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HOW ROOTS CONTROL THE FLUX OF CARBON TO THE RHIZOSPHERE

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Abstract. What determines the way in which roots provide carbon to and interact with other components of the soil? Roots lose metabolites and signal molecules to the soil at rates of significance to soil organisms, and we need to know if the mechanisms of passive diffusion identified in hydroponics apply in soil, and whether other, active mechanisms complement them. New insights from biosensors into the heterogeneity and localization of exudation are transforming our understanding of root–microorganism relations. We need to know more about compounds that are exuded at subnutritional rates in soil and may act as signal molecules modifying the biology of soil organisms. Insights into one suite of such compounds is coming from studies of border cells. These cells are lost from the root cap at a rate regulated by the root and secrete compounds that alter the environment of and gene expression in soil microorganisms and fauna. The amount of root places an upper limit on the effect roots can have; carbon flow to the rhizosphere is a function of root growth. Top-down metabolic control analysis shows that the control over the rate at which roots grow is shared between root and shoot, with most control being in the shoot.

Key words: biosensors; carbon flux; control analysis; exudation; rhizosphere; root border cells; root cap.

INTRODUCTION

Roots acquire nutrients and so deplete N and P locally; continued growth places new root in regions of soil richer in nutrient. Since roots are leaky, they also locally and continually enrich the soil with their exudates, which are rich in C and N. The rhizosphere is therefore nutritionally distinctive and is defined at least in part by the nature and amount of exudates from the root. The rhizosphere also affords the opportunity for both root and microbes to produce and release signal molecules that alter the physiology of adjacent organisms.

We have a huge challenge to understand the fluxes of C from the root, which place an upper limit on the growth of rhizosphere organisms, the flow and functioning (at the level of gene expression) of signal molecules, and the spatial variability of the root–rhizosphere system. This brief review summarizes current knowledge, showing how the root cap is spatially and functionally special, how the root apex is responsible for the bulk of carbon loss, what compounds are lost from the root and in what quantity, and where root growth itself is controlled. The evidence we present supports the view that the flux of carbon from root to rhizosphere is a key controller, both as a limiting substrate for growth and as a source of signal molecules.

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The root cap: a guiding principle

Roots grow by the activity of apical meristems, which also form a root cap distally. The root cap is a dynamic, specialized organ that facilitates root penetration of soil, senses threats as well as bounty, and responds by transmitting signals that alter growth patterns. Rhizosphere development is initiated through the controlled delivery of exudates from the apex of the root as it penetrates the soil. In hydroponic culture, 98% of the total extracellular material is derived from the root apex (Griffin et al. 1976). A large proportion of this material constitutes a complex ''tissue'' comprised primarily of detached root ''border'' cells encased within mucilage (Fig. 1) (Rougier 1981, McCully and Boyer 1997, Hawes et al. 1998). The border cell–mucilage capsule ensheaths the root tip, modulates the chemistry of the extracellular environment, and may define the ecology of the incipient rhizosphere as it forms (Hawes et al. 2000). Particular cell-wall degrading enzymes are needed for border cell separation and release of the capsule. Using antisense mRNA to inhibit the genes that encode these enzymes produces plants with pleiotropic changes in root structure and function (Wen et al. 1999).

The border cell–mucilage capsule is synthesized by the root cap. New cells generated in the cap meristem differentiate progressively through a series of distinctive morphological stages correlated with specialized functions, including synthesis of the starch grains which participate in gravity sensing (Moore and McClelen 1983, Feldman 1984). Ultimately, the starch

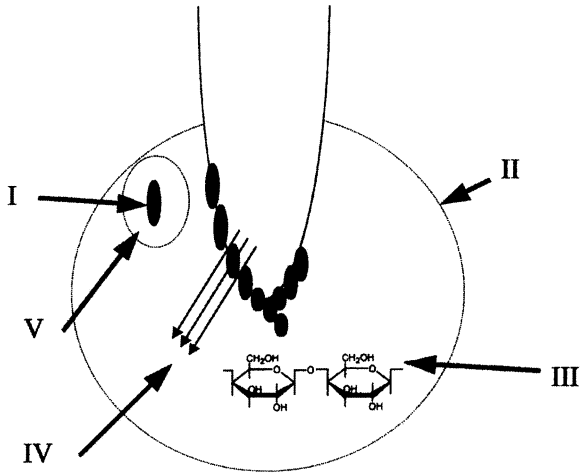

FIG. 1. Root tip capsule components: I, border cell populations; II, a high-molecular-weight mucilage secreted by the root cap; III, cell-wall breakdown products resulting from separation of thousands of border cells from each other and the root cap; IV, other extracellular products secreted by the cap; V, other extracellular products secreted by the border cells.

is degraded and a high-molecular-weight polysaccharide mucilage is synthesized and exported to form a water-soluble capsule surrounding the cap (Rougier 1981). As cells of the cap reach the periphery, they separate as a population of uniquely differentiated, metabolically active, border cells encased within the mucilage, forming a living interface with the soil (Hawes et al. 1998).

