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Review article

# Associative Nitrogen Fixation and Root Exudation – What is Theoretically Possible in the Rhizosphere?

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**Abstract**

Root exudation is a key driver of many rhizosphere processes including nitrogen fixation by diazotrophic bacteria residing in the soil. We critically review our knowledge of rhizosphere carbon flow and determine the extent to which rhizodeposition could fuel associative $N_2$ fixation by soil microorganisms. We conclude that most estimates of rhizosphere C flow are fundamentally flawed due to the use of inappropriate methodology combined with a poor mechanistic understanding of root C flow. Using a mathematical model, we predicted that rhizodeposition could under optimal conditions support the fixation of between 0.2 to 4 kg N $ha^{-1}$ $year^{-1}$ which is in good agreement with experimentally derived values for natural ecosystems (0.05 to 5 kg N $ha^{-1}$ $y^{-1}$). Our model indicated that fixation

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was highly dependent upon the number of potential $N_2$ fixers in the rhizosphere relative to the total microbial population. If $N_2$ fixer populations could be enhanced, we predict that fixation rates may reach up to 20 kg N $ha^{-1}$ $y^{-1}$ given highly optimal conditions which again agree with experimentally derived results. We conclude that whilst the potential for rhizodeposition-driven $N_2$ fixation in the soil is small in comparison to inorganic and symbiotic-$N_2$ fixation inputs, it may be of importance in N-limited ecosystems.

Keywords: Carbon, model, nitrogen fixation, rhizosphere, root exudates

## 1. Introduction

The rhizosphere is the zone of soil that surrounds the root and which supports a microbial population which is functionally and structurally different from that in the bulk soil (Curl and Trueglove, 1986). The physical characteristics of this zone of soil are also different from those in the bulk soil due in part to the release of carbon compounds from the root. This release of C directly affects the physical nature of the soil through the root secretion of mucilage which binds soil particles together, and also indirectly through the stimulation of bacteria which release their own polysaccharide gels and fungi whose hyphae bind soil particles together (Traore et al., 2000). The rhizosphere also differs in its chemical properties in comparison to the bulk soil, mainly as a result of root and microbial nutrient uptake, soil pH changes associated with this nutrient uptake and the release of metal complexing agents (e.g. organic acids that stimulate P and Al dissolution; Hinsinger et al., 2001). The behaviour of root exudates in soil is complex as shown in Fig. 1. Conventionally, it is believed that the majority of the root exudate C is employed to support growth of the soil microbial biomass (Newman and Watson, 1977; Lynch, 1990; Tinker and Nye, 2000). We will argue that this view is too simple.

Of key importance to issues of rhizosphere ecology, ecosystem functioning and agricultural sustainability is the extent to which this exuded carbon benefits the root and provides a competitive advantage to the plant. For example $N_2$ fixing bacteria residing in the rhizosphere and the release of their N to the plant can be stimulated by root exudates (Rao et al., 1998). Other examples of the positive and negative benefits of root exudates are shown in Fig. 2. The interactions of plants and microorganisms in the rhizosphere ultimately depend on the quality and quantity of root exudates (Pinton et al., 2000; Farrar et al., 2003). To understand these interactions requires that we can accurately assess the sources and sinks of C in the rhizosphere and the C fluxes between these pools. The aim of this study is to summarize our current understanding of

rhizosphere C flow, establish the knowledge gaps that exist, and to provide a theoretical estimate of the amount of $N_2$ fixation by free living bacteria that can be supported by rhizodeposition.

