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Root Development and Absorption of Ammonium and Nitrate from the Rhizosphere

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Abstract

Plant roots operate in an environment that is extremely heterogeneous, both spatially and temporally. Nonetheless, under conditions of limited diffusion and against intense competition from soil microorganisms, plant roots locate and acquire vital nitrogen resources. Several factors influence the mechanisms by which roots respond to ammonium and nitrate. Nitrogen that is required for cell division and expansion derives primarily from the apex itself absorbing rhizosphere ammonium and nitrate. Root density and extension are greater in nutrient solutions containing ammonium than in those containing nitrate as the sole nitrogen source. Root nitrogen acquisition alters rhizosphere pH and redox potential, which in turn regulate root cell proliferation and mechanical properties. The net result is that roots proliferate in soil zones rich in nitrogen. Moreover, plants develop thinner and longer roots when ammonium is the primary nitrogen source, an appropriate strategy for a relatively immobile nitrogen form.

Key words: ammonium; nitrate; roots growth; development; rhizosphere; pH redox potential

Introduction

Nitrogen is the mineral element that plants require in greatest amounts and whose availability often limits plant productivity in natural and managed ecosystems. To obtain nitrogen, plants may form symbiotic relationships with bacteria or fungi, scavenge amino acids from organic soils, or even practice insectivory. Nonetheless, most plants obtain the vast majority of their nitrogen through root absorption of the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-) from the soil solution.

The spatial and temporal availability of soil NH_4^+ and NO_3^- is highly heterogeneous. Within centimeters or over the course of a day, soil NH_4^+ and NO_3^- may vary by an order of magnitude (Jackson and Bloom 1990). This heterogeneity derives from several factors. The physical, chemical, and biological processes that release or remove NH_4^+ and NO_3^- from the soil are complex. The discontinuous gaseous, liquid, and solid phases in a soil limit movement of nitrogen compounds. To survive under such heter-

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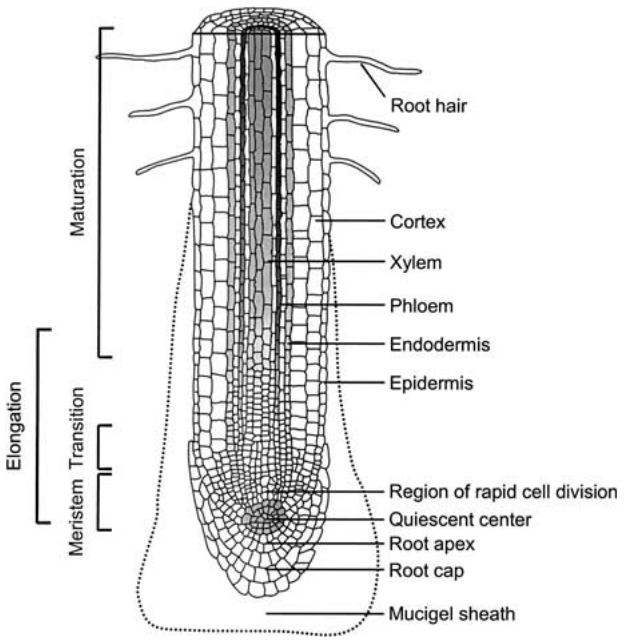

Figure 1. A diagram of a typical root showing the various developmental zones.

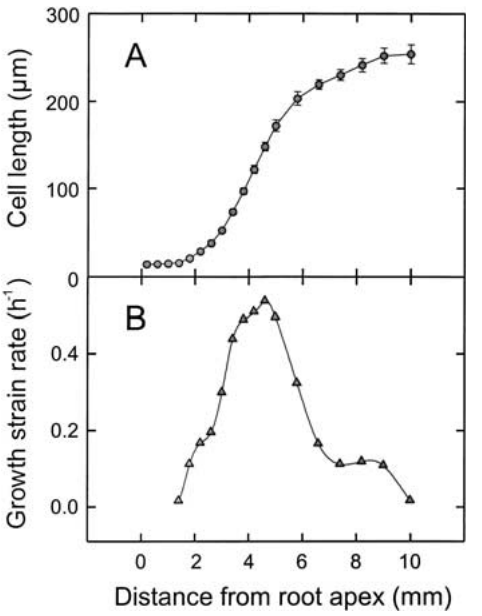

Figure 2. (**A**) Cortical cell length versus position along the root axis for 3-day-old maize seedlings grown in solution culture (mean ± SE, $n = 20$). (**B**) Growth strain rate (relative change in cell length per time) versus position as calculated from the data in **A** (Taylor and Bloom 1998).

ogeneity and under intense competition from soil microorganisms, plant roots must be in the right place, at the right time, and with the right mechanisms in place. Plant roots can sense soil patches enriched in nitrogen and, in response, alter their growth and development. The following discusses current research on the relationship between inorganic nitrogen in the rhizosphere and root growth and development.

Root Growth and Development

The root provides a relatively simple model for the study of plant organ development in that it has radial symmetry, is composed of cells organized into cylinders and sectors, and has relatively few differentiated cell types (Aeschbacher and others 1994; Rost 1994; Rost and Bryant 1996). A root tip is organized into the following regions: root cap, meristem, elongation region (which includes the meristem and a zone where division has ceased but elongation continues), and maturation region that overlaps the elongation zone (Figure 1). This view is a reasonable way to designate zones where different processes are mostly occurring, but distinct boundaries do not really exist, and each cell file or group of files tends to act independently. For example, the boundaries between the meristem may differ among cells in the cortex and cells in the epidermis or vascular sectors (Rost 1994).

The location of each region varies with root growth. For example, as root growth speeds up, the position where xylem vessel members mature moves farther away from the root apex (Rost and Baum 1988; Reinhardt and Rost 1995). Thus, the root can change the spatial relationships between cell division, elongation, and differentiation events.

Environmental conditions also influence the location of these regions. For instance, water stress reduces cell division rates without affecting cell elongation rates in the meristem, causing a shortening of the meristem and a lengthening of cells near the root apex relative to well-watered controls (Sacks and others 1997). In addition, water stress reduces cell elongation rates at the base of the elongation zone, causing a shorter elongation zone and a decrease in final cell lengths (Fraser and others 1990).

Primary root growth derives from the activity of the root apical meristem and the generation of lateral root primordia (Scheres and others 1996). In the meristem, cell division occurs both in the direction of the root base to form cells that will differentiate into the tissues of the functional root and in the direction of the root apex to form the root cap (Figure 1). Cell division at the boundary between the root body and cap is relatively slow at the quiescent center. After a few divisions in maize, when

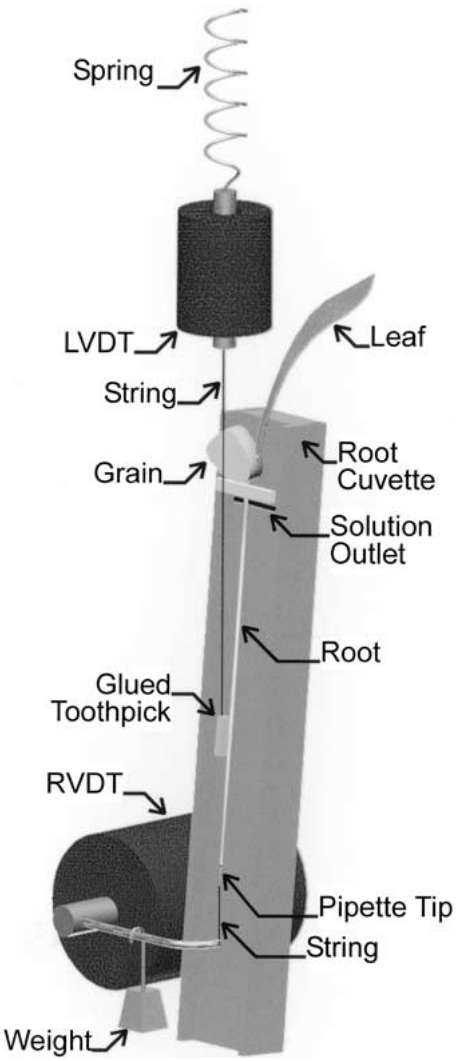

Figure 3. Schematic of the *in planta* root extensiometer that measures extension under stretching. A RVDT monitors the position of the root cap, while a LVDT monitors the position of the mature zone. A pipette tip is glued to the root cap and a toothpick is glued to the mature zone. Weights are placed near the tip to assess root plasticity and elasticity. Nutrient solution flows from an outlet near the top to bathe the root (J. Frensch and A.J. Bloom, unpublished).