Detachment of the border cell–mucilage capsule from the root cap facilitates passage of the root through soil, but little is known about the dynamics of the process (Bengough and McKenzie 1997). In the laboratory, a given cell generated in the cap meristem can traverse the entire cap and separate as a detached border cell within one day (Feldman 1984). In the absence of free water, the border cell–mucilage capsule remains tightly pressed to the root periphery, and mitosis and turnover of the cap cease. Existing border cells can remain at the cap periphery for days as root growth proceeds. Cells within the cap, such as gravity-sensing statocytes, remain static in a differentiated state. This inhibition of cap turnover occurs at least in part because border cells produce an extracellular signal that represses mitosis in the cap meristem when it accumulates in the mucilage to a threshold concentration (Brigham et al. 1998). While in this state, with an ever-aging border cell–mucilage capsule on its periphery, the root cap continues to function as a sensory organ to facilitate root growth responses to gravity and other signals but stops undergoing progressive differentiation and turnover. Upon immersion in water, the entire capsule detaches in less than a minute as the mucilage absorbs water and swells away from the root. Mitosis in the root cap meristem is induced, together with a global switch in gene expression throughout the cap (Brigham et al. 1998). Within one hour, several hundred new border cells and a proportional amount of cap-secreted mucilage are released at the cap periphery. Within 24 h, a new border cell–mucilage capsule is present, and differentiation within the cap again ceases. Synchronous cap development makes it possible to characterize numerous genes required for production of the border cell–mucilage capsule (Hawes et al. 2000).

As they separate from the cap periphery along with the extruded mucilage, border cells undergo changes in morphology and gene expression, which may play a role in regulating microbial community structure in the rhizosphere (Zhang et al. 1995, Hawes et al. 1998). When roots are pulled from soil, the root apex (~2 mm) is generally sterile. Components of the border cell–mucilage capsule can adsorb toxic metals like aluminum, and act as signals that specifically attract, repel, and control growth and gene expression in soil-borne organisms (Hawes et al. 1998, 2000, Miyasaka and Hawes 2001). The result is that nearly all pathogens and symbionts initiate relationships not at the tip where damage to the root meristem can halt further growth, but in the region of elongation.

Directional movement in roots

When and where rhizosphere development is initiated by delivery of exudates from the cap is a function of root growth. New cells are normally synthesized within the root meristem more or less continuously and proceed through a transition phase correlated with dramatic changes in gene expression, cell wall structure, shape, size, and organization (Baluska et al. 1996). Uptake of solutes into the vacuole results in a 10-fold increase in cell volume over 13 h following cell division. A given root tip can be found several centimeters past its point of origin from one day to the next. When the root meristem is removed, or damaged irreversibly, growth of that root stops.

Roots seldom grow in a straight line because they respond to external stimuli by rapidly changing the direction of growth. Directional changes in growth occur in the elongation zone, when cells on one side of the root elongate more rapidly than cells on the other. Bending in response to gravity is the most intensively studied model for directional root growth (Konings 1995, Barlow and Parker 1996, Chen et al. 1999), but roots also can change direction in response to water and nutrients, temperature, heavy metals, light, soil composition and texture, carbon dioxide and oxygen, electrical gradients, fungi and bacteria, and touch (Curl and Truelove 1986, Kochian 1995). The plant can respond in a hierarchical fashion to multiple stimuli; for example, a root growing on agar grows straight into the air if the agar contains a toxic level of aluminum (Hawes et al. 2000, Miyasaka and Hawes 2001).

The control center

The primary site of signal perception leading to directional movement is the root cap (Feldman 1984, Baluska et al. 1996, Sievers and Braun 1996). Removal of the root cap eliminates tropic responses even when the root meristem and region of elongation remain intact, although the root can still grow. The root meristem is programmed to synthesize a new cap when the existing one is excised, and if that fails, a proliferation of lateral and branch roots ensues (Tsugeki and Fedoroff 1999). Inhibiting specific genes needed for different stages of cap development with mRNA antisense technology slows root growth (Hawes et al. 1998, 2000) and genetic elimination of cells within the cap abolishes gravitropism, severely retards root growth, and completely alters patterns of root development (Tsugeki and Fedoroff 1999).

The root cap traditionally has been viewed as a mucilage-covered barrier functioning to protect the meristem physically and to lubricate its passage through the soil. In fact, the root cap is a multifunctional molecular relay station that detects, integrates, and transmits information about the environment to appropriate plant organs, and modulates properties of the soil habitat in advance of the growing root. It functions as a key, critically located, control point in rhizosphere development and function. But the rest of the root inherits that rhizosphere. Thus, defining the uniformity of nutrients within it is one key to an ecological understanding.

Using Microbial Biosensors to Define Nutrient Leakage from Roots

Soil, roots, and the rhizosphere are all heterogeneous. The composition of the rhizosphere is defined at least in part by the nature and amount of exudates from the root. As the root supports a temporally and spatially heterogeneous microbial community, it would be surprising to find a uniform pattern of nutrient leakage over the root surface. It would be satisfying to measure and model the contributions of local factors affecting root exudation. To achieve that goal, we need methods that assess root exudation over small spatial scales in realistic habitats such as soil.

Information is needed on the spatial and temporal pattern of exudation of sugars and amino acids from roots and the effect of these exudates on numbers of bacteria present in the rhizosphere. Sugars are important exudates because they are found in large quantity (Juma and McGill 1986) and are a labile source of C for microorganisms. Amino acids are also a large component of exudates (Juma and McGill 1986). As they provide both C and N to microorganisms, the proportion of sugars to amino acids largely determines the relative amounts of C and N available for microbial growth.