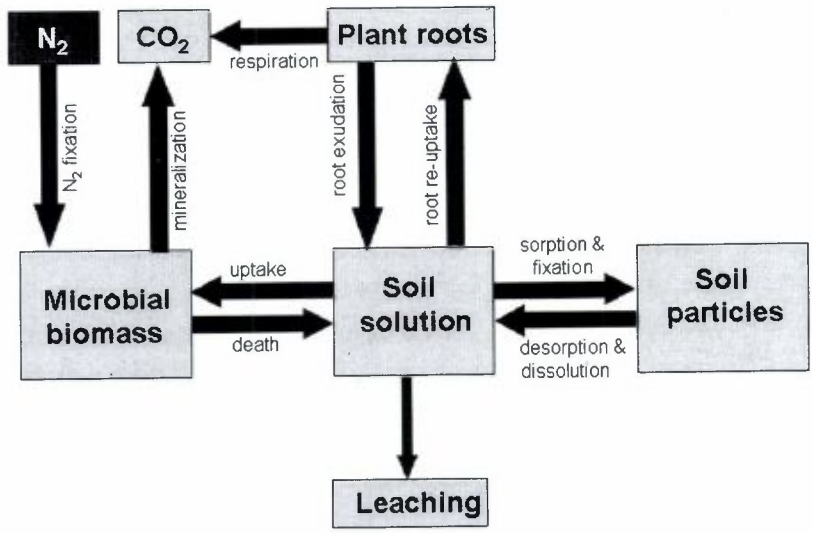


Figure 1. Schematic representation of the major carbon fluxes and pools in the rhizosphere.

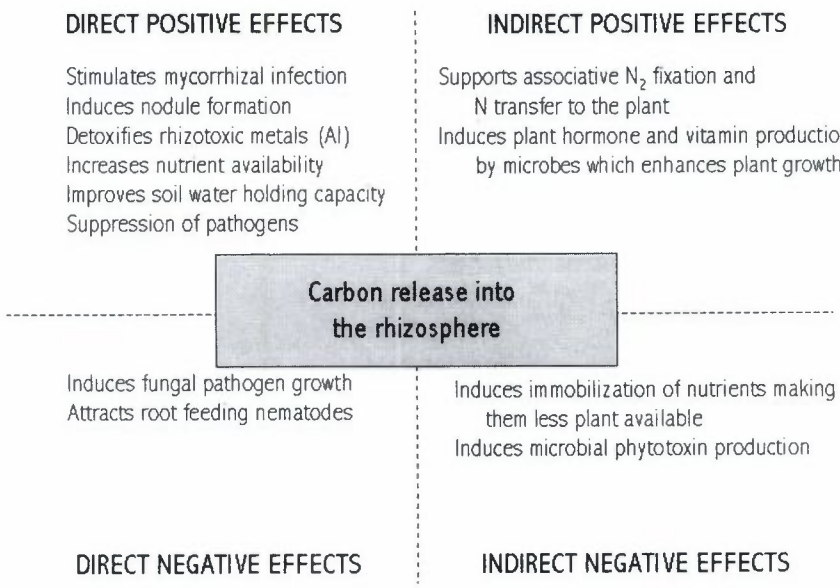


Figure 2. Schematic representation of the positive and negative direct and indirect effects of root exudates on plant growth.

## 2. Root Exudation: What Do We Know?

The loss of carbon from roots has been implicated as the key driver of the rhizosphere for over 50 years (Hale et al., 1971). During this period, many researchers have attempted to elucidate the factors regulating exudation and its impact on plant and soil interactions (Pinton et al., 2000). Unfortunately, as will be shown below, most of these studies are fundamentally flawed and their conclusions have to be treated with a great deal of caution. Since the pioneering work of Rovira, Vancura and others (Curl and Trueglove, 1986), our quantitative knowledge of rhizodeposition has increased little despite the publication of over one thousand articles on the subject. Early work clearly showed that root exudates contain a great variety of compounds differing in their abundance, but that the exudates were predominantly low molecular weight and reflected their dominance within the cytoplasm and cellular metabolism (e.g. sugars, amino acids, organic acids; Rovira, 1969). This early work also showed qualitatively that exudation was highly dependent upon the plant's physiological and developmental status and external environmental conditions (Rovira, 1959; Vancura, 1967). The lack of progress in the field of rhizosphere C flow is mainly due to (1) the use of inappropriate techniques, and (2) a lack of fundamental understanding of the processes leading to inappropriate analysis of the data collected. The main problem is how to experimentally quantify the rate of root exudation without unduly perturbing the system and making the measurements meaningless (Neumann and Römheld, 2000). Traditionally, this has been achieved by two approaches, the collection of exudates from plants grown in hydroponic culture, and by whole-plant $^{14}C$ labelling studies. The validity of these experimental approaches will be discussed later.

