

Teaching Plant–Soil Relationships with Color Images of Rhizosphere pH

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ABSTRACT

Plants and soils often do not receive their share of attention in the biology teaching laboratory despite their significance to human life and the environment. The opportunity to study complex living systems in the classroom is limited by many constraints. This laboratory exercise was developed to exhibit clear, graphic results within the normal class period using inexpensive materials and reasonable teacher preparation time. The exercise uses a simple imaging technique to illustrate the profound effects that living roots exert on the pH of the surrounding soil environment. Plants are grown in rhizotrons using a low organic matter content sandy loam soil with either nitrate (NO_3^-) or ammonium N (NH_4^+) added and with or without a nitrification inhibitor. After 4 wk of growth, the root system is exposed and treated with fluid agar containing bromocresol purple pH indicator. The color changes outlining the rhizosphere become visible within about 10 minutes. When N is absorbed as NO_3^- , alkalization of the rhizosphere occurs and is exhibited as purple. In contrast, when N is absorbed as NH_4^+ , acidification of the rhizosphere occurs and is exhibited as yellow. When NH_4^+ N is added without the nitrification inhibitor, differences in color (pH) between the rhizosphere and bulk soil are less pronounced. The exercise achieves visually stimulating results that can be used to reinforce lectures on rhizosphere pH, nutrient availability, plant tolerance of soil acidity, microbial activity, and plant susceptibility to diseases.

THE SCIENCE TEACHING LABORATORY relies on experimental work and demonstrations that must fit within many constraints. Relatively inexpensive materials, reasonable teacher preparation time, and production of clear, graphic results within a normal 1 to 2 h lab class period are all important components for successful laboratory experiences. Unfortunately, these constraints greatly narrow the variety of experiments that are feasible. A common casualty of many laboratory experiments is the opportunity to study complex living systems in their natural environment. We developed a laboratory exercise that meets all of the above criteria. The exercise uses a simple imaging technique (Weisenseel et al., 1979; Marschner et al., 1982) to illustrate

the profound effects that living roots exert on the pH of the surrounding soil environment.

The rhizosphere is that portion of the soil in the immediate vicinity of plant roots in which the chemistry and microbiology are influenced by the presence of plant roots. This paper describes the procedures for use of the bromocresol pH indicator method to exhibit differential pH changes in the rhizosphere of plants grown in soil with either NO_3^- or NH_4^+ nutrition. The exercise provides hands-on experience that helps to illustrate principles concerned with plant soil relationships, transport processes, charge balance, N nutrition and soil N transformations. We have "field tested" the laboratory exercise for classroom use with university undergraduate students in turfgrass science and report on its general appeal among grade school students.

Theoretical Considerations

Roots acquire nutrients as cations (NH_4^+ , Ca^{2+} , Mg^{2+} , K^+ , Na^+) and anions (NO_3^- , Cl^- , SO_4^{2-} , H_2PO_4^-). Relative differences in the uptake of cations and anions influence the pH in the zone of soil surrounding the root. The rhizosphere pH is largely a function of the cation to anion uptake ratio, root efflux of H^+ or OH^- , the soil buffering capacity, and the plant species. Changes in rhizosphere pH are brought about by the net excretion of H^+ or OH^- (probably HCO_3^-) from the roots. The efflux of the appropriately charged ion with respect to the imbalance in charges from differential cation and anion uptake, serves to maintain electroneutrality in root cells and the external soil solution. Nitrogen is often the nutrient absorbed in the greatest quantity. The form (NO_3^- vs NH_4^+) in which it is absorbed, therefore, largely determines the alkalizing or acidifying effect of nutrient uptake on rhizosphere pH (Marschner, 1986). Absorption of NH_4^+ -N promotes H^+ efflux and reduces the rhizosphere pH, whereas absorption of NO_3^- promotes OH^- efflux and raises the rhizosphere pH (Marschner, 1986). This effect occurs without an immediate change in the bulk soil pH. The rhizosphere pH may differ from the bulk soil pH by as much as two units.

Nitrogen added to soil in the NH_4^+ form, such as $(\text{NH}_4)_2\text{SO}_4$, will be available to the plant as NH_4^+ , NO_3^- or a mixture of the two sources depending on the degree of nitrification. Application of $(\text{NH}_4)_2\text{SO}_4$ in combination with a nitrification inhibitor (Hoeft, 1984), such as N-Serve¹, will slow or prevent nitrification (Adriaanse and Human, 1990).

