**Teacher Notes**:

 Experimental Protocol : Effect of Fertilizer on Carbon Sequestration

Below, you will find extensive notations on use of materials and procedures. Notes for teachers are in *italics.*

*The purpose of this experiment for students is fourfold:*

***To find and understand evidence for carbon moving from one reservoir to another (from the atmosphere to plants).*** *Students should understand that as carbon moves, it can change from one type of carbon compound into another. (The plant changes CO2 and H2O into different carbon compounds)*

***To construct an understanding of the ability of plants/trees to sequester carbon happens via photosynthesis.*** *Plants grow by sequestering carbon in the billions of carbon compounds they build. The source of that carbon is carbon dioxide taken in by photosynthesis. Light, water and temperature limit photosynthesis.*

***To understand that nitrogen (and other plant nutrients) is an important limiting factor for plant growth****. Approximately 5% of a tree’s mass is nitrogen and because nitrogen is needed to construct plants’ DNA and protein molecules, a lack of nitrogen will limit plant growth. Even if extra CO2 is added by applying CalCarb, nitrogen (and other plant nutrients such as phosphorus and magnesium) limits plant growth and thus carbon sequestration.*

***To understand that the ability of forests (and all plants) to sequester carbon and thus mitigate the climate warming effect of increased CO2 emissions is limited by the availability of nitrogen in the soil****. Students should understand that trees just aren’t going to just keep growing and growing as more CO2 is added to the atmosphere. Adding the CalCarb CO2 enhancer to Bottle 3 won’t make much of a difference in Biomass gain.*

*Materials and you will need per each class lab team.*

Materials:

* **Three hydroponic bottle set-ups**. **(*Bottle Cutting Directions*)** *You can make these from 2 liter plastic soda bottles. You will need to cut the bottle 1.5 inches* ***below*** *the curve of the neck. If you cut directly on the curve, the bottle top will fall in when you invert the bottle top into the bottom. Depending on your classes and time, it may be easier for you to make these yourself ahead of time. Draw a black line 1.5 inches below the curve of the bottle neck to serve as a cutting guide. Make an “incision” with a razor blade or box cutter, then use a good pair of scissors to cut completely around on the line. Invert the bottle top into the bottom part of the soda bottle which is now the water reservoir. Secure with two small strips of duck tape. A word to the wise – if you (or your students) wrap the duct tape all the way around, the bottle will take more time to take apart and rinse for next year’s use.*
* **3 handiwipe wicks** (*you will need to wash these before you use them to remove the soap and chemicals. Just throw them in a washing machine – no soap-. No need to dry them. You could also just soak them in a tub of water over night. Cut each individual towel in half. Students will then roll these up to create a wicking system*. *Insert ¾ of the wick into the water reservoir. Leave the remaining ¼ sticking up into the inverted bottle top.*
* **Radish seedlings – 7-8 per hydroponic bottle set up.** *Fast growing radish seeds like Small Round Red Rover F1, grow from seeds to adult plants in about 25 days. You don’t need to let them develop radish bulbs or flowers; however you do want enough growth to get reliable data. It would be great if you could harvest your data at the end of Lab 4 – Changes in the land. To do so, however, you will need to germinate seeds ahead of time in germination trays and allow a week’s growth. Students would then transplant the radish plant seedlings to the hydroponic bottle set ups*

**Germination Instructions and Materials.** *Germinate the radish seeds about 5-7 days ahead of Lab 1B.*

***Materials:***

***Plastic trays*** *with covers* ***or*** *zip lock bags. “Take-out” food trays or plastic salad containers work great as do any other plastic containers with a lid. The best ones have at least 4 inches of vertical growing space available. You need a lid to keep the moisture in the germination chamber.*

***Paper towels****, coffee filters or filter paper*

***Distilled water*** *– Important to prevent contamination by mold or chemicals in tap water.*

***Germination Instructions****: Plan on one germination tray per class lab group with about 20 radish seeds per tray. Line the bottom of the germination tray with two layers of paper towels. Dampen well with distilled water but do not flood. Place radish seeds on the wet towels. Snap lid down to keep moisture in. You may want to make a few extra germination trays just in case! DO NOT put germination trays in sunlight or direct light where temperature can become a variable. A shaded darker corner of the room is best. The seeds do not need light to germinate but do not cover with another towel. When most of the seeds have formed cotyledons, you can take the cover off, water with distilled water and provide some light. Here are two YOUTube videos that can give you some background info.*