One key misconception is that almost all authors believe that root C flow in the rhizosphere is a unidirectional flux (Lynch, 1990). This is clearly not correct as the re-uptake of low molecular weight carbon by roots, and particularly sugars and amino acids, has been recognized as a significant transport pathway by plant physiologists for many decades (Komor, 1973; Soldal and Nissen, 1978; Sauer and Tanner, 1989). This knowledge has somehow managed to escape the attention of nearly all researchers working on rhizosphere C flow. Most of the pioneering work on sugar and amino acid uptake by roots was originally concerned with characterizing the mechanistic basis of membrane transporters and understanding the internal regulation and partitioning of plant C (Bush, 1999; Ortiz-Lopez et al., 2000). These studies, although conducted at high exogenous C concentrations, indicated that roots have a large capacity for taking up externally applied low molecular weight C. Subsequent work by Jones and Darrah (1996) showed that at concentrations similar to those in the rhizosphere, the processes of carbon exudation (efflux)

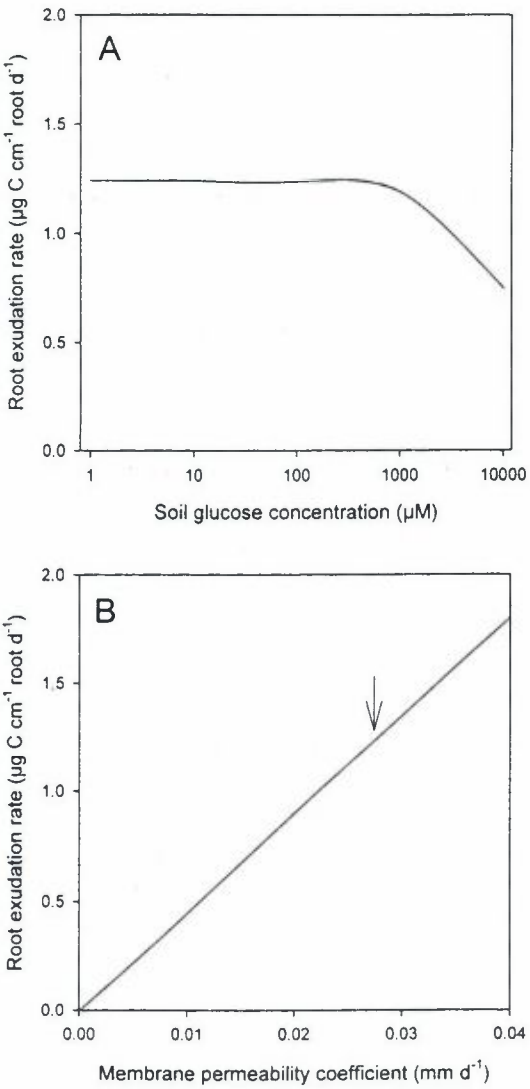


Figure 3. Calculated impact of (A) the soil glucose concentration on the rate of glucose exudation, and (B) changes in the plasma membrane permeability coefficient on the rate of glucose exudation. Note that the x-axis scale in Panel A is on a log scale. The arrow in panel B represents the predicted value for a maize root (Jones and Darrah, 1996). See text for details of the calculations.

and re-uptake (influx) were operating simultaneously within the same regions of the root. The insensitivity of amino acid and sugar efflux to metabolic inhibitors indicates that, at least in maize roots, exudation is a passive process