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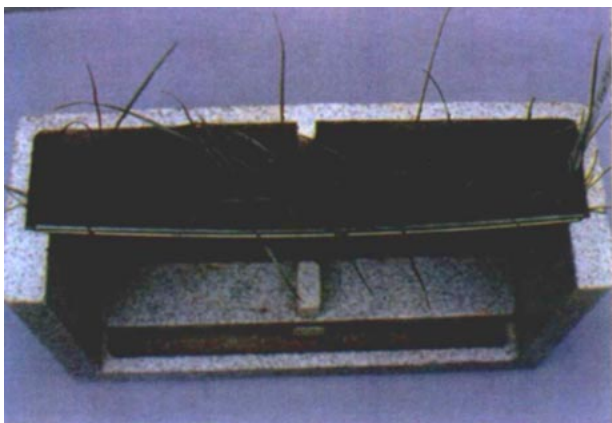


Fig. 1. Rhizotron with perennial ryegrass grown for 35 d.



Fig. 2. Rhizotron with cover removed to expose root systems.

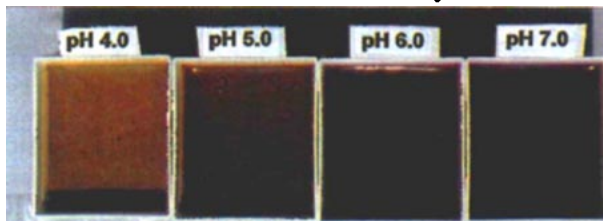


Fig. 3. Soil pH reference standards showing gradations in color change with bromocresol purple pH indicator.

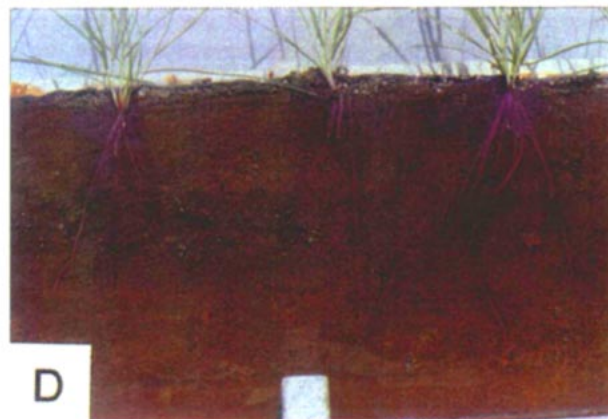
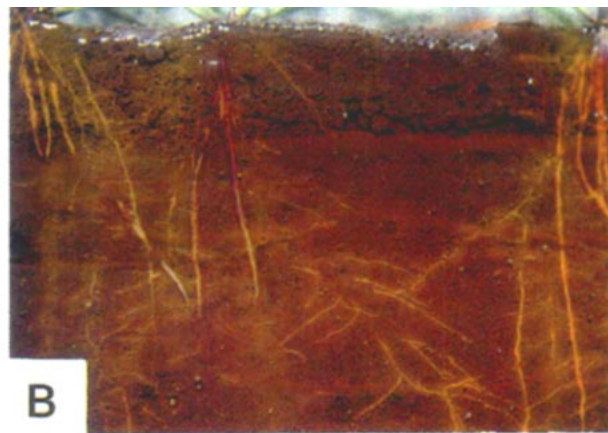


Fig. 4. Rhizosphere pH, as indicated by bromocresol purple, of root systems of plants grown with (A) $\text{Ca}(\text{NO}_3)_2$, (B) $(\text{NH}_4)_2\text{SO}_4$ + N-Serve, (C) $(\text{NH}_4)_2\text{SO}_4$, and (D) $\text{Ca}(\text{NO}_3)_2$ + N-Serve.

Ammonium uptake will then lead to acidification of the rhizosphere (Gijssman, 1990a). When nitrification is not blocked, the action of *Nitrosomonas* and *Nitrobacter* bacteria will cause release of NO_3^- and acidity in the bulk soil. Subsequent uptake of NO_3^- will lead to alkalization of the rhizosphere.

MATERIALS AND METHODS

Soil Preparation. A sandy loam soil with an organic matter content of 12 g kg^{-1} , a pH of 6.2 and low in available N was obtained in the fall from corn (*Zea mays* L.) plots that had not been fertilized with N. It was air dried and screened to pass through a 2-mm sieve. To ensure adequate nutrient supplies, the soil was amended with 0.44 g of CaHPO_4 , 0.19 g of KCl, and 1.0 g of CaSO_4 per kg of soil.

