Chapter 15

Plant Growth Responses to a Resource Gradient

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PLANT GROWTH RESPONSES TO A RESOURCE GRADIENT

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OBJECTIVES

1. To demonstrate and measure the growth responses of plants grown along a nitrogen gradient and/or light gradient.

2. To compare the growth responses of different plant species when grown along a nitrogen gradient and/or light gradient.

INTRODUCTION

Nitrogen is an essential mineral nutrient for plants. It is part of many plant molecules, including proteins, chlorophyll, and nucleic acids. Nitrogen deficiency is the most common mineral deficiency in plants. Two forms of nitrogen, nitrate (NO_3-) and ammonium (NH_4+), are absorbed by plants from the soil. Nitrogen–deficient plants generally exhibit chlorosis (deficiency of chlorophyll) and their leaves turn yellow.

Nitrogen in gaseous form (N_2) composes 78 percent by volume of the atmosphere. N_2 moves in and out of the leaves through the stomates, but plants cannot use nitrogen in this form. Some organisms are able to "fix" atmospheric nitrogen (N_2): they are able to take nitrogen from the air in the form of N_2 and reduce it to NH_4+ (ammonium), which is a form of nitrogen that can be used by plants. These specialized organisms include some species of soil bacteria, cyanobacteria, and bacteria that live symbiotically with the roots of higher plants. Many plants in the legume or pea family (Fabaceae) have root nodules where the symbiotic bacteria live.

Succession is a gradual and orderly process of ecosystem development brought about by changes in species populations: it is the replacement of populations in a habitat through a regular progression. It often culminates in a "climax community" that is characteristic of a particular geographic region. Succession occurs when a new habitat is created as a result of some process. Primary and secondary succession are the two types of succession. Primary succession occurs in a newly formed habitat, such as an area covered by volcanic ash or lava, an area exposed as a glacier recedes, or a sand dune that is exposed as the water level in a lake deceases. In secondary succession a new habitat is formed, but the area was previously covered by plant growth. Examples of secondary succession include abandoned farm fields and clear-cut forests. Generally, in primary succession there is little or no soil and, therefore, very low availability of soil nutrients. In secondary succession, the soil nutrient availibility varies from very low to relatively high, depending on the soil type and the use (or abuse) of the land before clearing. Either type of succession follows a general pattern. The earliest plants in the sequences are usually short grasses

and forbs. These are followed by taller grasses and forbs, and then by woody plants -- shrubs and often trees. In most of eastern North America, the "climax community" is a forest of some type.

Many theories on the mechanisms of ecological succession have been proposed. One of these theories, the resource competition theory, deals with the effects of plant competition for resources. Competition is the use of a resource by one individual that reduces the availability of that resource to other individuals. All plants require below-ground resources (soil nutrients and water) and above-ground resources (light). Below-ground resources are acquired by the roots, while above-ground resources are acquired primarily by the leaves and stems of a plant. In the resource competition theory of plant succession, there is a trade-off between the acquisition of below- and above-ground resources. When below-ground resources are limited, the plants that are most effective at acquiring these limited resources will out-compete the others. Large roots or an expansive root system are two ways that plants can maximize their uptake of limited soil resources. When the density of plants in an area increases, the above-ground resource, light, becomes limiting. Those plants which allocate more biomass to above-ground structures will then be better competitors for the limited amount of light.

In many plant communities, low availability of nitrogen limits plant growth. This is especially true in early stages of primary (and sometimes secondary) succession. Some of the non-legumes that have root. nodules for N_2 fixation are pioneer species that are found in early successional sites where the soil is very low in nitrogen. Therefore, they are better competitors for nitrogen because the bacteria in their root nodules fix N2 from the atmosphere, and possibly because they are also able to absorb the small amounts of NH_4^+ and NO_3^- in the soil.

As the early successional field gets "older", soil nutrients increase as decaying vegetation, bacteria, and other organisms add nitrogen to the soil. When the density of plants increases, the availibility of light to individual plants deceases. The resource competition theory of plant succession states that information on nutrient-limited growth rates should make it possible to predict the outcome of interspecific Competition. This theory predicts that plants dominant in younger, more nitrogen-poor fields should have a lower requirement for nitrogen, i.e., a greater ability to grow at low nitrogen levels, than plants dominant in later-successional, more nitrogen-rich fields. The plants growing in the later successional fields are better competitors for the above-ground limiting resource, light. In the later-successional fields, the soils are nitrogen-rich and the plants are not inhibited by low availibility of nitrogen in the soil. But the plants are now more crowded and shade each other. Therefore, the dominant plants will be better light competitors, i.e., those that can grow in the shade of others, and attain a greater height at maturity. A study of several

plants dominant along the successional gradient should reveal the following characteristics: early-successional plants - good nitrogen competitors, poor light competitors; mid-successional plants - average nitrogen and light competitors; later-successional plants - poor nitrogen competitors, good light competitors. (See Figure 1.)