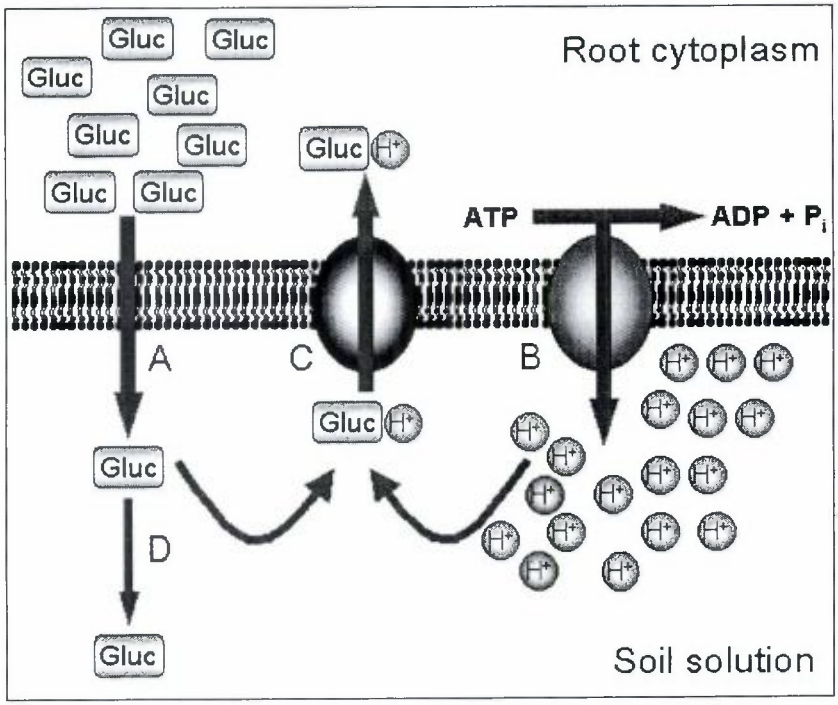


Figure 4. Schematic representation of the flow of glucose at the soil root interface. The key points are (A) the passive diffusive loss of exudates from the root, (B) the extrusion of $H^+$ from the root into the soil/apoplast by the plasma membrane $H^+$-ATPase, (C) the re-capture of glucose from the soil/apoplast by the plasma membrane $H^+$-cotransport protein, and (D) the diffusion of glucose away from the root into the soil.

which is regulated by the concentration gradient across the plasma membrane (Jones and Darrah; 1994, 1996). The rate of sugar and amino acid efflux (E) per unit area of root (A) can therefore be predicted from the internal ($C_I$) and external ($C_O$) solute concentrations and the permeability coefficient of the plasma membrane (P) where

$$E = P \times A \times (C_I - C_O)$$

Rhizosphere microorganisms may directly regulate the rate of C exudation by lowering the external concentration thereby stimulating exudation (Meharg and Killham, 1991). However, due to the size of the concentration gradient across the plasma membrane we hypothesize that reductions in soil solution

concentrations will have little effect compared with small changes in the permeability coefficient (Fig. 3). In the case of organic acids, a similar passive diffusion process operates in roots, but due to the negative charge of the organic anions at typical cytoplasmic pH values (7.0 to 7.5), this exudation process is also highly dependent upon the electrochemical potential gradient that exists across the plasma membrane (ca. –120 to –200 mV; Ryan et al., 2001). This electrical gradient stimulates the efflux of organic acids with the degree of flux enhancement dependent upon the anion's negative charge (e.g. $citrate^{3-}$ > $malate^{2-}$). In some plant species, the rate of organic acid exudation can be enhanced by the opening of uni-directional anion channels which facilitate the passive passage of the anions across the plasma membrane (Ryan et al., 2001). At present, it appears that the operation of these channels is highly spatially localized and dependent upon external environmental conditions. For example, in certain wheat cultivars malate-specific channels are triggered to open when the roots are exposed to rhizotoxic concentrations of $Al^{3+}$ (Zhang et al., 2001).