Individual soil particles may have important effects on localized exudation. Growing plants in a solid matrix, such as sand, soil, or simulated soil, from which exudates can be leached and chemically analyzed, offers an integrated view of how external particles affect root exudation, but does not provide sufficient spatial detail to evaluate the fine scale root–microbe interactions that may accelerate N cycling or other important processes in the rhizosphere. An alternative approach utilizes $^{14}CO_2$ labeling of plants in microcosms in natural soil to detect exudation from roots. This method can provide information on the rate of C flow through the plant into the soil as well as the quantity of C flow into the soil (Norton et al. 1990). When combined with autoradiography, general patterns of exudation can be observed. Such patterns, however, will be partially obscured by diffusion of $^{14}CO_2$ from both root respiration and microbial metabolism of root exudates, and this method does not define the composition of exudates.

We now have molecular tools to study the spatial patterns of nutrient exudation on roots and other plant parts. Bacterial cells harboring environmentally responsive promoters that are fused to appropriate reporter genes are introduced into the rhizosphere, and the resulting reporter gene product is measured as an indication that the cells have detected the appropriate effector molecules (Jaeger et al. 1999). These workers used a promoterless ice nucleation gene, *inaZ,* from the bacterium *Pseudomonas syringae* as a reporter gene. When *inaZ* is expressed, proteins with high ice nucleation activity are produced and incorporated into the outer membrane of bacteria, enabling the cells to catalyze ice formation. Bacterial ice nuclei are easily quantified by a simple droplet-freezing assay (Loper and Lindow 1994, Lindow 1995). Promoters from genes involved in sucrose catabolism in *Salmonella* as well as tryptophan-induced IAA production in *Erwinia herbicola* were fused to this reporter gene to yield sucrose or tryptophan-dependent ice nucleation activity in an *E. herbicola* host strain that could be inoculated onto roots to detect the availability of sucrose and tryptophan exudates in soil. Both biosensors exhibited up to 100-fold differences in ice nucleation activity in response to varying substrate abundance in culture. The biosensors were introduced into the rhizosphere of the annual grass *Avena barbata* and, as a control, into bulk soil. In the bulk soil, neither strain exhibited significant ice nucleation activity, but in the rhizosphere both tryptophan and sucrose were detected, and they showed different spatial patterns. Tryptophan was most abundant in soil around roots 12–16 cm from the tip, while sucrose was most abundant in soil near the root tip. The largest numbers of bacteria occurred near root sections with the highest apparent sucrose or tryptophan exudation. High sucrose availability at the root tip is consistent with the hypothesized movement of sucrose from the protophloem in the zone of root elongation to the meristem by apoplastic diffusion (Bret-Harte and

Silk 1994), and it is where recently assimilated C is localized (Farrar et al. 1995). *A. barbata* clearly supports different patterns of availability of exudate compounds along a root. A portion of the apoplastic sucrose may diffuse out of the root along the concentration gradient between the inside and the outside of the root. Sucrose does not leak from mature sections of the root, where the phloem is fully developed and diffusion between the root stele and the exterior is blocked by a suberized layer of endodermal cells. Tryptophan loss from older root sections may result from lateral root perforation of the root epidermis. Microbial biosensors thus allow the evaluation of exudation from roots growing through abrasive soil particles and native microbial populations, while factors such as soil moisture, soil nutrient availability, and shoot environmental factors are manipulated.

Other inducible-promoter reporter systems also have been used to evaluate the environment of rhizosphere bacteria. De Weger et al. (1994) used *lacZ* to evaluate phosphate limitation in rhizosphere bacteria, while van Overbeek and van Elsas (1995) used *lacZ* in *Pseudomonas fluorescens* to detect proline in root exudates. In both cases, the best results were achieved in nonsoil media. Using *lacZ* reporter genes for such studies can be complicated by the high background levels of β-galactosidase activity in soils, so the *inaZ* gene is better suited to yield bacterial population-level quantitative estimates of nutrient levels in soils. It can be easily measured in ''dirty'' biological systems and is also more sensitive than other genes, such as *lacZ, lux, or gus* (Lindow 1995, Miller et al. 2001). Similar genetic sensor systems in *P. fluorescens* have been used to map Fe^{+3} availability around roots in soil in microcosms (Marschner and Crowley 1997).

As bacterial and fungal colonization of the rhizosphere, and resources such as sucrose and tryptophan, are not evenly distributed, additional small-scale information on nutrient availability will help explain the processes of exudation and exploitation by rhizosphere microbes. Bacteria are more likely to be found clustered in crevices between epidermal cells, near emerging root hairs (Newman and Bowen 1974, Rovira and Campbell 1974). The factors that influence the availability of nutrients within or beyond these preferred sites remain elusive. Also, there is no information on nutrient availability at the scale most relevant to a root-colonizing bacterium, i.e., multiples of its own dimensions. It seems unlikely that nutrient abundance as averaged over even a small segment of root is of any relevance to an individual bacterial colonizer. For this reason, whole-cell biosensors that report nutrient concentration at the scale of individual cells have recently been described (Leveau and Lindow 2001). These tools were developed for analyzing mineral nutrients on leaves, but they should be equally valuable when they are transferred to bacterial species commonly found in rhizospheres.