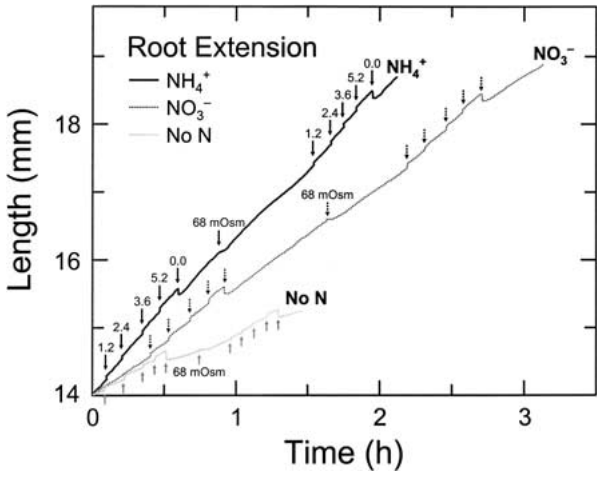

Figure 4. Length of the root elongation zone — the difference between the position of the root apex and a point on the root initially 15 mm from the apex—versus time for 3-day-old maize seedlings whose roots were exposed to l mM $CaSO_4$, 200 MM KH_2PO_4, and either 100 MM $NH_4H_2PO_4$ (NH_4^+), 100 MM KNO_3 (NO_3^-), or no nitrogen (No N). The labeled arrows indicate the times for the NH_4^+ treatment at which designated weights were added near the tip or 68 mOsm KCl was added to the nutrient solution. The same series of weights and osmoticant were applied to the NO_3^- and No N treatments at the times indicated by the shorter arrows. Adding 68 mOsm KCl to the nutrient solution applies a known stress of 0.17 MPa on the cell wall and provides an independent calibration of the stress induced by the weights (J. Frensch and A.J. Bloom, unpublished).

the root apex has grown away by about 0.1 mm, root cells begin to divide more rapidly. Cell division again tapers off at about 0.4 mm from the apex, and the cells may expand equally in all directions. Some have named this area the transition zone (Baluska and others 1996, 2001).

The elongation zone begins 0.7–1.5 mm from the apex (Figure 2). Here, cells rapidly extend in length and undergo a final round of divisions to produce the cylinder of endodermal cells. In the vascular cylinder, the phloem begins to differentiate within 3 or 4 mm of the apex, reflecting the importance of phloem function to cell division and elongation. Carbohydrates that flow through the phloem to the growing apices serve not only as an energy source but as carbon skeletons for newly synthesized organic compounds and as osmoticants. The phloem also supplies nitrogen to these tissues (Lazof and others 1992). In the youngest tissues where the phloem has not yet developed, namely, the meristematic and transition zones, translocation of carbohydrate and nitrogen must rely heavily upon symplastic diffusion, but symplastic diffusion alone is too slow to support apical growth (Bret–Harte and Silk 1994b). One explanation for the slow cell division in the quiescent center may be the limited quantities of carbohydrates or nitrogen reaching this centrally located region.

Nitrogen Limitations at the Root Apex

We have developed an *in planta* root extensiometer to monitor growth, plasticity, and elasticity of the

root elongation zone in response to different nutrient solutions (Figure 3). A pipette tip is attached to the root cap with surgical-grade cyanoacrylic glue and linked to a rotary voltage displacement transducer (RVDT) with a nylon thread. A piece of a toothpick is glued to the basal end of the elongation zone and linked to a linear variable differential transformer (LVDT) with a thread. The difference between the readings of the RVDT and the LVDT indicates the length of the root elongation zone. Changes in this length when weights are added near the apex reflect root plasticity plus elasticity, whereas the changes when weights are removed reflect only elasticity. The root cuvette is tilted a few degrees from vertical, and nutrient solution (1 mM $CaSO_4$, 200 MM KH_2PO_4, and either no nitrogen, 100 MM $NH_4H_2PO_4$, or 100 MM KNO_3 adjusted to pH 6.5 with KOH) continuously flows down its surface immersing the root. Our capability to assess root mechanical properties *in planta* is unique; the standard approach has been to assess tissue segments that have been frozen, thawed, abraded, and then boiled (for example, see Wu and others 1996).

In preliminary experiments on 3-day-old maize seedlings, the root elongation zone extended 1–3 mm h^{-1} (Figure 4 shows typical traces for three treatments; the extension rates were calculated once they reached a steady value after a perturbation), rates comparable to those of plants growing in vermiculite (Sharp and others 1990) or solution culture (Taylor and Bloom 1998). This indicates that the root extensiometer does not significantly damage the root. Extension under NH_4^+ was significantly faster (2.16 ± 0.07 mm h^{-1}, $n = 6$) than under NO_3^- (1.90 ± 0.13 mm h^{-1}, mean ± SE, $n = 6$), which in turn was significantly faster than under nitrogen deprivation (1.67 ± 0.08 mm h^{-1}). Exposure to 68 mOsm KCl diminished root extension under NO_3^- (1.56 ± 0.12 mm h^{-1}, $n = 6$), but not under NH_4^+ or N deprivation. Applying a load of 1.2–5.2 g had no significant effect on the rate of root extension and the roots exhibited only an elastic response (data not shown).

These results imply that growth of the root elongation zone, even for plants with large nitrogen reserves, is nitrogen limited. The meristem and transition zones differ from more mature root zones in that they lack fully differentiated phloem tissue and, thus, cannot rapidly import nitrogen from more mature tissues. For example, little of the NO_3^- absorbed in the maturation zone moves toward the apex (Siebrecht and others 1995). Consequently, the nitrogen required for cell division and isotropic cell expansion derives primarily from nitrogen that the apical zones themselves absorb and assimilate. Assimilation of NH_4^+ and NO_3^- to glutamine consume the equivalent of about 2 ATPs per NH_4^+ and 12 ATPs per NO_3^-, respectively (Bloom and others 1992). In the carbohydrate-limited apical meristem (Bret–Harte and Silk 1994a), the lower-energy requirement for NH_4^+ assimilation may permit dividing cells to maintain energy reserves above a critical threshold. Absorption and assimilation of NH_4^+ and NO_3^- also alter the redox poise of a cell and, thereby, its division rate. This is discussed more fully below.

The presence of NO_3^- stimulated root elongation under 0 mOsm KCl, but not under 65 mOsm KCl. This suggests that NO_3^- may serve as an osmoticant, a possibility that is also discussed below.

Root Hairs and Lateral Roots

The maturation zone of a typical root starts between 5 and 20 mm from the apex (Figure 1). In this zone, the xylem develops the capacity to translocate substantial quantities of water and solutes to the shoot. Root hairs also first appear in this region. Although root hairs have become a model system for the study of tip growth and associated ion fluxes (see, for example, Gassmann and Schroeder 1994; Ridge 1995; Felle and Hepler 1997; Schiefelbein 2000; Bibikova and Gilroy 2003), their primary physiological function is still in question (Peterson and Farquhar 1996; Raven and Edwards 2001).