Current evidence about exudate influx suggests that both sugars and amino acids are taken into the root by a range of compound-specific $H^+$-cotransport systems which are powered by the plasma membrane $H^+$-ATPase (Rausch, 1991; Fig. 4). For organic acids, under certain conditions, monocarboxylic acids such as lactate and acetate can be taken up passively into roots when uncharged but not when charged (Jones, 1998). The uptake of di- and tri-carboxylic acids by plant roots appears to be very small in comparison to the rate of efflux (Jones, 1998). We speculate that this is because (1) organic acids are largely released for positive benefit and therefore reuptake is counterproductive, (2) the passive uptake of organic acids is prevented by the charge gradient across the plasma membrane, and (3) there are no specific inwardly directed active transport systems.

## 3. Carbon Exudation Measured in Hydroponic Culture

At its simplest, this technique involves the placing of roots in a container of solution for a known period of time followed by analysis of the solution for specific carbon compounds. Whilst this approach sounds straightforward, there are a large number of caveats. To enable extrapolation to field conditions, the plant should ideally be grown from seed under sterile conditions in an aerated nutrient solution that reflects the chemistry of the soil solution and in which the roots have some structural support (Neumann and Römheld, 2000). Preferably, the nutrient solution should flow over the roots in a one-pass system to prevent exudate recapture and not recirculated (Jones and Darrah, 1993; see later). The shoots should be maintained with sufficient light to reflect field conditions and the establishment of normal plant source-sink relationships. A

lack of consideration of one of these factors can potentially lead to the production of large artifacts (Jones and Darrah, 1993). For example, if the nutrient solution is not aerated the roots will excrete large quantities of excess carbon produced from anaerobic respiration into the external solution (e.g., lactic acid; Xia and Roberts, 1994). In addition, the morphology of the roots grown in hydroponic culture is often different from those grown in soil making extrapolation to soil conditions difficult (e.g. root hair production is often suppressed in hydroponic culture). If the root-bathing solutions are not sterile the carbon released by the roots can be rapidly consumed by the microorganisms present on the rhizoplane and in the root bathing solution (Neumann and Römheld, 2000). This is shown in Fig. 5 where glucose, a dominant root exudate, was added to the aerated root-bathing medium of sterile and non-sterile wheat roots at a concentration of 10 μM. There were no visible differences between the root systems and no visual microbial contamination. Most of the glucose was rapidly assimilated by the microbes in the non-sterile cultures leading to a gross underestimation of C exudation under these conditions. From Fig. 5, it can also be seen that the concentration of glucose in the external solution falls in the plants grown under sterile conditions. This loss of carbon is not due to glucose sorption to the container walls but to the active root uptake of glucose from the solution.

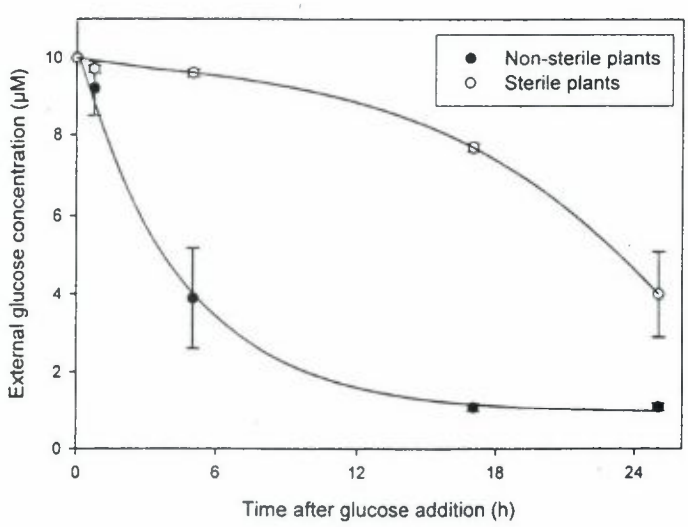


Figure 5. Impact of microbial contamination of roots on the recovery of root exudates from hydroponic culture experiments. The root bathing solution of sterile and non-sterile maize plants were supplemented with glucose (10 μM) and its concentration monitored over time. See text for further discussion. Values represent means ± SEM (n=4).