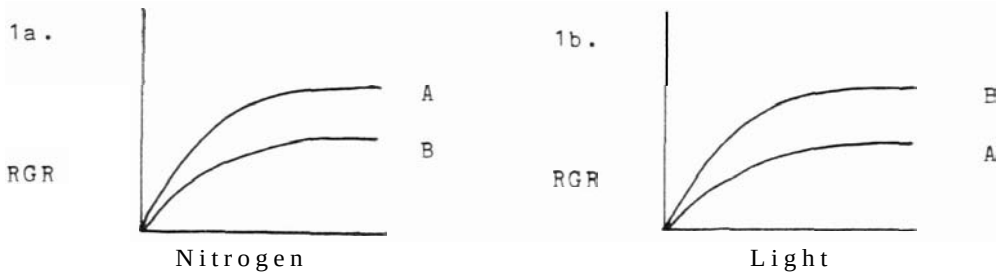

Figure 1 - Relative Growth Rate as a function of nitrogen (1a) and light (1b) where species A is an early successional plant and species B is a late successional plant.

In this experiment, you will be studying the growth responses of two plants grown at seven different nitrogen levels. Growth characteristics of the better nitrogen competitors, i.e., those dominant in early succession, should include: (1) higher relative growth rates (RGR), (2) higher root:shoot ratios, (3) shorter height at maturity, and (4) earlier reproduction. Because of the length of the experiment, you will probably not be able to make comparisons using the last two characteristics (height and time of first reproduction).

Relative growth rates are used to compare the growth of two or more species during a specific time period. Relative growth rates can be calculated using the following equation:

$$RGR = \frac{\ln (W_4 / W_1)}{t_4 - t_1}$$

where W_4 = total plant weight at final harvest; W_1 = seed weight; t_4 = day of final harvest: and t1 = day of planting. You may also wish to determine RGR's during other periods of growth, e.g., between day 1 (seed weight) and day 14, between day 14 and day 70, etc. The early-successional plants should exhibit higher relative growth rates than the late-successional plants because they are able to grow more quickly under conditions of low nitrogen.

Root:shoot ratios can be calculated by dividing the total below-ground biomass per pot by the total above-ground biomass per pot. Because nitrogen is a resource acquired through the roots, plants adapted to low nitrogen soils should maximize their ability to absorb whatever nitrogen is available by allocating more of their biomass to roots, i.e., by having higher root:shoot ratios.

MATERIALS

1. Soils -
In the nitrogen gradient experiment, you will use seven soil mixtures that have been pre-mixed by the instructor or lab assistant. Each soil mixture is clearly labeled. Nutrients other than nitrogen have been added.
In the light gradient experiment, a soil rich in nutrients is used. Additional fertilizers have been added if necessary.

2. Pots and trays -
Each group will need 56 pots and trays.

3. Coarse gravel.

4. Seeds -
The instructor will tell you the amount (in weight or volume) of seeds you need to use. This will vary from species to species and from year to year because of variations in viability. You will also need exactly 100 seeds of each species to determine the weight of an individual seed. (See step 3 under Methods.)

5. Soil moisture meter -
A soil moisture meter measures the percentage of water in the soil.

6. Materials for data collection -
These include: data sheets, ruler, paper bags, markers,

7. Harvesting equipment -
This includes: sink or basin with running water (preferably warm), sieve, basin to collect soil (not necessary if harvested outdoors), paper bags, scissors, drying oven, balance

METHODS (The schedule below may be altered by your instructor.)

Day 1 -

1. Label each pot with nitrogen level, species, replicate. E.g., **N1 - A - 1; N1 - A - 2;** etc., where N1 = lowest nitrogen level, A = species A, and 1 (or 2) = replicate 1 (or 2), etc.

2. Put a layer of coarse gravel in the bottom of each pot. Add the soils. Add sufficient water to the pots so that some water drains out the bottom. Let the soils drain for 1 day.

3. Count out exactly 100 seeds of species A and weigh the seeds. Divide this weight by 100 to get the average weight of one seed of species A. Record this information. Repeat for species B.