Root hairs increase root surface area and should enhance root water and nutrient absorption. They have the capacity to absorb nutrients (Jungk 2001). Exposure to low levels of nutrients may stimulate root hair extension (nitrate, Föhse and Jungk 1983, but see Ewens and Leigh 1985; phosphate, Bates and Lynch 1996, but see Gahoonia and others 1999). Moreover, wild-type *Arabidopsis* plants acquire phosphate more efficiently than mutants lacking root hairs (Bates and Lynch 2001).

In contrast, root hairs may not enhance nutrient absorption. They may be situated where the root apex has already depleted the rhizosphere of water and nutrients (Clarkson 1985; Jungk 2001) or may grow so slowly and so close together that their own depletion zones overlap (Clarkson 1991). This would limit their effectiveness in absorbing water from drier soils or immobile nutrients such as NH_4^+. Cytoplasmic streaming is also relatively slow in root hairs, impeding rapid transfer to the remainder of the plant (Raven and Edwards 2001).

Root hairs may be involved in anchorage. Although their ability to anchor an entire plant is limited (Bailey and others 2002), they may serve to anchor the root in the mature zone and provide a

firm base from which the root apex can extend into new soil. This may explain why roots in hydroponics tend to be deficient in root hairs.

Lateral roots initially emerge anywhere from 5 to 200 mm from the apex and vary greatly in number. This variability probably reflects the fact that lateral root development requires the three relatively independent steps: initiation, organization, and emergence (Hinchee and Rost 1986). Several lines of evidence suggest that lateral root initiation occurs near the root apex:

- The first visual evidence of lateral roots is usually the formation of a small bump on the surface of the root a few millimeters from the apex.
- Excising primary roots at different distances from the apex produces a similar regeneration response in maize (Feldman 1976), long pod bean (Francis 1978), and pea (Rost and Jones 1988; Reihman and Rost 1990). In peas (Reihman and Rost 1990), if the excision was 0.5 mm or closer to the apex, regeneration of a new root tip occurred without any swelling or evidence of wound repair. With excision at up to 1.5 mm, regenerated root tips formed but at an angle less than 90° from the primary root longitudinal axis. Excision at greater than 1.5 mm resulted in the formation of lateral roots at right angles to the root axis. This means that the competency to form a lateral root in peas occurs at approximately 1.5 mm from the root apex. The state of cell differentiation at that level in the root is apparently such that inductive cell divisions occur at right angles to the root axis, resulting in a lateral root. This observation establishes that the initiation of lateral root primordia can occur as close as 1.5 mm from the root apex.
- Pea seedlings grown at 32°C formed no lateral roots at all (Gladish and Rost 1993). If these seedlings were transferred to 25°C, lateral roots formed after a delay. The most likely reason for this delay is because new initiation sites have to be formed by the root apex. Apparently, a developmental window or site (state of initiation competency) is localized just basal to the root apex; if lateral roots are not initiated in that window, then lateral roots can not be initiated at all.

The next step of lateral root development, namely, organization, involves postembryonic organogenesis in which mature pericycle cells first rapidly divide and then redifferentiate into functional lateral root primordia (Laskowski and others 1995; Malamy and Benfey 1997). The last step, emergence, is where the lateral root primordium cells divide and push their way through the surrounding tissues. Early on during the emergence step, the lateral root develops a root cap and apical meristem. Only much later, after the xylem and phloem fully differentiate, do the vascular cylinders of a lateral root and its parent join (McCully 1975; Raven and others 1992).

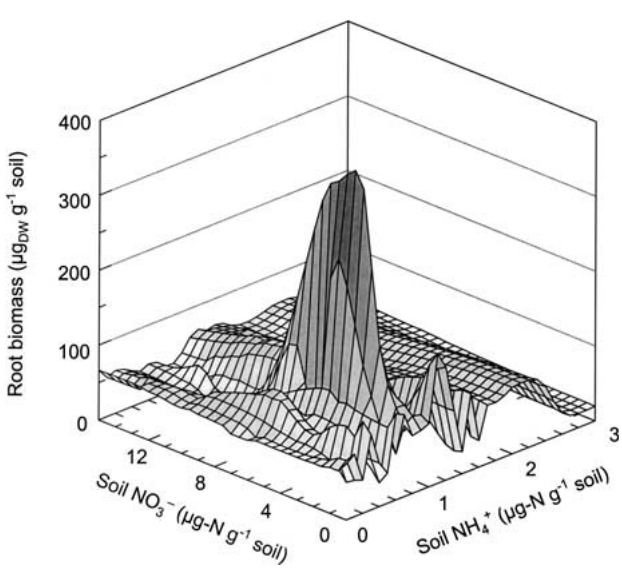

Figure 5. Root biomass (Mg root dry weight g^{-1} soil) versus KCl-extractable soil NH_4^+ and NO_3^- (Mg extractable N g^{-1} soil) for *Lycopersicon esculentum* L. Mill. cv. T-5, a fresh-market tomato cultivar growing in an irrigated but unfertilized field that had been fallow the previous two years. Biomass showed a hyperbolic response to soil levels of inorganic nitrogen: very few roots were found in soil blocks depleted in inorganic nitrogen, roots proliferated as soil inorganic nitrogen increased, and root growth declined in soils with the higher levels of inorganic nitrogen (Bloom and others 1993).

Root Development and Nitrogen

The regulation of root developmental processes is poorly understood, in part because of the differential response of each tissue. For example, auxin at low levels promotes cell expansion in the transition zone (Ishikawa and Evans 1995), inhibits cell extension in the elongation zone (Ishikawa and Evans 1995), initially inhibits root hair growth (Felle and Hepler 1997), but greatly stimulates the organization of lateral root primordia (Taiz and Zeiger 2002). Another confounding factor is that root development depends upon a broad range of external factors including inorganic nitrogen, pH, and redox potential.

The importance of rhizosphere nitrogen in plant mineral nutrition has often diverted attention from its regulatory role in root development. Experi-

Table 1. The Plant Parameters (mean ± SE, $n = 4$) of Shoot and Root Biomass, Total Root Length, Root Branching, and Root Area for *Lycopersicon esculentum* L. Mill. cv. T5[a]

Treatment[b]	Shoot biomass (mg_{DW})	Root biomass (mg_{DW})	Root length (m)	Root branching (roots m^{-1})	Root area (cm^2)
100 μM NH_4^+	34.8 ± 1.5	13.2 ± 0.6	4.27 ± 0.19	34.9 ± 1.6	17.3 ± 0.8
200 μM NO_3^-	35.4 ± 3.0	9.1 ± 0.8	3.00 ± 0.23	28.1 ± 0.8	12.9 ± 1.5

[a]*Plants were grown in solution culture for 12 days under constant levels of nitrogen nutrition and pH 6.0 ± 0.3.*
[b]*The two treatments developed similar shoot biomass indicating that nitrogen was not limiting (aBloom and others 1993).*

ments in which roots grow through compartments containing different levels of NH_4^+ or NO_3^- (Robinson 1994) demonstrate that lateral roots proliferate only within the highly localized region directly exposed to these ions (Hackett 1972; Drew and others 1973; Drew 1975; Drew and Saker 1975; Grime and others 1986; Sattelmacher and Thoms 1989; Bingham and others 1997; Dunbabin and others 2001). These results are inconsistent with strictly nutritional effects. Lateral root primordia, given their location, should have access to nitrogenous compounds transported in the stele from either the shoot or other root zones. Nitrogen absorbed as NO_3^- in more basal regions may be translocated to the maturation zone (Lazof and others 1992). Hence, even when a root apex grows into nitrogen-depleted regions, the lateral root primordia are likely to be well nourished if more basal parts of the root are exposed to high NO_3^- concentrations. External levels of NO_3^-, therefore, seem to serve as a signal in lateral root development (Tischner 2000; McIntyre 2001; Forde 2002).