A strategy for small-scale estimates of sugar availability on plants involved the use of reporter gene technology in which the plant colonist *E. herbicola* 299R (*Eh*299R) harbored a sugar-responsive element that was fused to a gene for green fluorescent protein (GFP). The GFP content of single cells thus becomes a measure for local sugar availability and can be readily determined by fluorescence microscopy. The promoter region of the *fruBKA* operon from *Escherichia coli* (Reizer et al. 1994) drove expression of GFP. This gene cluster codes for the metabolism of fructose, and its expression is controlled by the catabolite repressor/activator or Cra protein (FruR; Saier and Ramsier 1996) in response to fructose 1-phosphate, the first intermediate in the fructose metabolic pathway (Ramsier 1996). Because *E. coli* and *E. herbicola* are closely related bacteria, Leveau and Lindow (2001) anticipated that the *fruB* promoter would function properly in *Eh*299R. *Eh*299R can metabolize sugars such as fructose, so that its ability to report the presence of fructose is a function of both local fructose abundance and fructose consumption. Instead of the original GFP from *Aequorea victoria* (Tsien 1998), they used variant GFP[AAV] (Andersen et al. 1998) which matures faster, yields a brighter fluorescence, and has a reduced stability, which allows for real-time monitoring of gene expression (Sternberg et al. 1999, Ramos et al. 2000). Analysis of individual *Eh*299R fructose bioreporter cells showed that there was substantial heterogeneity in the availability of fructose (Leveau and Lindow 2001). Clearly, the *Eh*299R (pP_{fruB} − *gfp*[AAV]) fructose bioreporter system offers tremendous potential for the study of microbe–microbe and host–microbe interactions in the rhizosphere, awarding us with an exciting opportunity to view such interactions from the unique perspective of the microbes themselves.

Current biosensors detect only a fraction of the microbially relevant components of root exudation—a few sugars, metals, amino acids, and galacturonides (Loper and Lindow 1994, Joyner and Lindow 2000, Bringhurst et al. 2001); others that detect important mineral nutrients such as NH_4^+ or NO_3^- are under development (S. Lindow and M. Firestone, *personal observations*). Additional biosensors could be easily developed for other components of root exudates. Thus biosensors, in conjunction with equivalent localization of metabolites and gene expression to the cell level in higher plants (Korolev et al. 1999, Koroleva 2001), will revolutionize our understanding of spatially complex processes in the rhizosphere.

Root Exudation

In addition to detailed spatial information, we need to know the spectrum of compounds lost from the root, the rate at which they are lost, and what controls that rate, since these compounds provide the driving force for rhizosphere development (Curl and Truelove 1986). Discussions elsewhere in this Special Feature argue

TABLE 1. Typical concentrations (μmol/L) of low-molecular-weight C-containing compounds in the root cytoplasm and soil solution.

Compound	Root cell cytoplasm	Soil solution	References
Sugars	5000	20	Jones and Darrah (1996)
Amino acids	10 000	0.1–10	Jones and Darrah (1994), Stevenson (1982)
Organic acids	5000	20	Jones (1998)

that signal molecules released by plants are also important for determining rhizosphere community structure. The release of C compounds from roots into the surrounding soil, exudation, is a ubiquitous phenomenon, but we know little about the genetic regulation of exudation. Here, we hypothesize about the fundamental nature of the exudation process and identify significant knowledge gaps.

Mechanisms of C loss from roots

The continual day and night release of C from the root into the soil occurs due to the inherent leakiness of root cell plasma membranes and the large concentration gradient between the root cytoplasm and the soil solution (Table 1). Diffusional loss of low-molecular-weight C compounds from the root is accelerated by continual removal of the exudates from the soil solution by the soil microbial community. In addition, high-molecular-weight constituents such as proteins appear in the rhizosphere primarily as products of border cell lysis or cortical cell damage associated with disease or lateral root formation. Thus the passive C flux will be dominated by low-molecular-weight solutes which are present in the root cytoplasm at high concentration. While membrane permeability, and hence exudation, could be controlled by altering the membrane lipid and protein composition, we hypothesize that in healthy roots membrane permeability is optimized and conserved among plant species making further regulation difficult without compromising other aspects of cellular function such as ion uptake. Under myriad biotic and abiotic plant stresses, however, membranes typically become compromised resulting in increased membrane permeability, diffusional C losses and presumably rhizosphere microbial growth (Ratnayake et al. 1978, Cakmak and Marschner 1988). Therefore the plant exerts little control over much of its C efflux. However, C efflux can be increased (1) to prevent toxic compounds, such as aluminum, from entering the root, (2) to remove potentially toxic chemicals from the root (e.g., lactic acid during root hypoxia), and (3) to enhance mobilization and uptake of limiting nutrients. In these three cases, it is likely that C efflux is still primarily passive diffusion with exudation enhanced by membrane transport proteins to increase membrane permeability.