The following four treatments were used to establish NO_3^- , NH_4^+ , or mixed nutrition:

- A. $\text{Ca}(\text{NO}_3)_2$ at 0.59 g kg^{-1} soil
- B. $(\text{NH}_4)_2\text{SO}_4$ at 0.47 g kg^{-1} and N-Serve 24E, nitrapiyridin [2-chlor -6-(trichloromethyl) pyridine] at 0.01 g kg^{-1} soil
- C. $(\text{NH}_4)_2\text{SO}_4$ at 0.47 g kg^{-1} soil
- D. $\text{Ca}(\text{NO}_3)_2$ at 0.59 g kg^{-1} and N-Serve at 0.01 g kg^{-1} soil

Precautions were taken when handling N-Serve. Direct contact of concentrated N-Serve with skin and inhalation of the fumes was avoided. A dilute stock solution (200 mg N-Serve in 1000 mL of distilled water) was prepared by working under a fume hood. Amendments were broadcast over the soil and thoroughly blended. Those treatments receiving N-Serve were amended by dribbling 50 mL of the prepared stock solution over each kilogram of soil and mixed.

Plant Culture in Rhizotrons. This exercise used ROOT-VUE FARM (Edmund Scientific Co., Barrington, NJ) styrofoam rhizotrons (30 by 20 by 12 cm) with slanted plexiglass faces and opaque plastic covers to keep light out. Rhizotrons were filled within 2 cm of the rim with the treated soils and were tapped on the sides to allow uniform soil density. Ten perennial ryegrass (*Lolium perenne* L.) seeds were sown in a row 0.5 cm below the soil surface and 0.5 cm from and parallel to the slanted plexiglass face. Water was added carefully to moisten the soil to the bottom of the rhizotron. The opaque cover was then reinstalled and tightly secured with paper clips against the plexiglass face to keep out light. After germination, the ryegrass was thinned to about five plants per rhizotron and allowed to grow for 30 to 35 d in the greenhouse (Fig. 1). During this period, root development was periodically observed by lifting the opaque cover.

Agar Preparation. The method employed here is similar to that of Weisenseel et al. (1979) and Marschner et al. (1982). Distilled water, 1000 mL, was added to 7.5 g of agar, 0.06 g of bromocresol purple, and 0.136 g of CaSO_4 and heated to a boil. The boiled agar was then cooled to 38°C and maintained as liquid at that temperature by holding the flask in a warm water bath. About 500 mL of liquid agar was required per rhizotron.

Embedding of Roots and Soil in Agar. The rhizotrons were oriented horizontally, with the plexiglass covers facing

up. The covers were then carefully removed to minimize disturbance of the root system (Fig. 2) while exposing the soil face and root system. The soil and roots were then slowly covered with the liquid agar at 36 to 38°C by pouring on a few milliliters at a time. Once the agar was solidified in the soil around the roots, the soil-root surface was covered by another 1 to 2 mm thick layer of agar. Within about 5 min the agar had sufficiently solidified to permit a partial upward tilting of the rhizotrons for photography. Within 10 min, the color changes outlining the rhizosphere appeared and were fully developed within 20 to 30 min.

At the completion of the experiment, the pH of the bulk soil was measured using a pH meter with a glass electrode (1:1 ratio of soil to distilled water volume).

Soil pH Reference Standards. The same sandy loam soil with an initial pH of 6.2 was used to make pH 4.0, 5.0, 6.0, and 7.0 reference standards. The soil was divided into four equal portions to which HCl or NaOH was added to lower or raise soil pH to the desired level. Each pH adjusted soil was then placed into a small plastic box and covered with a 3-mm thick layer of agar (Fig. 3) for comparison purposes.