The role of NO_3^- as a signal has been most thoroughly explored in *Arabidopsis.* In this species, high NO_3^- levels in the medium inhibit lateral root elongation (Zhang and Forde 1998; Zhang and others 1999; Linkohr and others 2002). If NO_3^- enrichment is limited to a small zone, lateral root elongation doubles in that zone. Mutants deficient in NO_3^- reductase showed a similar response to local NO_3^- enrichment indicating that the NO_3^- stimulation of lateral root growth can be independent of nitrogen nutrition (Zhang and Forde 1998). Studies on mutants, which are compromised in auxin production or reception, indicated that this hormone might (Zhang and others 1999; Marchant and others 2002) or might not (Linkohr and others 2002) mediate the influence of external NO_3^- on lateral root elongation. An analogous study with ABA mutants suggested that *Arabidopsis* has both ABA-sensitive and -insensitive pathways that mediate this response (Signora and others 2001).

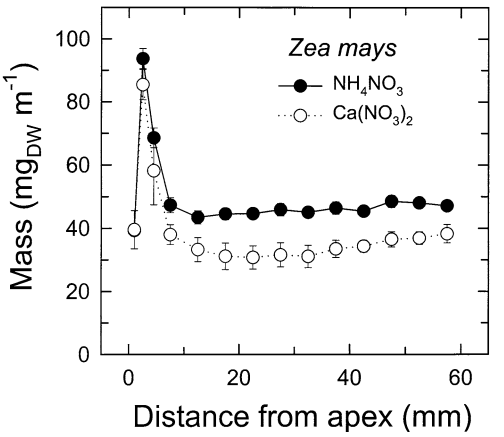

Figure 6. Mass (mg dry weight per m root length) of tissue at various distances from the apex of a maize seminal root grown in medium containing either NO_3^- or NH_4^+ plus NO_3^-. Sections from 10 roots were cut, dried, and weighed in four separate experiments. Shown are the mean ± SE for the four replicates (Taylor and Bloom 1998).

Evidence for developmental coordination in the response to local concentrations of nutrients comes from several sources. For plants grown in split-root hydroponic systems, raising nutrient concentrations in one chamber increased the number of lateral roots in that chamber and decreased the number in the other chamber even though nutrient concentrations in the second chamber remained unchanged (Gersani and Sachs 1992; Bingham and others 1997). In two field experiments (Bloom and others 1993), root growth of tomato showed a hyperbolic response to soil levels of inorganic nitrogen: Very few roots were found in soil blocks depleted in inorganic nitrogen, roots proliferated as soil inorganic nitrogen increased, and root growth declined in soils with the higher levels of inorganic nitrogen (Figure 5). The optimal levels for root growth were 2 Mg NH_4^+–N g^{-1} soil and 8 Mg NO_3^-–N g^{-1} soil.

Root development responds not only to the quantity of inorganic nitrogen in the rhizosphere,

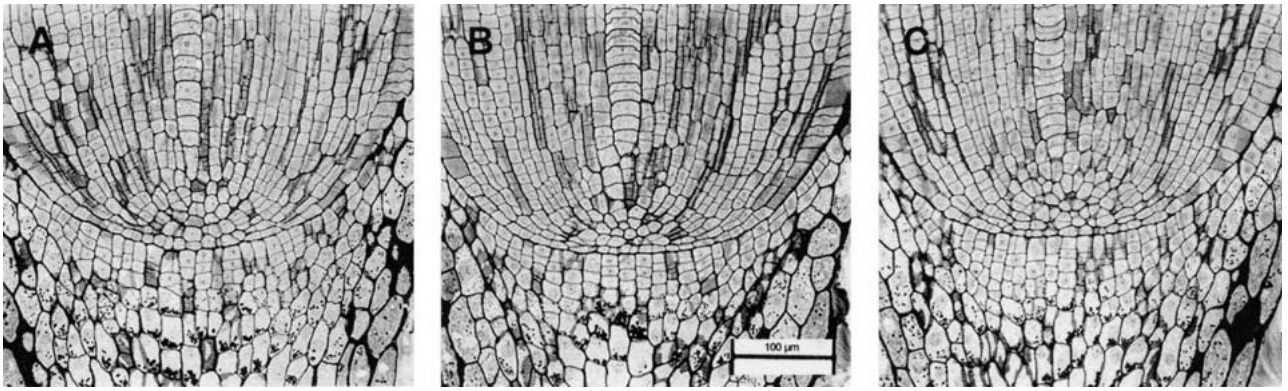

Figure 7. Micrographs of root apices from 4-day-old maize seedlings grown on (**A**) 100 MM NH_4^+, (**B**) 100 MM NO_3^-, or (**C**) a medium without nitrogen. These tissues appear similar in their organization. The bar in the middle frame indicates the scale for all three micrographs. The photomicrographs were prepared by Dr. Carol Wenzel.

but to nitrogen form NH_4^+ or NO_3^- (Bloom 1997). In a solution culture system that controlled NH_4^+ or NO_3^- levels (Bloom and others 1993), shoot growth of tomato was similar under both nitrogen forms, but root growth (biomass, length, branching, or area) was enhanced by NH_4^+ (Table 1). Root extension (Figure 4) and mass (Figure 6) of maize seedlings were greater in nutrient solutions containing NH_4^+ than in those containing NO_3^-. Root apical organization was independent of nitrogen source (Figure 7). These results are consistent with the hypothesis that NH_4^+ nutrition accelerates cell division. We are currently testing this hypothesis.

Nitrogen as a Signal

We conducted an experiment to determine whether NH_4^+ and NO_3^- themselves serve as signals that determine root proliferation. Maize seeds (*Zea mays* cv. Dekalb) were surface-sterilized in 1% NaClO, rinsed thoroughly with water, and placed in rows on germination paper, a thick paper toweling that is difficult for roots to penetrate. The germination paper was rolled into a tube, placed upright with one end in l mM $CaSO_4$, and left in the dark at 25°C for 2 days. Seedlings with a primary root of 30 ± 1 mm were transferred to a controlled environmental chamber held at 22°C and 95% relative humidity. The shoots received 600 Mmol quanta m^{-2} s^{-1} PAR from metal-halide HID lamps over a 14-h day. The roots were placed in light-impermeable boxes held at 10° from vertical so that the roots grew down at a slant along a Plexiglas surface covered with germination paper. A second strip of germination paper was draped over the root. The bottom ends of both strips of germination paper were immersed in a

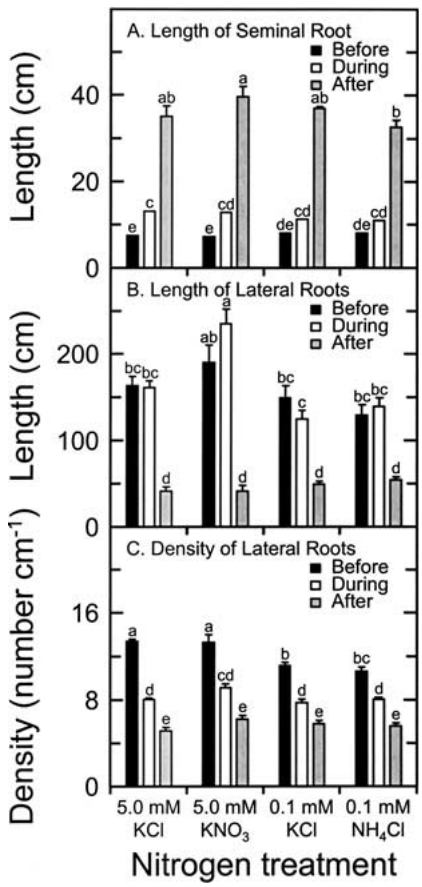

Figure 8. (**A**) Seminal root length, (**B**) lateral root length, and (**C**) lateral root density (number per unit length of seminal root) in maize plants that were treated for 48 h with either 5 mM KC1, 5 mM KNO_3, 0.1 mM KCl, or 0.1 mM NH_4Cl. Basal, treated, and apical refer to the root regions of the seminal root that initiated before, during, and after the treatments. Shown are the mean ± SE for 12 plants (A.J. Bloom and P.A. Meyerhoff, unpublished).

reservoir that contained a nutrient solution that was refreshed on a daily basis. For 2 days this solution contained either 0.1 mM NH_4Cl, 0.1 mM KCl, 5.0 mM KNO_3, or 5.0 mM KCl. The lower concentrations of NH_4^+ were to avoid ammonium toxicity. Subsequently, all of the treatments received the same nutrient solution [one-tenth strength of a modified Hoagland solution (Bloom 2002)] for an additional six days.