Total C efflux vs. net C efflux

Carbon losses from roots are accompanied by C influx. Low-molecular-weight solutes are taken up actively by roots (Jones and Darrah, 1993, 1994, 1996) and so C uptake may reduce the amount of C available to the rhizosphere food web, with important implications for both ecologists and root physiologists. Indeed roots can control the levels of low-molecular-weight components accumulating in the rhizosphere by regulating either C efflux or C influx.

Net root exudation regulated by C influx

The efflux of uncharged or net-neutral C compounds such as sugars and amino acids as a result of the concentration gradient between the cytoplasm and apoplast/soil solution can be described by the equation

$$E = AP(C_i - C_o)$$

where E is efflux (μmol·cm^{-1} root·h^{-1}), A is root area (cm^2/cm root), P is the solute-specific membrane permeability coefficient (cm/h), C_i is cytoplasmic concentration (μmol/cm^3), and C_o is soil solution concentration (μmol/cm^3). Similarly, influx (I, μmol·cm^{-1} root·h^{-1}) can be described by Michaelis-Menten kinetics where

$$I = V_{max}C_o/(C_o + K_m)$$

and where V_{max} is the maximum uptake rate (μmol C·cm^{-1} root·h^{-1}) and K_m is the Michealis constant (μmol/cm^3). Therefore, net flux (J) across the root membrane is simply

$$J = E - I.$$

If we assume, for a maize root, a steady-state cytoplasmic glucose concentration of 40 μmol/cm^3, soil solution concentration of 0.01 μmol/cm^3, permeability coefficient of 1.15×10^{-4} cm/h, V_{max} of 0.02 μmol·cm^{-1}·h^{-1}, K_m of 0.8 μmol/cm^3, and A of 0.37 cm^2/cm root, net efflux is 0.35 nmol·cm^{-1} root·h^{-1} (Bresseleers et al. 1984, Xia and Saglio 1988, Jones and Darrah 1996), with efflux of 1.70 nmol·cm^{-1} root·h^{-1}, and influx of 1.35 nmol·cm^{-1} root·h^{-1} indicating a root recapture efficiency of 80%. Approximately 2% of the glucose in roots is lost into the soil each day, assuming the cytoplasm occupies 10% of the cell volume. We lack the data to calculate net exudation for other solutes in this way.

While it is likely that efflux cannot be down-regulated (except for physical barriers like the exodermis), the question remains as to whether influx can be regulated. Solute influx from the soil solution is mediated via relatively solute-specific plasma membrane H^+-

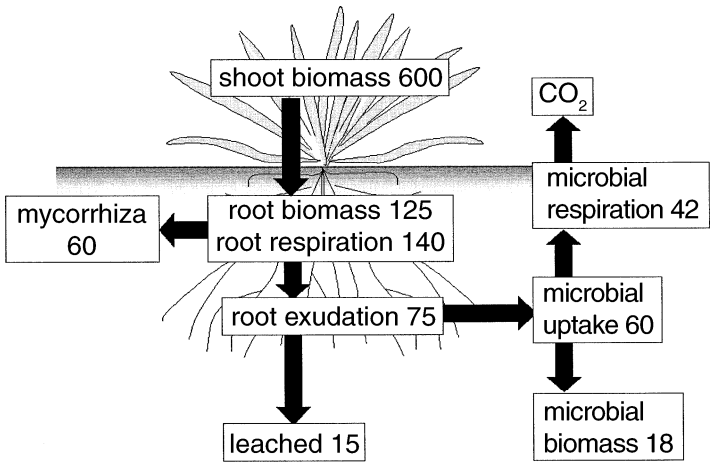

FIG. 2. The partitioning of C within the plant and the distribution of root exudates within the soil after the net fixation of 1000 C units (Martin 1977, Haller and Stolp 1985, Heulin et al. 1987, Whipps 1990).

ATPase driven, proton co-transporters (Fig. 2; Jones and Darrah 1993, 1994, 1996). The synthesis of these inwardly directed transport proteins is obviously under genetic control, but little is known about whether their in situ activity can be up- or down-regulated. Do these transporters play a central role in plant and rhizosphere ecology? Recapturing lost C probably increases plant C efficiency, and recovering exudates may benefit roots by lowering external C concentrations and thus reducing the growth of pathogens and other microorganism that compete for mineral nutrients.

Exudate recapture can occur along the whole root length and the kinetics of the transporters are similar to those for inorganic nutrients (Jones and Darrah 1994, 1996). In certain ecosystems, the direct uptake of organic solutes, particular amino acids, has been linked with plant fitness (Chapin et al. 1993). In N-limiting environments plants may satisfy their N requirement partly through the direct uptake of amino acid-N which is generated in large quantities by soil microorganisms during the breakdown of soil organic matter. The rate of amino acid influx may even exceed the rate of efflux although typically only unidirectional influx is measured rather than net influx, and comparative experiments with NO_3^- and NH_4^+ have not been performed under field conditions. As these influx and efflux processes will vary spatially along the length of the root, are their rates spatially coordinated? Further, food web organisms might alter influx and efflux of C from the roots through the production of substances that control plant membrane transporter activity and membrane potential. More research is required.