RESULTS AND DISCUSSION

Rhizosphere Observations. The agar method afforded rapid visualization of qualitative changes in the behavior of root systems in response to the form of N absorbed. Bromocresol purple exhibits gradual changes in color from yellow at pH 4.0 to purple at pH 7.0 (Fig. 3). With NO_3^- as the source of N, a distinct zone of alkalization was exhibited along the length of the root system. The color intensified from a pale red (pH 6.2) exhibited in the bulk soil to a deep purple (pH > 6.8) in the rhizosphere (Fig. 4A). In contrast, a distinct acidification of the rhizosphere was exhibited with NH_4^+ as the source of N when it was added with N-Serve. In this case, the color changed from light red in the bulk soil (pH 6.2) to yellow (pH < 5.0) in the rhizosphere (Fig. 4B). Comparing the NH_4^+ -N vs. NO_3^- -N sources, the rhizosphere pH differed by as much as two units, while the pH of the bulk soil remained relatively unchanged at about 6.2. The responses here were similar to those observed by Marschner and Romheld (1983). The agar method also demonstrated that N-Serve effectively blocked nitrification (except for a few localized zones, as indicated by a pink rhizosphere, where the N-Serve may not have been adequately blended with the soil). This was supported by the observation that the pH of the bulk soil (pH 6.2) exhibited little change when N-Serve was added. When $(\text{NH}_4)_2\text{SO}_4$ was added without the nitrification inhibitor, the bulk soil was acidified (yellow agar) and measured pH 4.8 (Fig. 4C). Plants grown with $(\text{NH}_4)_2\text{SO}_4$ but without N-Serve generally had less discernable differences in pH between the rhizosphere and bulk soil. In one instance, however, when the plants were allowed to grow for several weeks longer before the agar method was initiated, rhizosphere alkalization (purple agar) was more strongly exhibited. This situation can be attributed to near complete nitrification of the added NH_4^+ , which then permitted N to be absorbed predominately as NO_3^- . The degree of rhizosphere pH change when $(\text{NH}_4)_2\text{SO}_4$ is added to soil without N-Serve may depend on several factors: (i)

the amount of acidity produced by the nitrification process that occurs within the rhizosphere soil; (ii) the length of time that roots have been present for N uptake; and (iii) the relative amount of N uptake before and after the N fertilizer is nitrified.

Because rhizosphere pH is primarily determined by the supply of NO_3^- and NH_4^+ , the root-induced pH changes in the rhizosphere, as reflected by the pH indicator, were in agreement with the expected effects of NO_3^- vs. NH_4^+ nutrition. Hence, NO_3^- nutrition led to soil alkalization from excess anion uptake and OH^- excretion (to maintain root cell electroneutrality), and NH_4^+ nutrition led to soil acidification from excess cation uptake and H^+ excretion.

Observed changes in rhizosphere pH did not differ when $\text{Ca}(\text{NO}_3)_2$ was added with or without N-Serve (Fig. 4A and D). Because the soil was low in available N at the beginning of the experiment, almost all the N that was absorbed originated from fertilizer. In addition, mineralization of organic N to NH_4^+ in this low organic matter content soil did not appreciably influence the mixture of N sources available to the plant. Contrasting the $\text{Ca}(\text{NO}_3)_2$ plus N-Serve treatment with the others demonstrates to students that the purpose of using a nitrification inhibitor is to maintain the presence of NH_4^+ . The bulk soil pH for this treatment measured 6.2.

Although the technique is not well adapted to determining pH patterns within root systems, subtle variations in color along roots, illustrating pH levels, were observed during the development of the images in the agar. These variations were presumably related to the nutrient uptake pattern along the root (Fig. 4B and C). Differences in rhizosphere pH in the meristematic, elongation, and maturation root development zones may be related to differences in the cation to anion uptake ratio, root exudation, rate of nitrification, and root respiration patterns (Gijsman, 1990b). Microelectrodes may be used to more clearly resolve differences in pH in the rhizosphere (Gollany and Schumacher, 1993).

TEACHING VALUE

This experiment addresses the recently expressed concern that plants receive insufficient attention in biology courses, relative to their huge significance in the global environment (Hershey, 1993). The same is true of soil and its importance for human life. The opportunity of examining an organism in the living state and contact with its natural environment is significant. Soil preparation and planting are ideal preparatory steps for students to perform. Plant growth requires about 4 wk. These preliminary stages serve to generate anticipation and provide the opportunity to introduce background information and develop the theoretical implications of the experiment with an involved, motivated audience, before immersing students in the details of the results. The subject of N nutrition is especially rich in this respect. Classroom discussions of the responses of plants to NO_3^- vs. NH_4^+ nutrition, soil pH changes, soil microorganisms and nitrification, nitrification inhibitors, NO_3^- contamination of drinking water and other current environmental themes of interest allow students to realize that agronomy is an environmental science.

The use of rhizotrons permits students to observe details of the root system as it develops. Further, the experiment clearly demonstrates to students that plant roots can alter their immediate environment in response to the form of N nutrition. Lectures focused on how changes in rhizosphere pH can modify nutrient availability, plant tolerance of soil acidity, and microbial activity and survival. As an example, the uptake of P, Fe, and Mn may be enhanced by rhizosphere acidification, particularly in an alkaline soil (Marschner, 1986; Gillespie and Pope, 1990). Additionally, take-all disease of wheat (*Triticum aestivum* L.) (Smiley and Cook, 1973) and summer patch disease of turfgrass (Thompson et al., 1995) have been found to be suppressed by rhizosphere acidification that is associated with NH_4^+ nutrition. Students were impressed by the vivid color changes of this experiment. Some university undergraduate students stated that the visual images served to reinforce lecture points about the importance of rhizosphere chemistry and plant-soil relationships in general.