We measured the roots three times: first, before the different treatments were applied; second, when the different treatments ceased; and finally, six days after the treatments. These measurements permitted us to identify which parts of the seminal root initiated before, during, or after the treatments. Seminal root length and number of lateral roots were assessed under a dissecting microscope. Some of the roots were cleared under vacuum in ethanol:acetic acid (3:1) to count unemerged lateral root primordia. We evaluated lateral root lengths using a video camera and an image analysis system (AgVision Root and Leaf Analysis, Decagon Devices, Pullman, WA) on roots that were stained with Toluidine Blue to increase contrast.

There were several differences among the treatments (Figure 8). In the root zone initiated during the NO_3^- treatment, lateral roots were longer (Figure 8B). This response appeared to require direct exposure of young laterals to external NO_3^- because the lengths of lateral roots that initiated in the absence of NO_3^- were similar among all treatments (Figure 8B). Our results on maize are consistent with those on *Arabidopsis* introduced above in which exposure of root patches to NO_3^- stimulated lateral root elongation in the patch (Zhang and Forde 1998; Zhang and others 1999; Linkohr and others 2002). We also found in maize that exposure to the higher osmotic treatments (5.0 mM KCl or 5.0 mM KNO_3) enhanced lateral root density in zones initiated before the treatments began (Figure 8C). All of the lateral root primordia eventually emerged (data not shown). Seminal root lengths were similar under all treatments (Figure 8A). Altogether, these observations—higher lateral root densities, all lateral roots emerge, and similar seminal root lengths—imply that higher osmotic strengths stimulated maize lateral root initiation.

Stimulation of root growth and development by NO_3^- may derive in part from its role as an osmoticant (McIntyre 2001). In maize, NO_3^- stimulated extension of the seminal root under low osmotic conditions but not under high (Figure 4). Exposing a section of an *Arabidopsis* root to NO_3^- stimulated lateral root elongation in proportion to the concentrations from 0.05 to 10 mM NO_3^- (Zhang and others 1999; Linkohr and others 2002). Extension of cells in the root 'elongation zone' depends upon osmotically driven uptake of water. Cells in the elongation zone generate the required osmotic potential through accumulating carbohydrates and K^+ (Sharp and others 1990), but the counterions for this K^+ remain uncertain. Organic acids, such as malate generated from dark CO_2 fixation, may be involved (Osmond 1976), but Cl^- and particularly NO_3^- are perhaps more suitable because accumulation of these inorganic anions entails less metabolic energy. Plant cells function normally under high levels of NO_3^- (Goyal and Huffaker 1984). Moreover, NO_3^- stored in the cells of the elongation zone can later serve as a nitrogen source as the tissue matures (Lazof and others 1992). As an osmoticant, NO_3^- would not behave like a typical chemical signal. In the standard model of a signal, once the signal exceeds a certain low threshold, a specific receptor in the cell membrane changes state and starts a transduction cascade that generates a response at a larger scale.

Another possible signal of inorganic nitrogen in the soil is nitric oxide (NO). Soil microorganisms readily convert NH_4^+ and NO_3^- into NO. It serves as a potent signal for many other animal and plant responses (Beligni and Lamattina 2001; Wendehenne and others 2001). To examine the influence of NO upon root development, we grew tomato for eight days on a complete nutrient solution containing 100 MM NH_4NO_3 and then bubbled either air or 0.8 ppm NO (a level that roots might encounter in the soil; D. Smart, personal communication) through the solution for 10 h. We analyzed a number of root parameters immediately after the treatments and four days later and found no significant differences between the treatments (Table 2).

These results argue that neither NH_4^+ NO_3^-, nor NO serves as a direct signal for root development rather some secondary effects of NH_4^+ and NO_3^- may be the actual stimulus perceived by the plant. For example, NH_4^+ and NO_3^- alter the pH and redox potential of the rhizosphere, and roots may respond to such alterations.

Acid Growth of Roots

The 'acid-growth hypothesis,' originally postulated to explain auxin-induced growth (Cleland 1971; Hager and others 1991; Rayle and Cleland 1992), may apply to nitrogen-induced growth (Bloom 1997). Plant nitrogen metabolism alters rhizosphere

Table 2. Relative Change per Day* for 8-day-old Tomatoes Exposed to Air or 0.8 ppm NO for 10 h Measured 0 and 4 Days after Treatment (mean ± SE for 6 plants)

		Lateral roots		
Treatment	Primary root length (cm cm^{-1} d^{-1})	Total length (cm cm^{-1} d^{-1})	Avg. length (cm cm^{-1} d^{-1})	Mass (roots cm^{-1}) $()^{-1}$ d^{-1}
Air	0.105 ± 0.013	0.399 ± 0.020	0.286 ± 0.021	0.008 ± 0.027
NO	0.093 ± 0.008	0.438 ± 0.023	0.325 ± 0.010	0.020 ± 0.028