Net root exudation regulated by C efflux

The efflux of negatively charged solutes such as organic acids is directly regulated via the driving force for transport, the plasma membrane electrochemical potential gradient (Jones 1998). Due to the direction of the charge gradient across the membrane created by the plasmalemma H^+-ATPase (Fig. 2), influx of anions by passive diffusion is so small that net C loss is essentially unidirectional efflux. As the exterior face of the plasma membrane is positively charged, we hypothesize that solutes such as citrate (cit^{3-} at a cytosolic pH of 7.1–7.4) will be drawn out of the cell at a rate greater than that of noncharged solutes such as sugars. The flux across the membrane can be predicted using the net flux density equation (Nobel 1991) where the net flux (J) is described as

$$J = E - IA\left(\frac{PzE_mF}{RT}\right)\left(\frac{1}{e^{zE_mF/RT} - 1}\right)(C_o - C_i e^{zE_mF/RT})$$

using z as solute charge, E_m as the membrane potential, F as the Faraday constant, R as the gas constant, and T as temperature. We use the following parameters: a root area of 5.7×10^{-6} m^2/mg fresh mass root, P of 4.32×10^{-4} cm/h, E_m of -120 mV, F of 9.649×10^4 $J \cdot mol^{-1} \cdot V^{-1}$, R of 8.3143 $J \cdot mol^{-1} \cdot K^{-1}$, T of 293 K, C_o of 0.01 $\mu mol/cm^3$, C_i of 0.500 $\mu mol/cm^3$ and assuming a cytosolic pH of 7.2, net charge of malate (z) to be -2 (Lüttge and Smith 1984, Nobel 1991, Delhaize et al. 1993, Papernik and Kochian 1997). The net efflux of malate is then 0.6 $nmol \cdot cm^{-1}$ $root \cdot h^{-1}$, close to experimentally derived rates for wheat root tips that range from 0.25 $nmol \cdot cm^{-1}$ $root \cdot h^{-1}$ under normal conditions to 5.9 $nmol \cdot cm^{-1}$ $root \cdot h^{-1}$ in the presence of toxic levels of Al and when membrane permeability transport is enhanced through the opening of anion channel proteins (Ryan et al. 1995, 2001). The gating of the anion channel for the Al-associated malate efflux in wheat appears to be under the control of a single, unidentified genetic locus (Ryan et al. 2001). Channel-mediated malate release is almost instantaneous and thus cannot involve de novo synthesis of proteins. Genes controlling malate release (e.g., *Alt1*) may encode not only the organic acid transport proteins but also signaling elements necessary to trigger the release of malate and citrate. The signaling protein may be an Al^{3+} receptor located in the plasma membrane (Kochian 1995). A

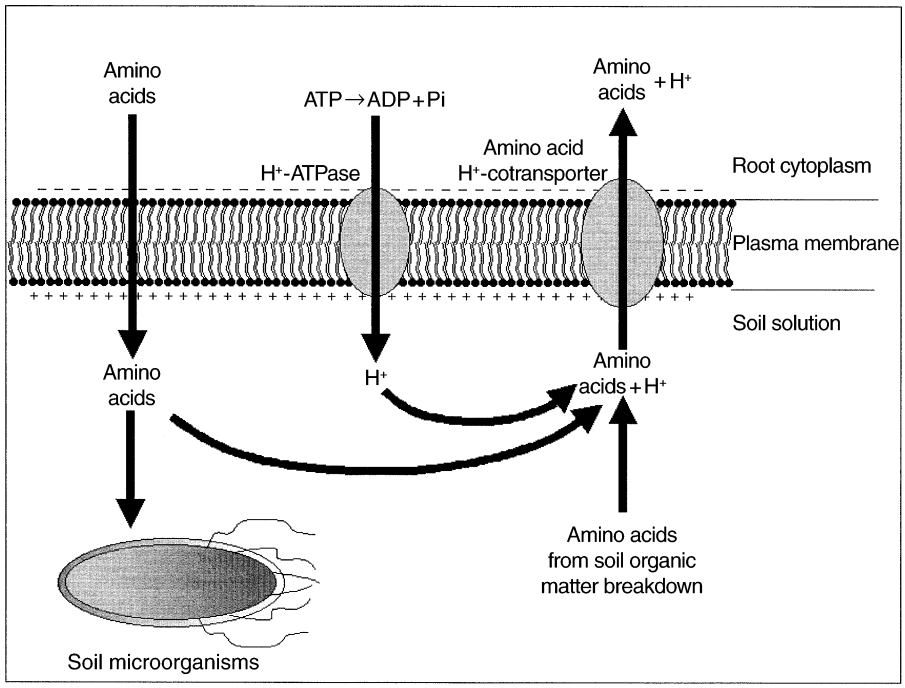

FIG. 3. Schematic representation of the efflux and influx of low-molecular-weight C compounds at the soil–root interface. Although amino acids are shown here, the process is also characteristic of most sugars.

similar channel-enhanced efflux of organic acids also may occur in some plants under P deficiency where citrate is released to enhance P mineral dissolution (Johnson et al. 1996).

How large is the root exudation C flux?

Although exudation can be predicted using simple transport equations, measurement of C loss from roots can also be determined by (1) $^{14}CO_2$ feeding of shoots of soil-grown plants followed by separation of ^{14}C fractions and (2) collecting specific exudate compounds in the culture medium of hydroponically grown plants. Although both techniques are fraught with problems (Jones and Darrah 1993, Meharg 1994), it is clear that large amounts of C are lost from roots. A summary of 95 whole-plant ^{14}C-labelling studies performed on a broad range of plant species indicates that ~5–10% of the net fixed C can be recovered in soil (Fig. 3).