Day 2 -

4. Add the pre-determined amount of seed to the top of each pot

and cover the seeds with a 5–10 mm layer of coarse sand. In an experiment using a nitrogen gradient, the soil mixtures at the different nitrogen levels may be different colors and, therefore, heat up differently. The uniform color of the sand removes this variability.

Days 3 - 70 -

5. Damping off is a disease of planted seeds or young seedlings caused by fungi and resulting in the death of the plants. To reduce the possibility of damping off, do not thoroughly water again until the seeds have germinated and are at least 1 cm tall. If the soil appears dry, mist the surface.

6. Weed and water as necessary. In this experiment, we want to insure that the plants are not water-limited. The seven soil mixtures have different percentages of sand, silt, clay, and organic matter. Since the range of available water varies in soils with different compositions, you must use the soil moisture meter to determine the percentage of water in each pot. Your instructor should have information on the lowest and highest readings required for each soil level. In this experiment, sandy soils may retain water for a longer period. Also, more water is available for use by the plant in sandy soils because the sand particles do not bind to the water as much as clay and silt particles do. (See a soils science text for more information.)

Days 14, 28, 42, and 56 -

7. Collect and record data on the height of individual plants in each pot.

Day 14 -

8. Harvest one replicate of each species at each nitrogen level (a total of 14 pots). Use the following procedure to harvest the plants from one pot: remove the plants from the pot, knock off any loose soil, place the plants in a sieve under a water supply, and wash the soil off of the roots. Separate the above- and below-ground portions of the plants, and place them into labeled bags. Repeat for the other 13 pots.

9. Dry the plants at 50° C until all of the moisture is removed from plants. The amount of time required to dry the plants will depend on the size and type of the plant. Small plants (under 10 cm tall) will only require one or two days of drying.

10. Weigh the plants during the next lab period (Day 21).

Day 42 -

11. Repeat steps 8 - 10. (Harvest one replicate of each species at each nitrogen level.)

Day 70 -

12. Repeat steps 8 - 10. (Harvest the remaining plants -- 28 pots total.)

For lab report:

Plot any or all of the following:
growth rate as a function of nitrogen level;
height as a function of nitrogen or light level;
weight as a function of nitrogen or light level;
root:shoot ratio as a function of nitrogen or light level.

QUESTIONS

1. Define the following terms:
   a. succession (including the difference between primary and secondary succession)
   b. competition

2. Which of your species had the higher relative growth rate at the lowest nitrogen level? at the highest nitrogen level? Is this what you expected? Why or why not?

3. How do the root:shoot ratios of the two species compare?

4. Give some general growth characteristics of plants that you would expect to find in: (a) a farm field that has recently been abandoned, (b) a 50-year old tall grass prairie, (c) the area within a 1-mile radius of Mt. St. Helens today.

REFERENCES

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Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, N.J.

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INSTRUCTOR'S PREPARATION MANUAL

INTRODUCTION

This lab, as written, is designed for an introductory or advanced ecology course, but it may be appropriate for an introductory plant science course, or a plant physiology or agronomy course. The biggest drawback for its use in an introductory biology course is the fact that the experiment takes at least 10 weeks to complete, and this would be a lot of time devoted to a plant- and/or ecology-related topic in an introductory biology course. Also, some preliminary information on plants and basic ecological principles, while not necessary, would be helpful.

This lab is written as a greenhouse or growth chamber exercise. If you teach a summer course and have the appropriate outdoor space, you may wish to expand this experiment by using larger pots and conducting the experiment outdoors.

MATERIALS

1. Soils -

For the soil nutrient gradient experiment, you will need 2 soils (one low and one high in nitrogen) or 3 soils (one each of low, medium, and high nitrogen availability). These soils may be: 1) topsoil from a nearby field - the quality of this can vary greatly; 2) a black loam or potting soil which you purchase - this will probably have a relatively high nitrogen content; 3) coarse beach sand - this will probably be very low in nitrogen. (See below for soil analysis and construction of nitrogen gradient.)

For the light gradient experiment, it is best to use a soil with a high nutrient content to ensure that the plants are not limited by any resource absorbed through the roots.

2. Fertilizer -

In the nitrogen gradient experiment, prior to planting, add sufficient amounts of all macro- and micronutrients, except nitrogen, to the soils.

In the light gradient experiment, prior to planting, add sufficient amounts of all nutrients that may be limiting based on the soil analysis (see below).

3. Pots -

In the greenhouse or growth chamber, use small plastic pots (7 to 15 cm in diameter) and grow one to several plants per pot. Small plastic trays, such as meat trays, should be placed under each pot to collect water that seeps through the pot after watering. This is to insure that there is no transfer of nutrients from a pot of high to low availability.