Germinating seeds in a plastic bag <http://www.youtube.com/watch?v=To2DlJwErao>

Radish seeds growing: <http://www.youtube.com/watch?v=d26AhcKeEbE>

* **Distilled or filtered water**. *You will have less problems with mold if you use distilled water but filtered water works fine.*
* **Fertilizer solution made with distilled or filtered water.** Use typical houseplant/vegetable fertilizer that you can dissolve in water such as MiracleGrow. DO NOT use slow release pellets like Osmocote. Follow the instructions on the fertilizer box to make the correct solution concentration.
* **Laboratory mass balance – to milligrams**
* **Soil-less Planting *Mixture****. Soilless mixes are readily available at commercial gardening centers. Examples of good ones to use include:*

 *1. PRO- Mix,*

 *2. ½ Perlite + ½ Vermiculite mixed together*

*3. Coconut fiber*

*You just need to be sure that the soil-less mix you are buying has NO NUTRIENTS like nitrogen, phosphorus, magnesium etc. Why use a soilless mixture and not soil? This is an important question. A misconception that students have is that the plant makes itself from the soil. It does take nutrients from soil fertilizer (nitrogen etc), but this amounts to a very small percentage of the plants mass (typically about 5% of the plant is nitrogen) If you use hydroponics, it is easier to eliminate this misconception. ALSO – if you use potting soil, compost or soil from your garden, there are nutrients in the soil. You would have to pre-prepare soil that has been rinsed for quite a long time to flush out the nutrients. You can easily purchase soilless mixtures at a local commercial gardening center or on-line.* [*www.GYOstuff.com*](http://www.GYOstuff.com) *is a good on-line hydroponics store.*

* **Plant light source.** *You may have access to a plant growing stand and/or PLANTGRO lights. If not, use 100 watt bulbs in lamps or a sunny window. Have students make sure that plants are equidistant to light source. If you grow plants in a sunny window, you will have to keep an eye out for drying of the growing medium especially until the radish form long enough roots to access the water being wicked up. You will probably have to add some water every other day for the first week.*

**Calcarb solution in Spray Bottle Mist Bottle.** *One tablespoon of Calcarb dissolved in one/half gallon of water in a spray bottle will provide more than enough CalCarb solution for your classes. CalCarb is a carbon dioxide enriching molecular delivery system. Gardners and farmers who grow extreme size pumpkins and other vegetables use this type of foliar carbon enhancement product. You can find information on CalCarb at any of these sites.*

 <http://xtreme-gardening.com/videos/>

<http://xtreme-gardening.com/calcarb/what-is-it/>

<http://www.parsource.com/products/fertilizers/calcarb-foliar-spray>

* **Box cutter/sissors to cut the bottles**. *See bottle cutting instructions above.*
* ***Duct Tape:*** *See bottle cutting instructions above.*

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Student Instructions with *Teacher Notations in Italics*

**Research Question:**

Is the ability of radish plants to sequester carbon limited by soil nutrients such as nitrogen, phosphorus and magnesium?

**Experimental Design.**

Radish seedlings will be planted in three different experimental conditions.

After approximately two weeks of growth, 3-5 of the healthiest plants from each bottle will be harvested and massed to determine the GREEN MASS and BIOMASS of the plants. Then, % change in biomass and the approximate amount of carbon sequestered will be calculated and graphed.

**Experimental Bottle Set-Ups:**

Hydroponic Bottle # 1. The Control. The variables for the control are radish seedlings, planting medium, water, fertilizer, light.

Hydroponic Bottle #2. The variables for Bottle #2 are radish seedlings, planting medium, water, light but NO Fertilizer

Hydroponic Bottle #3. The variables for Bottle #3 are radish seedlings, planting medium, water, light, NO Fertilizer with Enhanced CO2 using CalCarb.

**IMPORTANT - BEFORE STUDENTS BEGIN PLANTING -** *Find the mass of one hundred radish seeds and calculate the average mass of one radish seed. This is an important step because it allows students to choose only those plants that are the healthiest. This will eliminate plants that are crowded out from light and/or living space by faster growing plants. It will also eliminate plants that die due to root damage, mold etc. An average mass for a typical radish seed is 0.20 g*

**Materials:**

*Each class lab team should gather the following materials:*

* **Three hydroponic bottle set-ups**.
* **3 handi-wipe wicks**
* **Radish seedlings – 7-8 per hydroponic bottle set up.**
* **Distilled or filtered water**. You will have less problems with mold if you use distilled water but filtered water works fine.
* **Fertilizer solution made with distilled or filtered water.**
* **Laboratory mass balance – to milligrams**
* **Soil-less Planting Mixture.** Most potting soil has fertilizer already in it so you will use a hydroponic soil-less planting mixture that has no fertilizer.
* **Plant light source –** Plant GRO Lights, 100 Watt bulbs or a sunny window.
* **CalCarb solution in Spray Bottle Mist Bottle. CalCarb solution in Spray Bottle Mist Bottle.** CalCarb is a spray that farmers and gardeners use to increase the amount of CO2 that plants get. Gardners and farmers who grow extreme size pumpkins and other extreme size vegetables use this type of CO2 enhancement product. You can find information on CalCarb at any of these sites.

