemical changes will not affect your results:

Soil Test Kit

Test kits for analysis of soil pH can be used without modification for compost samples. Simply follow the manufacturer's instructions.

pH Paper

If your compost is moist but not muddy, you can insert a pH indicator strip into the compost, let it sit for a few minutes to soak up water, then read the pH using color comparison.

Compost Extractions

Using a calibrated meter or pH paper, you can measure pH in a compost extract made by mixing compost with distilled water. It is important to be consistent in the ratio of compost to water and to account for the initial moisture content of the compost, but there is no universally accepted protocol specifying these procedures.

One approach is to read the pH in oven-dried samples that have been reconstituted with distilled water.

1. Spread compost in a thin layer in a pan, and dry for 24 hours in a 105-110°C oven.
2. Weigh or measure 5 g samples of oved-dried compost into small containers.
3. Add 25 ml distilled water to each sample.
4. Mix thoroughly for 5 seconds then let stand for 10 minutes.
5. Read the pH with a calibrated meter or with pH paper and record as compost pH in water, or pHw.

An alternative is to measure pH in samples that have not been dried. In this case, the amount of water that you add will need to vary to compensate for the varying moisture content of the compost. You will still need to dry some of the compost in order to measure moisture content, but you can take the pH readings on samples that haven't been altered by drying.

1. Calculate the % moisture of your compost:
   a) Weigh a small container.
   b) Weigh 10 g of compost into the container.
   c) Dry the sample for 24 hours in a 105-110°C oven, or for 5 minutes in a microwave oven. If you use a microwave oven, place a beaker containing 100 ml of water in the oven during the drying to protect the oven's magnetron.
   d) Reweigh the sample, subtract the weight of the container, and determine the moisture content using the following equation:$M = ((W_w-W_d)/W_w) \times 100$
   in which:
   M = moisture content (%) of compost sample
   W_W = wet weight of the sample, and
   W_d = weight of the sample after drying.

2. Use the % moisture to figure out how much water to add.
   For example, if your compost sample is 40% moisture, you will compensate by adding only 60% of the water you would need if the sample were air dried (0.60 x 5 ml = 3 ml water needed).
3. Weigh or measure 5 g samples of compost into small containers.
4. Add the calculated amount of distilled water to each sample.
5. Mix thoroughly for 5 seconds.
6. Let stand for 10 minutes.
7. Read the pH with pH paper or a calibrated meter and record as compost pH in water, or pHw.

Monitoring Compost Odors

A well-constructed compost system should not produce offensive odors, although it will not be odor-free. You can use your nose to detect potential problems as your composting progresses. For example, if you notice an ammonia odor, your mix probably is too rich in nitrogen (the C/N ratio is too low), and you should mix in a carbon source such as leaves or wood shavings. If you smell a musty odor, it may be because the mix is too moist, which you can correct by adding more of your bulking agent. Left uncorrected, compost that is too wet may go anaerobic, producing a foul sulfurous odor that is hard to ignore. If this occurs in indoor bioreactors, you may wish to take them outside or vent them to the outside, then aerate or mix thoroughly and add additional absorbent material such as wood chips or sawdust. In an outdoor compost pile, turning the pile may be sufficient to correct the anaerobic condition, although initially this may make the odor even more pronounced.

Observing Compost Invertebrates

by Elaina Olynciw

Background

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Composting Health Considerations

by Tom Richard (modified)

Health concerns relating to compost are dependent both on the individual and on the material being composted. Dog and cat manures can contain harmful pathogens and should be avoided. While few human pathogenic organisms are found in vegetative wastes, normal sanitary measures (i.e., washing hands before touching food, eyes, etc.) are important. Although most people are unlikely to have any problems, there are a few concerns which place some individuals at risk.

Just as individuals vary in their resistance to disease, a few individuals may be particularly sensitive to some of the organisms in compost. The high populations of many different species of molds and fungi in an active compost process can cause allergic responses in some people, though most experience no adverse reaction. One of these fungal species, Aspergillus fumigatus, can infect the respiratory system of a sensitive person who is heavily exposed. Conditions that may predispose individuals to infection or an allergic response include: a weakened immune system, allergies, asthma, some medications such as antibiotics and adrenal cortical hormones, or a punctured eardrum. People with these conditions should avoid turning compost piles or take precautions to minimize exposure.

To minimize these potential risks, common OSHA approved dust masks can be worn under dry and dusty conditions, especially when the compost is being turned. If, following these precautions, individuals still develop an infection or have an allergic reaction to compost, they should consult a medical professional.

See also Cornell Waste Management Institute fact sheet, "Health and Safety Guidance for Small Scale Composting".