*$(\ln L_1 - \ln L_2)/(t_1 - t_2)$

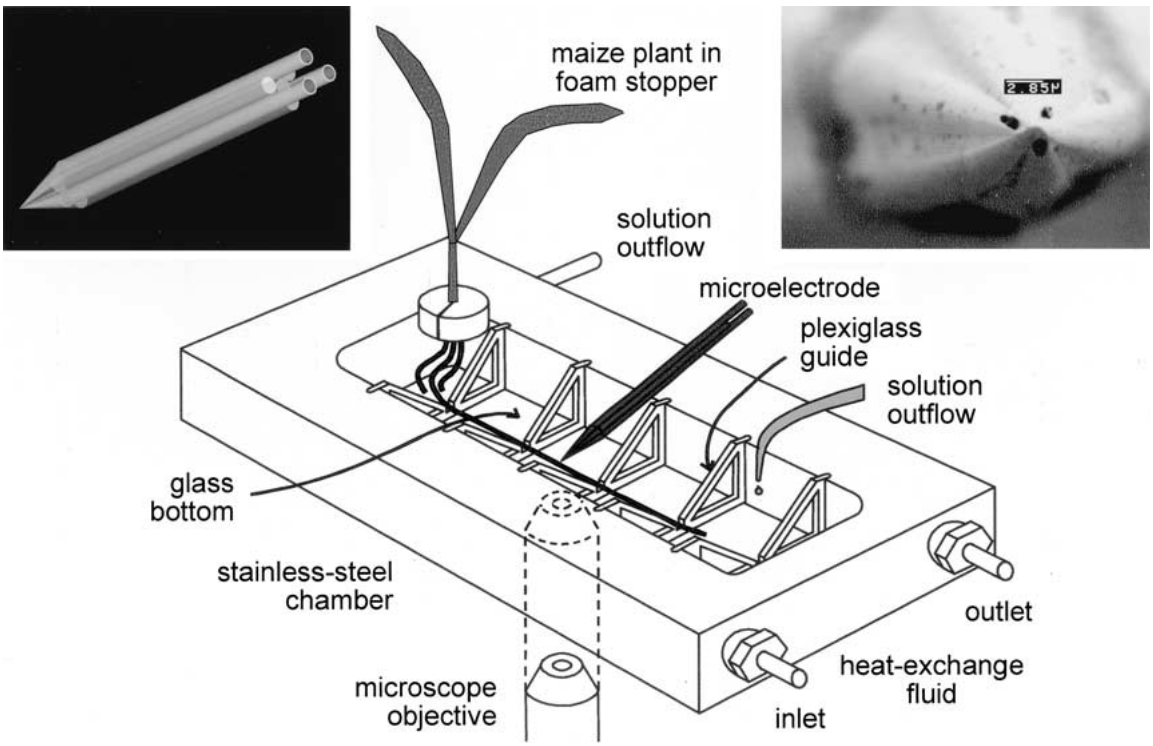

Figure 9. Stainless-steel and glass root cuvette for the microelectrode experiments. The cuvette is positioned on the stage of an inverted microscope and the root and microelectrode are viewed from below. Experimental solutions flow into the chamber at one end and out at the other. When the flow is stopped, a ion depletion zone develops. A micromanipulator positions the microelectrode tip at various distances from the root surface to monitor this zone. The left inset depicts a multibarrel microelectrode. The three longer barrels are pulled from thin-wall glass capillaries: one contains NH_4^+-selective LIX (liquid ion exchanger), another contains NO_3^- LIX, and the last contains a salt solution and serves as a local reference electrode. The four shorter barrels (that is, three outer barrels and the center) are pulled from solid glass rods. The right inset shows a scanning electron micrograph of the tip from a multibarrel ion-selective microelectrode. Each of the three barrels with holes is filled with a liquid ion exchanger or salt solution. The total tip diameter to enclose the open barrels is about 3 Mm (Colmer and Bloom 1998).

pH: NH_4^+ assimilation releases protons, whereas NO_3^- assimilation produces hydroxide ions (Raven and Smith 1976; Allen 1988). Plants supplied with NH_4^+ as the N source strongly acidify and those supplied with NO_3^- slightly alkalinize the rhizosphere (Smart and Bloom 1998). Through such pH changes, rhizosphere NH_4^+ and NO_3^- may affect cell wall expansion. Acidity in the root apoplast loosens the cell wall matrix (Taiz 1984; Edelmann and Fry 1992). Root growth zones correlate well with rhizosphere acidification as detected with pH indicators (Marschner 1995) and microelectrodes (Taylor and

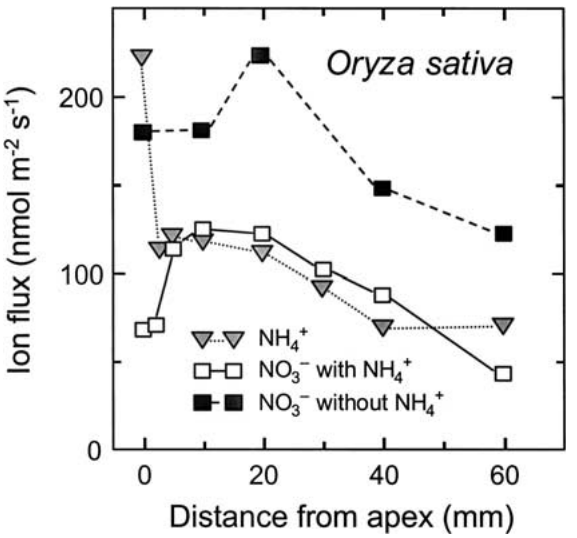

Figure 10. Absorption of NH_4^+ and NO_3^- at various distances from the apex for nodal roots of rice. NH_4^+ and NO_3^- were provided as sole N sources or in combination as NH_4NO_3. The presence of NO_3^- did not influence NH_4^+ absorption so that data for $(NH_4)_2SO_4$ and NH_4NO_3 were pooled (Colmer and Bloom 1998).

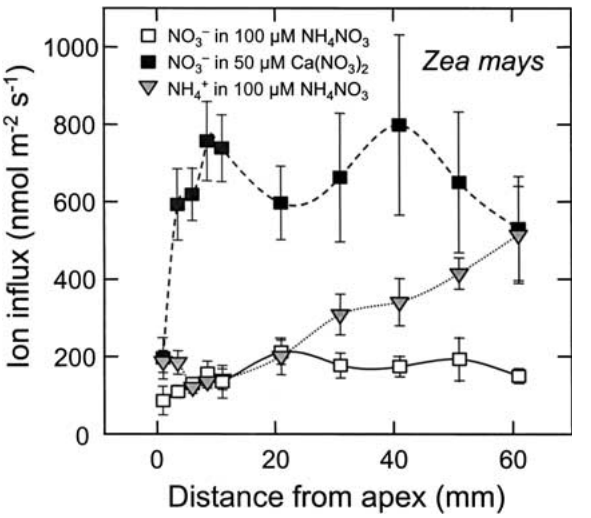

Figure 11. Absorption of NH_4^+ and NO_3^- at various distances from the apex of a maize seminal root. NH_4^+ was provided as NH_4NO_3; NO_3^- was provided as $Ca(NO_3)_2$ or NH_4NO_3 (Taylor and Bloom 1998).

Bloom 1998). Enhanced H^+ efflux is detected on the outer curved surface of roots responding to gravity and is thought to contribute to more rapid cell expansion (Mulkey and Evans 1982).

To quantify the relationships among inorganic nitrogen assimilation, rhizosphere pH, and root development, we developed multibarrel ion-selective microelectrodes that can simultaneously monitor electrical potential and the activities of two ions with high temporal and spatial resolution (Figure 9). Each of the ion-selective barrels is filled with a liquid ion exchanger (LIX) consisting of an ionophore that is a lipophylic ion channel, a plasticizer to solubilize the ionophore, and PVC dissolved in a volatile solvent. A salt solution containing the ion of interest is then backfilled behind the LIX, and a chloridized silver wire is placed in the salt solution. The silver wires from the three barrels fit into a circular Teflon IC socket that fits into the head stage of a multichannel, differential electrometer amplifier (Bloom 1989).

With these electrodes, we monitor ion gradients between 20 and 100 Mm from the surface of a root to determine which areas along the root axis are most active in NH_4^+, NO_3^-, and H^+ fluxes (Colmer and Bloom 1998; Taylor and Bloom 1998). This approach, which assumes radial symmetry and ion diffusion through water, pinpoints fluxes to within 800 Mm along the root axis with 95% certainty (Henriksen and others 1992).

In both rice (Figure 10) and maize (Figure 11), NH_4^+ absorption was higher at the root apex, whereas NO_3^- absorption reached a maximum in the area where root hairs emerge. In both species, NO_3^- absorption was greater when NH_4^+ was absent. These results indicate that NH_4^+ is the preferred form of nitrogen to support protein synthesis in the root apical meristem and that NO_3^- accumulation may provide a metabolically benign osmoticant to support cell expansion in the zone of elongation near the apex (Bloom 1996, 1997).