In contrast, experiments performed in hydroponics demonstrate that typically only 0.5–1.5% of a plant's net fixed C is lost as rhizodeposition. The disparity may be due to the elimination of bidirectional C fluxes in hydroponic studies and the inclusion of dead root material and symbiont cells (e.g., mycorrhizas) in ^{14}C labeling studies. A conservative estimate is that the net fixed C lost by roots is within the range of 0.5 to 5%. The corollary is that 95–99.5% of the C entering soil from roots does so following root turnover and death—but not directly to the rhizosphere. We clearly need to combine our knowledge of total fluxes with spatial and temporal variation in those fluxes, and how these link to microbial growth and root physiology. Understanding the total flux to soil and its control demands a knowledge of root growth, which we discuss next.

CHARACTERIZING AND QUANTIFYING THE CONTROL OF CARBON FLUX

The total flow of C to the rhizosphere will depend on both how much each apex and each unit length of root release and also how much root there is. This section addresses three questions: what determines the amount of root growth? Where is the control over the C flux, which underlies root growth, exercised? Can we be objective and quantitative about identifying control points? An answer to these questions would greatly narrow the search for mechanisms and for the genes that are most important in control. We argue that control is shared or distributed—that is, part of the control over root growth is located in the root itself, and part in the shoot (Farrar and Jones 2000). We contrast shared control with hypotheses that suggest control either entirely by photosynthetic supply of assimilate or by demand from processes within or outside the root. By analogy, shared control will extend to fluxes of C, N, and P in the rhizosphere. It certainly occurs in the leaf (Gunn et al. 1999*b*), sinks in general (Farrar 1996), and in whole plants (Farrar 1999).

Control analysis

Direct evidence for shared control comes from applying the principles of metabolic control analysis (Fell 1997). Its central tenet is that in complex, multistep

TABLE 2. Top-down metabolic control analysis of C flux in single-rooted soybean leaves.

Parameter	Plantlet age (d)	
	10	12
Leaf/root dry mass	2.02	1.42
Shoot control coefficient	0.64	0.76
Root control coefficient	0.36	0.24

Notes: Control coefficients sum to 1 for this two-block system. One block consists of a source leaf, the ''shoot,'' which produces sucrose; the second, an adventitious root system, consumes the sucrose (S. Gunn, J. Pritchard, and J. F. Farrar, *personal observations*).

linear or branched systems, every step contributes to the control of flux, and so control is distributed throughout the system. Its purpose is to objectively measure the control attributable to each step.

For each reaction in a pathway, $C_x^J = (\delta \ln J)/(\delta \ln E_x)$, where C^J is a flux control coefficient, J the flux through step x, and E the activity of the machinery (typically an enzyme) which catalyzes step x (Fell 1997). The flux control coefficient is the dimensionless ratio of the proportional change in flux to the proportional change in amount of machinery that alters that flux. Further, for a linear pathway, $C_1^J + C_2^J + C_x^J = 1$, and the flux control coefficients sum to unity. In a branched system, flux-control coefficients can still be derived for each step, but they no longer sum to 1. When some steps in a pathway are not known, blocks of reactions can be treated by a variant called top-down metabolic control analysis.

We have applied top-down metabolic control analysis using a leaf blade of unchanging photosynthetic area supplying assimilate only to a root system growing at a constant rate (a single-source/single-sink system), by rooting a soybean leaf from the base of the lamina. Our data ascribe 25–50% of control to the root (Table 2). The amount of control in the root decreases as the root grows larger relative to the shoot.

Sweetlove et al. (1998), using transgenic potato with multiple sinks and ^{14}C to estimate flux, conclude that ~80% of control resides in source leaves and the remainder within sinks. The broad agreement between our results and theirs is probably fortuitous, because control coefficients can change rapidly. When we exposed single rooted leaves of cotton to 0.8 ppm ozone for 45 min, the ozone shifted control away from the root: C_C^J was 0.5 before, and 0.1 after, applying ozone (S. Gunn, D. Grantz, and J. Farrar, *personal observations*). We need to extend this type of analysis to field-grown plants, since the implications for ecosystem processes are considerable. The application of control analysis to rhizosphere fluxes would produce an objective and quantified analysis of flux-based control points. For example, we calculate that if exudation of sugars from roots and uptake of sugars by soil microorganisms both follow Michaelis-Menten kinetics, but the root sugar concentration exceeds the K_m for exudation while the soil sugar concentration is lower than the K_m for uptake, more control of this flux from root to microorganism will reside with exudation from the root than with microbial uptake.

The lessons of shared control are therefore (1) all steps in the pathway of carbon from photosynthesis to metabolism in the rhizosphere contribute to control of the overall flux, (2) observations to date suggest that the bulk of control will be associated with plant source leaves, and (3) within the root–rhizosphere system, exudation from the root may exert more flux control than utilization by soil microbes.