The experiment was also carried out as a demonstration to a group class of 3rd through 8th grade science students, who had previously been exposed to a 2-wk unit on pH and its meaning. Another class was devoted to root uptake of nutrients. The clear, concrete color change phenomenon greatly assisted the students' comprehension, and especially helped those who had difficulty with the theory of ion exchange across root membranes. Throughout the demonstration, students showed avid interest and speculated on what color change would occur for each treatment. After the demonstration, students were given a homework writing assignment in which they were asked to explain what had happened during the experiment and why. Although the quality of the responses was highly varied, it showed that about 25% of 3rd graders understood the basic concepts. The percent of students who demonstrated an understanding of the basics increased with grade level, reaching nearly 100% among eighth graders.

GENERAL CONSIDERATIONS

1. Soil properties, such as texture, buffering capacity, available mineral N, and pH, can influence the expression of root-induced pH changes in the rhizosphere. We found that the procedure exhibits vivid images when a sandy loam soil that is slightly acidic (pH 5.8–6.4) and low in mineral N and organic matter is used.

2. Although we have obtained similar results using corn, the procedure may produce different results using other plant species. Legumes, for example, often decrease the rhizosphere pH even with NO_3^- nutrition. The use of a legume could provide an interesting variation in the experiment.

3. The effects of other N fertilizers could be studied using the procedure. We also experimented with urea and observed effects similar to those obtained when using $(\text{NH}_4)_2\text{SO}_4$. The effect of NH_4NO_3 may be intermediate between NH_4^+ or NO_3^- only treatments.

REFERENCES

- Adriaanse, F.G., and J.J. Human. 1990. The effects of nitrate:ammonium ratios and nitrapyrin on the nitrogen response of *Zea mays* L. under field conditions. *Plant Soil* 122:287–293.

- Gijsman, A.J. 1990a. Nitrogen nutrition of douglas-fir (*Pseudotsuga menziesii*) on strongly acid sandy soil. *Plant Soil* 126:53–61.
- Gijsman, A.J. 1990b. Rhizosphere pH along root zones of douglas-fir (*Pseudotsuga menziesii*), as affected by source of nitrogen. *Plant Soil* 124:161–167.
- Gillespie, A.R., and P.E. Pope. 1990. Rhizosphere acidification increases phosphorus recovery of black locust: I. Induced acidification and soil response. *Soil Sci. Soc. Am. J.* 54:533–537.
- Gollany, H.T., and T.E. Schumacher. 1993. Combined use of colorimetric and microelectrode methods for evaluating rhizosphere pH. *Plant Soil* 154:151–159.
- Hershey, D.R. 1993. Plant neglect in biology education. *Bioscience* 43:418.
- Hoefl, R.G. 1984. Current status of nitrification inhibitor use in US agriculture. p. 561–570. *In* R.D. Hauck (ed.) *Nitrogen in crop production*. ASA, CSSA, and SSSA, Madison, WI.
- Marschner, H. 1986. *Mineral nutrition in higher plants*. 1st ed. Academic Press, San Diego, CA.
- Marschner, H., and V. Romheld. 1983. *In vivo* measurement of root-induced pH changes at the root–soil interface: Effect of plant species and nitrogen source. *Z. Pflanzenphysiol.* 111:241–245.
- Marschner, H., V. Romheld, and H. Ossenberger-Neuhaus. 1982. Rapid method for measuring changes in pH and reducing processes along roots of intact plants. *Z. Pflanzenphysiol.* 105:407–416.
- Smiley, R.W., and R.J. Cook. 1973. Relationship between take-all of wheat and rhizosphere pH in soils fertilized with ammonium vs. nitrate nitrogen. *Phytopathology* 63:882–890.
- Thompson, D.C., B.B. Clarke, and J.R. Heckman. 1995. Nitrogen form and rate of nitrogen and chloride application for the control of summer patch disease in Kentucky bluegrass. *Plant Dis.* 79:51–56.
- Weissensteil, M.H., A. Dorn, and L.F. Jaffe. 1979. Natural H⁺ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiol.* 64:512–518.◆