<http://xtreme-gardening.com/videos/>

<http://xtreme-gardening.com/calcarb/what-is-it/>

<http://www.parsource.com/products/fertilizers/calcarb-foliar-spray>

**Part 1: Planting**

1. Set up hydroponic bottles by rolling up handiwipe wick and pushing ¾ of the wick into the water reservoirs in EACH bottle.

2. Label Bottle #1 – Control,

 Label Bottle #2 – No Fertilizer

 Label Bottle #3 – NO fertilizer /Enhanced CO2

3. In Bottle # 1 **ONLY**, fill reservoir ¾ full with fertilizer solution.

4. In Bottle #2 and 3, fill the water reservoirs ¾ full with distilled or filtered water but NO FERTILIZER.

MAKE SURE THAT ALL THREE BOTTLES HAVE THE SAME LEVEL OF WATER IN THE RESEVOIR.

5. Fill the top of the EACH bottle (the growth chamber) approximately 2/3 full of planting mixture.

6. Get your germination tray with the radish seedlings from your teacher. Gently remove the healthiest seedlings from the tray. Try not to tear the roots. If you do, do not plant that seedling. Plant 7-8 seedlings in the bottle growth chamber. Use a pencil to create a hole then gently plant the seedling. Carefully spread some more around the roots of the seedlings. Make sure the roots are covered by at least 1 inch of planting mixture. *Students may have some difficulty with handling these small seedlings. If they injure the roots taking the seedling off the towel, that plant may not recover. If seedlings are firmly rooted in the towel, cut the section of towel with the seedling and plant both.*

7. Water the growth chamber with 25 ml of distilled or filtered water. If you don’t have enough, you can use tap water from now on.

8. Place near a plant light source. Make sure plants are equidistant from the light source. It might be a good idea to rotate the plants to ensure that they get the same amount of light.

 9. In two days, spray CalCarb solution on the underside and tops of the leaves - ON **BOTTLE #3 ONLY**. Do this every 3-4 days throughout the experiment. This process will introduce higher amounts of CO2 to the plants in this bottle. It is very important to spray the underside of the leaves because they contain more openings (stomata/stoma) where CO2 enters the leaf. MAKE SURE you shake the spray bottle before spraying. A white powder may appear on the leaves but it is not harmful to the plants.

Read how CalCarb works by clicking on this Calcarb link [link <http://www.parsource.com/products/fertilizers/calcarb-foliar-spray> “Calcarb link’ new]

 *You may want to review the structure of a leaf with them and how CO2 enters the leaf.*



**Part 2: Observations**

1. Observe your radish plants every 2-3 days. Make note of any changes by writing, drawing and/or taking photographs.

2. Make sure there is ample water in the reservoirs and test the moisture in the planting mixture. If dry, add 25 ml of distilled or filtered water to ALL three bottles. *All students have to do here is stick their finger into the mixture. It should feel moist. If not, then water.*

**Part 3. Harvesting the PLANTS - Data Collection.**

***Your teacher will give you a separate data table to fill-in and questions to answer.***

Approximately 10-14 days of growth after planting, its time to harvest your plants. You may see that some plants are taller and more robust than others in the bottle. There could be several reasons for this. For example, a faster growing plant may shade the other smaller plants from the light. Less light means less growth. Additionally, some of the smaller plants may have broken roots or a fungus that may slow down their growth. Choosing the 3-5 most robust and healthy plants will help to eliminate this source of error.

1. Carefully remove 3-5 of the healthiest plants from the Control bottle to weigh. Pinch plants off at a node right above the roots. Don’t include the roots when you weigh the plants. The reason for doing this is because the roots will be tangled up in the handiwipe wicks. By pinching the bottom of the plant stem off at the node right above the roots, you will eliminate this problem. ***RECORD the number of plants you are massing in your DATA TABLE.***

2. Gently pat plants dry with a paper towel and weigh the plants on the lab balance. This will give you the **GREEN MASS** of the plants, which includes both the plant’s carbon compounds and it water. Radish plants are approximately 45-50% water – the rest are carbon compounds. Record the GREEN MASS in your data table in ROW C. Repeat steps 1 through 3 with bottles #2 and # 3.

**Part 4. Making Calculations in Your Data Table – BIOMASS, % CHANGE in BIOMASS and Sequestered CARBON.**

Now that you have determined the GREEN MASS of your plants, you are ready to do some simple calculations that will give you data that you can graph and analyze.