We have also examined net H^+ efflux along the root axes of maize when the medium contained NH_4^+, NO_3^-, or both NH_4^+ and NO_3^- (Taylor and Bloom 1998). Net H^+ efflux was fastest in the presence of NH_4^+ alone and slowest in the presence of NO_3^- alone (Figure 12). The rates for net H^+ efflux under NH_4^+ nutrition (Figure 12) and for net NH_4^+ influx (Figure 11) were similar, supporting the fact that NH_4^+ assimilation entails the excretion of an H^+. Net H^+ efflux under NO_3^- nutrition suggests that NO_3^- influx involves the efflux of one to two H^+ and that NO_3^- assimilation entails the influx of at least one H^+ (Glass 1988; Garnett and others 2001). For all treatments, net H^+ efflux was slowest at the very apex and increased in the zone of elongation 2–10 mm from the apex (Figure 12). In fact, the surface of the elongation zone was consistently 0.4–0.8 pH units more acidic than the surrounding medium regardless of nitrogen source (Figure 13). This suggests that roots maintain an acid environment around the elongation zone in spite of nitrogen treatments that differentially alter H^+ extrusion

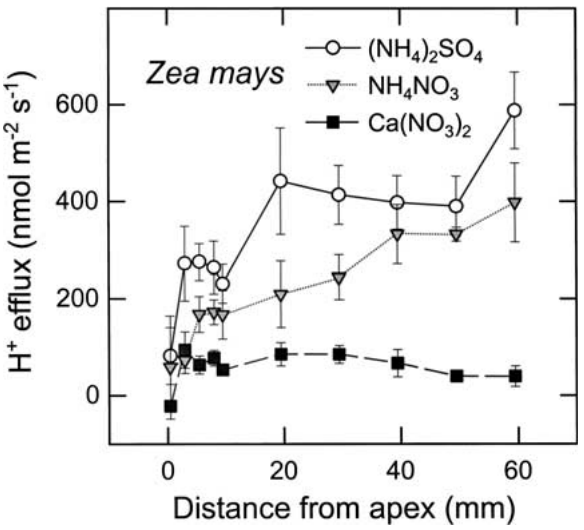

Figure 12. Net proton efflux at various distances from the apex of a maize seminal root when the medium contains NH_4^+, NO_3^-, or both forms of nitrogen (Taylor and Bloom 1998).

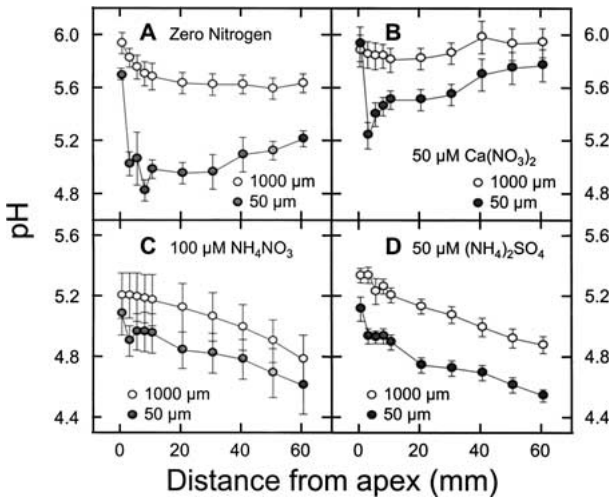

Figure 13. The pH at 50 or 1000 Mm from the root surface at various distances from the apex of a maize seminal root. The root was bathed in a medium that lacked nitrogen or contained NH_4^+, NO_3^-, or both forms of nitrogen (Taylor and Bloom 1998).

from the root. In comparison to the NH_4^+ treatments, the surface of the root was more alkaline when NO_3^- was the sole nitrogen source (Figure 13) so that the more limited proton pumping under NO_3^- (Figure 12) still produced a similar decline in pH (Figure 13).

Shifting the nutrient solution that bathed a root in an *in planta* root extensiometer (Figure 3) from pH 6.5 to 5.6 increased root elasticity by 50% (data not shown). The rate of root extension, however, declined from 1.67 ± 0.08 to 1.51 ± 0.06 mm h^{-1} (mean ± SE, $n = 6$). Peters and Felle (1999) found that lowering the pH of the medium stimulated root elongation only in slowly growing maize plants. We must conclude, as did these authors, that cell wall pH may contribute to the control of root growth but that other factors can override its influence.

Redox Potential and Root Development

The redox potential of the rhizosphere may reflect not only which nitrogen compounds are present and in what amounts, but how they were generated. As nitrogen cycles through various inorganic and organic compounds in the soil, its oxidation number ranges from −3 to +5. NH_4^+ is a moderate reducing agent (E_h = −0.35 V), whereas NO_3^- is a strong oxidizing agent (E_h = +0.74 V). Conversion of NO_3^- to N_2 may generate highly reactive oxidizing agents such as O_3 or O_2^- (Paul and Clark 1996). In addition, ammonification (the process through which soil microbes convert organic nitrogen into NH_4^+) occurs under both reducing and oxidizing conditions, whereas oxidizing conditions favor nitrification (microbial conversion of NH_4^+ to NO_3^-) and reducing conditions favor denitrification (microbial conversion of NO_3^- to NO, N_2O, or N_2). The net results are that soil NO_3^- is likely to accumulate only when the soil redox potential is high and that the relative availability of NH_4^+ should increase as the soil redox potential declines with the maximum absolute availability at moderate redox potentials.

Root activity alters rhizosphere redox potential through respiratory oxygen consumption and ion uptake or exudation. In particular, root absorption and assimilation of NH_4^+ and NO_3^- consume 0.31 mol O_2 mol^{-1} NH_4^+ and 1.5 mol O_2 mol^{-1} NO_3^-, respectively (Bloom and others 1992). Thus, when roots use NO_3^- as a nitrogen source, the rhizosphere redox potential declines more rapidly than when they use NH_4^+.

Redox potential influences a wide range of cell functions including the detoxification of free radicals, the activity of many enzymes, and the expression of many genes (Lüthje and others 1997; Bérczi and Møller 2000; Pfannschmidt and others 2001). To regulate internal redox potential, plant cells use both a complex suite of cytosolic redox buffers such as glutathione or ascorbic acid (May and Leaver 1993; Noctor and others 2000) and several plasma membrane oxidoreductases that transfer electrons from cytosolic donors to extracellular acceptors in the apoplast. These oxidore-