Plasticity of the shoot:root ratio

Shared control results in partitioning a fraction of new photoassimilate to roots, with the remainder staying in the shoot. The result is described by the ratio of shoot to root (S:R). The instantaneous value of S:R, together with plant size, will determine the amount of C available for roots and the rhizosphere food web. Remarkably, S:R ratio or leaf area:fine root length (Korner 1994, Farrar and Gunn 1998) is conserved both in controlled environments and in the field, the range of values typically being between 0.1 and 5 (Farrar and Gunn 1996), far smaller than the range of absolute plant size (Korner and Renhardt 1987, Larcher 1995). The relative constancy requires explanation. If the function of the root is overwhelmingly to acquire resources, then the plant should produce just enough root to acquire sufficient soil-based resources to balance carbon fixation by the shoot. Since the ratios of C:N and C:P in plant tissues are rather invariate, we would predict that natural selection has resulted in a conserved S:R, assuming the capacity of each unit of root to acquire N and P is similar.

Within the broad range of conserved values, S:R within and between species is plastic. Differences in S:R between species and environments may be partly due to differences in capacity for resource acquisition per unit of root and partly due to the plasticity of responses (Wilson 1989). Since atmospheric CO_2 and soil potassium have little effect on the S:R ratio (Wilson 1989, Gunn et al. 1999*a*), it probably is regulated by rather specific mechanisms (Brouwer 1983, Farrar and Gunn 1998). Regrowth occurs to suit the current environment. Thus, when partial shoot removal is combined with transfer to a low-N medium, plants regrow to a S:R typical of a plant maintained intact in low N (Fig. 4). Thus, the capacity of the soil to supply resources for plant growth will modify within-plant partitioning and in turn the ability of the plant to interact with soil biota.

The mechanism by which S:R is regulated must involve sensing resources. Resource status is sensed within the plant, not externally in the soil. Sucrose may play this role (Farrar 1996, Farrar et al. 2000, Smeekens 2000), but it cannot do so alone. At least one sensor of resources acquired by the root is also needed. Nitrate

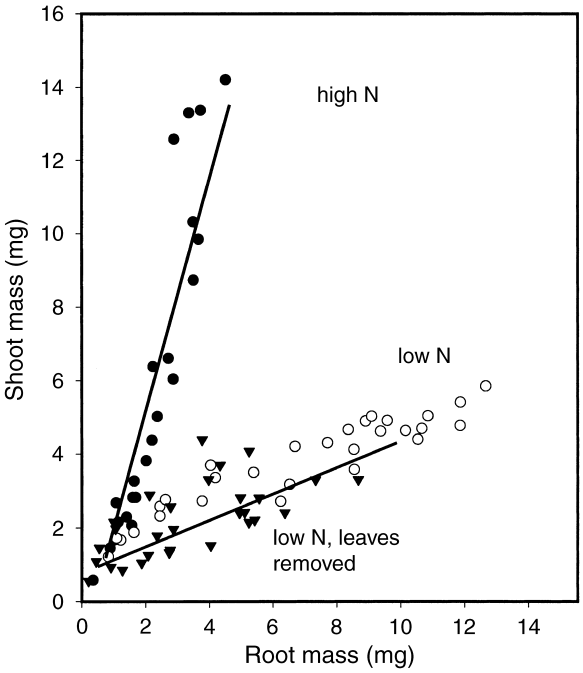

FIG. 4. Shoot–root relations in vegetative plants of barley grown hydroponically in high or low (2 or 0.02 mmol/L) nitrate, and with half of the leaf area of one set previously grown in high N removed at the same time as they were transferred to low N (J. F. Farrar and L. Thurlow, *personal observations*). Filled circles indicate intact plants with high N supply, open circles indicate intact plants with low N supply, and filled triangles indicate plants with reduced leaf area and low N supply.

can alter growth of roots, and it is sensed internally to modify expression of a MADS-box gene when altering root growth (Zhang and Forde 1998) and within shoots for the regulation of S:R (Scheible et al. 1997, Andrews et al. 2001). The status of P, another determinant of S:R and the architecture of the root (Wilson 1989, Ciereszko et al. 1999), is detected within young leaves (R. Solbe, C. Marshall, and J. F. Farrar, *personal observations*).

Is there such a thing as ''root demand''?

Root growth is thus controlled by each step regulating C flux in the whole plant, and processes within the root or outside it in the soil are only partly responsible for control of root growth. Therefore, no measurement can be made on a root or its environment that will predict how it will grow, so the concept of root demand has little meaning. Regulation of the genes controlling C flux is likely to be central to the mechanisms regulating the shared control of root growth (Farrar et al. 2000, Smeekens 2000). Our analysis makes it clear that, whatever the demand from the world outside of the root, this can never completely control the flux of C from root to soil; a rhizosphere partly limited by C is a real possibility.

CONCLUSIONS

The flux of C into and through plant roots to the rhizosphere is regulated in part by processes within the root and in part by processes such as photosynthesis in the shoot. Root exudation represents a small but significant C flux in plants involving a wide variety of compounds. It is a spatially and temporally complex process that is highly dependent upon the root's physiological status and the nature of its surrounding environment. It is a complex of multidirectional fluxes operating simultaneously. These fluxes can be highly localized; thus sucrose efflux occurs predominantly at the root apex. The apex, and in particular the cap, is critical in determining the direction of root growth and the development of new rhizosphere.

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