 **Calculating BIOMASS**.

 You now want to determine the BIOMASS of your seeds and your harvested plants. All living things are made primarily of water and carbon compounds with small amounts of vitamins and minerals. Because we want to know how much carbon is being sequestered in the radish plants as they grow, we need to find the approximate mass of the carbon compounds – without the WATER! This measurement is called a plant’s BIOMASS.

Finding the BIOMASS of the radish seeds is easy – it’s just a direct measurement. Radish seeds have very little water in them, so when we mass them, we are directly measuring their BIOMASS. You already determined the average biomass per radish seed when your teacher massed out 100 seeds and divided that mass by 100.

Finding the BIOMASS of the harvested plants requires another step. You can determine BIOMASS of the plants in one of two ways:

 1. You could let your harvested plants dry out. This would remove the water while leaving carbon compounds behind.

 OR

 2. You could do a simple calculation. Scientists have determined that radish plants are approximately 45-50% water. So, calculating 50% of the GREEN MASS will give an approximate BIOMASS for the plants.

 (*Drying the plants in a 99 degree oven in a typical stove overnight will dehydrate the plants enough to get a biomass measurement. You may be lucky enough to have a drying oven in your lab. If you have the equipment to do this as opposed to using the percent calculation it will make more sense to students. You will have to wrap plants up in a square of parchment paper so they can label them with a pencil. If you have them cut out a square of parchment and mass that ahead of time, they can measure the plants and parchment together the next day.*

*Its very important to talk with the students about the difference between the Green Mass and the Biomass. They will need to have a firm grasp of the difference. )*

**% CHANGE In BIOMASS:**

Determining % change is a very simple but important calculation. The **% CHANGE in BIOMASS** calculation will give you a measure of how much **BIOMASS (g)** the plants have gained since first starting out as a seed. Calculating the % change allows you to compare the amount of growth between the three experimental bottles.

The general formula for finding % change is as follows:

The final measurement - beginning measurement X 100

 Beginning measurement

For your radishes -

The final measurement = the **BIOMASS**(g) of the harvested plants

The beginning measurement = the **BIOMASS (g) of the SEEDS**.

To calculate this, you need to multiply the average mass of a radish seeds X the number of plants you massed. Example: If the average biomass of a radish seed is 0.20g and you massed five plants, then you multiply 0.25g X 5 plants = 1.25 g. This gives you the **biomass of the seeds**.

**Calculating Grams of Sequestered CARBON**

 Recall that a plant grows by taking in carbon dioxide and water and using both to make carbon compounds. The BIOMASS gives us a measurement of carbon compounds, BUT not CARBON by itself. How much CARBON did these plants actually absorb from the atmosphere and sequester in their cells and tissues? Scientists have determined that approximately 40% of the BIOMASS of radish plant is comprised of only CARBON. Thus….

BIOMASS (g) X .40 = # of grams of CARBON sequestered

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When you feel that you are ready to move on, **FOLLOW** steps A-F below as you in-put your data and carry out your calculations. Note that each lettered step below matches a box in the DATA TABLE given to you by your teacher. Make sure that you correctly and completely fill –in each of the boxes under the **Control, No Fertilizer and No Fertilizer/Enhanced CO2 columns.**

First, write down the average biomass of one radish seed \_\_\_\_\_\_\_\_\_\_\_g

1. Fill-in the number of plants you massed.
2. Find the beginning BIOMASS of your radish plants by multiplying the # of plants you massed X the average biomass of a radish seed.
3. Fill-in the GREEN MASS of the plants if you haven’t done so already. Recall that plants are approximately 45-50% water, so the GREEN MASS include both carbon compounds and water.
4. Calculate the approximate BIOMASS of the plants. Multiply the GREEN MASS X .50 This will give you the approximate BIOMASS.
5. Calculate the % CHANGE in BIOMASS. The formula is as follows:

BIOMASS of harvested plants - BIOMASS of the seeds  **X 100**

 BIOMASS of seeds

 F. Calculate the number of grams of carbon sequestered in the harvested plants

 BIOMASS of harvested plants (g) X .40 = grams of CARBON

 G. Optional Calculation. *(Some students will be able to easily figure out that this is a % change in amount of carbon sequestered problem. Thus, they would use the same formula as for the % change in Biomass.* )

 **Part 5: Graphing, Data Analysis and Questions.**

Follow the graphing and analysis instructions on the Data Table Sheet. Then answer the **Stop and Think** Questions. *You may want to consider having students put all of their data into a class data table and then have the students graph from a larger data set. You will also need to decide whether to use two separate graphs for % Change in Biomass and Carbon Sequestered. Expect to see a large percent % change especially if you let the plants grow until the radish bulbs start to form. You can have students write this up as a formal lab report.*