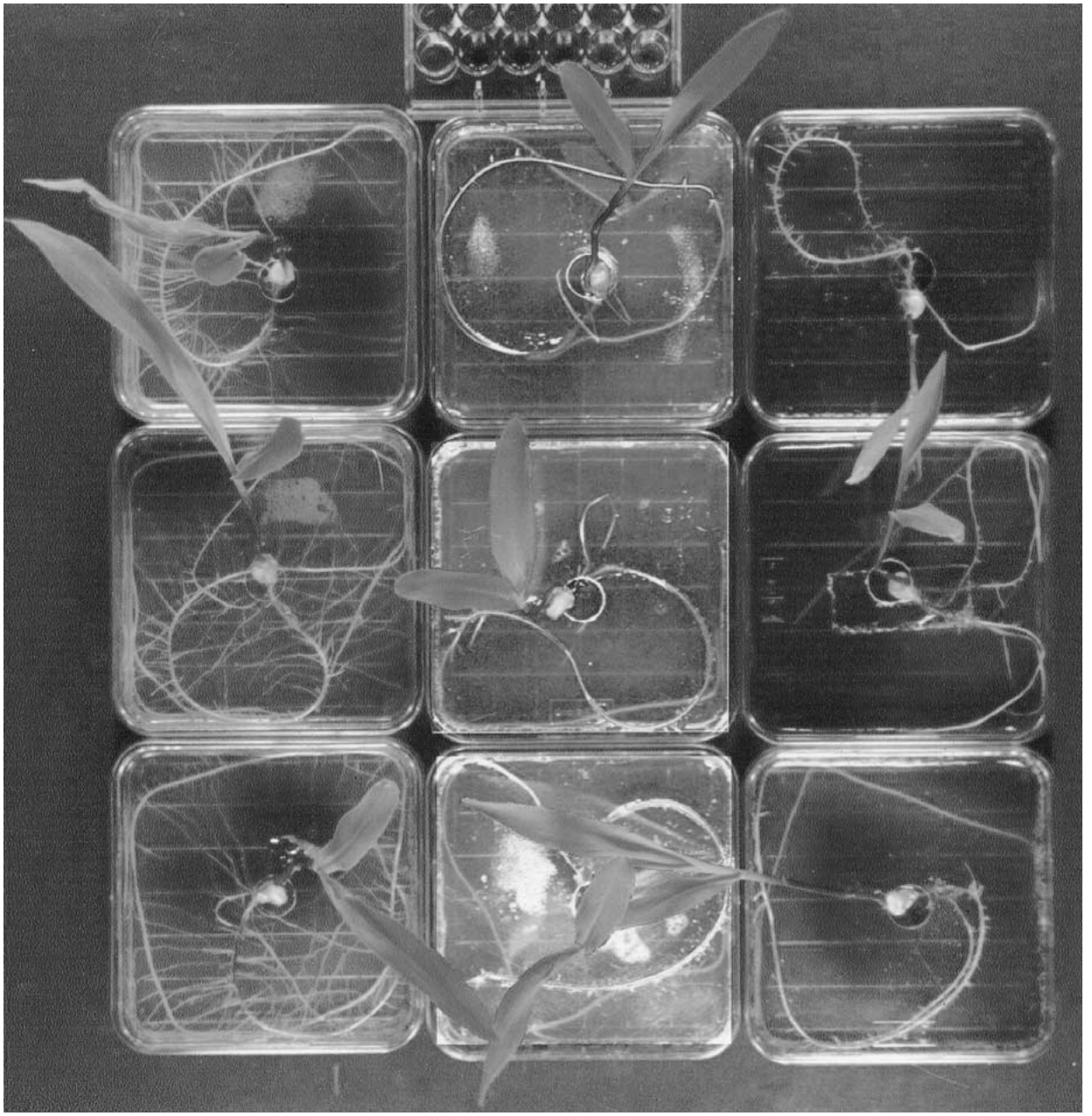

Figure 14. Maize growing in 0.8% agar that contains 1 mM $CaSO_4$, 1 MM KH_2PO_4, and either a pH indicator dye (0.12% Bromocresol purple, left column), a redox indicator dye [800 MM $K_3Fe(CN)_6$ with 1 mM $FeCl_3$, middle column], or both dyes (right column). The rows contain replicates. Usually, the plates were covered with aluminum foil. Yellowing of the pH dye and darkening of the redox dye indicate H^+ extrusion and root reduction of the rhizosphere, respectively. The shoots grew normally under all treatments, but the presence of the redox indicator dye inhibited lateral root development (A.R. Taylor and A.J. Bloom, unpublished).

ductases are involved with signal transduction of light (Gautier and others 1992, but see Taylor and Assmann 2001; Mullineaux and Karpinski 2002), membrane polarization and H^+ excretion (Marré and others 1988), generation of oxidative bursts in response to wounding and pathogen attack (Auh and Murphy 1995), hormonal regulation of cell growth (Böttger and Hilgendorf 1988), and modification of cell wall structure (Bradley and others 1992).

In plant roots, plasma membrane oxidoreductases have been detected and isolated based on their ability to reduce artificial impermiant electron acceptors such as ferricyanide (HCF). All

plant roots studied so far have a constitutive NADH oxidase or 'standard oxidoreductase system' (Crane and others 1991). This system can reduce external electron acceptors of high redox potential and may use O_2 as the terminal electron acceptor. In dicots and nongraminaceous plants, iron stress induces a 'turbo' ferric reductase system, but it remains unclear whether the turbo system is distinct from or reflects enhanced expression/activity of the standard oxidoreductase system (Beinfait 1988).

Plasma membrane redox enzymes may regulate cell elongation via structural changes in the cell wall. Low extracellular concentrations (MM) of artificial electron acceptors, such as ascorbate free radical or HCF, stimulate the standard oxidoreductase, H^+ secretion, and root growth, presumably through an acid growth mechanism and enhanced nutrient absorption (Crane and others 1991; Gonzáles–Reyes and others 1995). Higher levels (mM), however, inhibited the growth of *Lepidium* roots (Crane and others 1991) and the development of maize lateral roots (Figure 14). This indicates that regulation of cell wall loosening by pH and redox activity is complex. Cell wall stiffening occurs as part of plant pathogen defenses (hypersensitive response) when membrane-associated peroxidases generate H_2O_2 that crosslinks cell wall proteins (Bradley and others 1992). Redox reactions also diminish cell plasticity in the elongation zone of coleoptiles (Bradley and others 1992).

Redox activity influences root elongation also through cell proliferation. Cell division in *Arabidopsis* root apical meristems depended upon the redox potential of the cytosol (Sánchez–Fernández and others 1997). Apical initials had high cellular levels of the reductant glutathione (GSH), whereas the quiescent zone had low levels. Artificially increasing GSH pools or exogenously applying GSH or ascorbic acid stimulated cell division and growth. In maize, root apical meristems showed a similar pattern; actively dividing meristematic cells had higher levels of the cytosolic reductant ascorbic acid than cells in the quiescent zone (Kerk and Feldman 1995).

We propose the following scenario to integrate these phenomena: The redox potential of the rhizosphere influences the relative availability of NH_4^+ and NO_3^- (moderate potentials favor NH_4^+) and the redox potential of the roots. Root redox potentials and membrane-associated redox activities regulate cell proliferation and extension and, thus, root growth (moderate potentials favor growth). The nitrogen demands of root growth stimulate NH_4^+ or NO_3^- assimilation that, in turn, influences redox potentials (NO_3^- rapidly lowers potentials). The interplay between NH_4^+ and NO_3^- and rhizosphere redox potential may be partially responsible for the observed large fluctuations in the relative availability of soil NH_4^+ and NO_3^- and in root growth (Jackson and Bloom 1990).

Summary

Although NH_4^+ and NO_3^- absorption and assimilation by plants are only some of the many processes that influence rhizosphere pH and redox potential or that regulate root growth and development, root nitrogen acquisition is a primary determinant of plant productivity and, as such, should be a central process to integrate the responses of roots to their environment. The following factors influence the mechanisms by which roots respond to NH_4^+ and NO_3^-:

1. Growth at the root apex may be nitrogen limited. In maize seedlings with ample nitrogen reserves, extension of the root apex increased in solutions containing NH_4^+or NO_3^-. The apex differs from mature root zones in that it lacks fully differentiated phloem tissue and, thus, cannot rapidly import nitrogen from other tissues. This suggests that the nitrogen required for cell division and expansion in the apex derives primarily from its own absorption of rhizosphere NH_4^+ and NO_3^-.
2. Cell division in the root apical meristem may be more rapid under NH_4^+ nutrition. Root density and extension of maize seedlings were greater in nutrient solutions containing NH_4^+ than in those containing NO_3^- as the sole nitrogen source. Perhaps in the carbohydrate-limited apical meristem, the lower energy requirement for NH_4^+ assimilation versus NO_3^- assimilation permits dividing cells to maintain energy reserves above a critical threshold.
3. Root nitrogen acquisition alters rhizosphere pH and redox potential, which in turn may regulate root cell proliferation and mechanical properties. Assimilation of NH_4^+ releases protons, whereas that of NO_3^- produces hydroxide ions; plants supplied with NH_4^+ strongly acidify and those supplied with NO_3^- slightly alkalinize the rhizosphere. An acid pH in the cell wall matrix increases its elasticity. Moreover, NO_3^- assimilation depletes reducing equivalents more than NH_4^+ assimilation. This may shift cellular redox potential to a level that diminishes cell proliferation.

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