Instead of round or square plastic pots, you can use small,

cone-shaped containers (cone-tainers). These are suitable if you are planning to grow one plant per container. You can buy holders for the cone-tainers. Cone-tainers are available from some greenhouse and nursery supply centers, or you can order them directly from the distributor (cone-tainer Nursery, 1500 North Maple, Canby, Oregon 97013, 503-266-3333)

If the experiment is to be done in the field, large plastic pots (Zarn pots or poly-pots) should be used. The larger pots will allow more space for the rapid root growth. Pots that are 30 cm in diameter and 30 cm deep weigh approximately 23 kg (50 #) when filled with soil. Use smaller pots if you wish to harvest plants before the end of the growing season.

All of the above pots are available through garden supply centers.

4. Coarse gravel or two layers of cheesecloth to line the pot, to allow for drainage and to prevent soil loss.

5. Soil moisture meter - (available from Forestry Supplies)

A soil moisture meter measures the percentage of water in the soil. The range of available water varies in soils with different proportions of clay, silt, sand, and organic matter. When conducting a nutrient gradient experiment, make sure you have a soil moisture meter that can be calibrated so that you can adjust it to measure the percentages of available water in all of your soil mixtures.

6. Seeds -

You will need at least two species of herbaceous plants for this experiment. It may be interesting to have each group of students use a different set of two species. If possible, use species commonly found in your area. The strongest interspecific differences will be seen if you choose species which a) have very different growth forms (e.g., rosettes v. grasses), or b) are dominant during different stages of ecological succession ("weedy" colonizers v. "climax" species). Since the experiment will be relatively short if it is part of a course taught during the regular school year, you may wish to use annual species. However, perennial species can be used successfully if you refrain from using those that produce tubers or large tap roots during their early growth. (See below for information on seed collection and viability.)

7. Materials for data collection -

These include: data sheets, ruler, paper bags, markers,

8. Harvesting equipment -

This includes: sink or basin with running water (preferably warm), sieve, basin to collect soil (not necessary if harvested outdoors), paper bags, scissors, drying oven, balance

PRELIMINARY ANALYSES AND PROCEDURES

A. Soil analysis -

Collect 10 small samples (20-30 ml) of each soil type that you plan to use. Mix all the samples from one soil type together in a paper bag. Label the bag and send it to a soils lab for analysis.

Soils analysis can be done as part of class project in a soils class, or by the soils department at your school, or by a soil analysis service run by your state. Have the soils tested for percentage of organic matter and all macronutrients (N, P, K, Mg, Ca, S). It is probably not necessary to have analysis done on the micronutrients unless you have reason to believe that one of these may be lacking. (A soil scientist in your area can advise you in this matter.) Avoid soils with a high percentage of organic matter (> 20%) because these soils will decompose relatively quickly and release more nutrients, including N. This will cause complications in your analyses because a soil mixture with more of the soil that is high in organic matter may have a different total nitrogen value than originally intended. (Note: Regular potting soil that you buy for use at home has a high percentage of peat moss, and therefore, is high in organic matter. Greenhouse supply personnel can assist you in finding soil that has a lower organic material content.)

B. Calibrating the soil moisture meter -

A few soil science definitions may be useful before explaining this next section. When a soil is at "field capacity", it essentially contains the maximum amount of water that can be held in its pore spaces. When the soil moisture content is so low that a plant remains wilted both night and day, the soil has reached the stage that is called the "wilting coefficient". "Available water" is the range of soil moisture conditions where there the soil moisture content is above the wilting coefficient and below the field capacity. Because of the variation in water holding abilities in different soils, you will need to calibrate the meter so that your meter readings cover the range of available water in all of the soils. For example, the range of "available water" in a typical sandy soil is 3 to 9 per cent by weight; in a sandy loam the range is 7 to 15 per cent. See a soil science textbook for more information on water availability in different soils. (Brady, N.C. 1974. The Nature and Properties of Soils, 8th Edition. MacMillan Publishing Co., Inc., New York, NY.)

Once you determine the range of available water for each of your soil mixtures (they will probably be fairly close), you will need to calibrate the soil moisture meter. A soil moisture meter will give you a reading from 0 to 10 with the low value relating to little or no water in the soil. The procedure for determining

the percentages of water per dry weight soil in each of your soils follows:

1. Take a small sample (30–50 ml) of each soil.
2. Soak the soils until you think they have reached field capacity. Set aside for approximately one hour.
3. Take a meter reading. Record.
4. Weigh the sample. Record. (Make sure you have weighed the container beforehand so you can subtract that weight in your calculations.)
5. Place the containers in a drying oven for 30 - 60 minutes.
6. Remove the containers from the drying oven, then repeat steps 3 - 5.
7. Keep repeating steps 3 - 6 until the soils are very dry. (This should take about 3–4 hours.)
8. Using the last reading for dry weight, calculate the percentage of water in each soil sample for each reading. Match this percentage with the reading on the meter and determine the proper meter readings for the range of available water for each soil.

C. Constructing a nitrogen gradient -

Materials: soils, scale, cement surface for mixing, shovels, large heavy–duty plastic bags or other containers for storing soil mixtures

Procedure:

Determine the range of nitrogen availability that you will use. Your highest value should be at least as high as the richest soils in your area.

Determine the number of nitrogen levels you will use. The weight and height of plants should be expected to increase along the nitrogen gradient, but eventually the response will level off. The shape of the response will be a Monod growth curve (or Michaelis-Menten curve). To get adequate data for statistical analysis of the results, you should use at least 6 nitrogen levels.

Using the soil nitrogen analysis data, mix soils in different proportions to obtain sufficient soil for each nitrogen level. If this experiment is done in a greenhouse or growth chamber, you will need relatively small amounts of soil for each nitrogen level. To reduce variability, mix a sufficient amount of soil for each nutrient level at one time. Under most circumstances, the mixing can be done on a cement surface (greenhouse floor or parking lot) using shovels. Add fertilizers at this time.

The example below shows the percentages (by weight) of three soils in each soil mixture in a gradient using seven nitrogen levels (Cowan, 1986).

NITROGEN LEVEL	BLACK LOAM (5000 ppm N)	TOP SOIL (350 ppm N)	SAND (25 ppm N)
1 - 125 ppm	1.50%	4.50%	94.0%
2 - 250 ppm	3.00	9.00	88.0
3 - 375 ppm	6.25	18.75	75.0
4 - 575 ppm	9.75	29.25	61.0
5 - 825 ppm	13.25	39.75	47.0
6 - 1275 ppm	18.25	54.75	27.0
7 - 1825 ppm	25.00	75.00	0.0

D. Seed Collection -

Seeds may be collected at a local field site or purchased from a nursery supply center. Throughout the Midwest, prairie grasses and forbs can be purchased from nurseries that specialize in native species. While purchased seed requires much less work on your part, if you wish to make predictions about responses of plants at your field site, it is best to collect seed from your site.

In the upper Midwest, mature seeds can be collected from most species in August and September. Some species, such as the "cool season" grasses, have mature seeds earlier in the season. Collect the seeds by stripping them from the stem or cutting off the entire flowering stalk. Store in a cool, dry place in paper bags.

Some seeds need to be stratified (stored at cold temperatures to simulate winter conditions). These seeds can be stored in a refrigerator. Some seeds have an impermeable seed coat and need to be scarified. This can be accomplished by placing the seeds in a solution of sulfuric acid for 1–5 minutes, or by rubbing the seeds on sandpaper. Consult an agronomist or botanist about the correct procedure for the particular species you are using. Some plant labs have equipment for scarifying seeds and for cleaning seeds (removing stems and other extraneous plant parts).

E. Seed Viability -

If you purchase seed, it normally has been tested for viability, and this value is given. If you collect your own seed, you should do germination trials to insure sufficient germination during the experiment. First, you should clean the seed by removing stems, etc. Next, count out 50 or 100 seeds, weigh them, and determine their volume. Place these seeds on

moist paper toweling in a petri dish and cover them. Observe daily and record the number of seeds that germinate. Do not let the seeds mold or dry out. Some seeds will germinate in 3–5 days, others will take several weeks. It may be best to use species that germinate in approximately the same amount of time when conducting a short–term experiment.

Decide how many seeds you want to grow in each pot. Determine the amount of seed (by weight or volume) you will need to use to achieve this density. If you are only planning to grow one plant per pot, it is best to plant extra seeds and thin the plants after germination.

F. Setting up a light gradient -

Determine the range of light availability using a light meter and decide the number of light levels you will use. If the experiment is conducted in a greenhouse or growth chamber, determine which area receives the greatest amount of light throughout the day. Use this area as the highest light level. To make lower light levels, use cheesecloth, shading material available through garden centers, or some other material that can be layered to cut out light. Depending on your set–up, you may want to place the shading material above and/or on the sides within each light level. Allow space between each light level to take into account shading from adjacent levels.