## 4. Carbon Exudation in Whole-Plant $^{14}C$ or $^{13}C$ Isotope Labelling Studies

This technique for estimating rhizodeposition relies on feeding the above ground portions of the plant with isotopically labelled $CO_2$ which is then incorporated by photosynthesis into the rest of the plant. Isotopic labelling can be done either by a continuous feeding of $^{13}C/^{14}CO_2$ to the leaves or by the introduction of a single isotopic pulse (Meharg and Killham, 1998). Although this technique is simple to perform both in the laboratory and field, the interpretation of the results remain controversial (Meharg, 1994). Despite the many papers published using this technique, there has been no quantification of the errors involved in the experimental procedure. Whilst a measurement of the amount and distribution of the isotopic label into the aboveground plant parts is relatively straightforward, the fractionation of the below ground isotopically labelled components is contentious. Some authors have attempted to quantify the amount of isotope contained within the root, the microbial biomass (rhizoplane and rhizosphere) and the soil's soluble and insoluble C pools (Whipps, 1990). Realistically, and despite the claims made in the papers, there is no way to know whether these pools are being accurately separated and quantified. One particular problem is that the physical separation of roots and soil is impossible to perform without causing loss of root C into the soil (e.g. breakage of root hairs and bursting of epidermal cells) or getting some soil adherence to the roots. As the concentration of solutes within the root cell is much greater than present in the external soil solution small losses could lead to a gross overestimation of root exudation. Further, in most long-term labelling studies, no account is made for the C entering the soil from root turnover. Typically, the entire C recovered in the soil is assumed to be due to rhizodeposition, but even with short labelling times both root turnover and direct transferred of C from the root to the soil via mycorrhizas will also be measured. Experiments attempting to quantify the amount of isotope contained within the rhizoplane and rhizosphere and their associated microbial biomass are also fundamentally flawed as the techniques used, which typically involve shaking or sonicating soil with water are not suitable. For example, using this approach isotopic label contained within fungal hyphae and some bacteria will inevitably be released into solution by the physical disruption caused by the extraction procedure while studies in our laboratory have shown that exudates in solution may be degraded during extraction by removing diffusion barriers and allowing microbial access to previously protected C. The accurate quantification of C contained within rhizoplane microorganisms and soluble and insoluble C on the rhizoplane surface C are also impossible to physically achieve and measurements of this type published in the literature should be treated with a high degree of caution. Some studies have employed

isotopic labelling in both sterile and non-sterile soils in order to distinguish between plant and microbial respiration (i.e. rhizodeposition C = soil C + microbial respiration C). Again this approach is flawed for a number of reasons including the knowledge that sterilizing soil causes the production of phytotoxic compounds which affects root growth and metabolism, and that under sterile conditions the root has ample time to recapture its exuded C by the mechanisms described above.

A second major problem is that of dealing with isotopic dilution of $^{14}C$ in its transfer between leaf and soil, a problem which is as real in hydroponic as in soil-based experiments. Ideally the specific activity of the individual compounds being exuded would be known in each part of the plant, but particularly in the cytosol of cells in the root from which exudation occurs. In practice this is very hard to measure, especially as the commonest exudates such as sugars and organic acids are in both cytosol and vacuole of different cell types in the root. Nevertheless the ideal must be to measure the specific activities and calculate the true flux of exudate using compartmental analysis.

Generally, although simple, isotopic labelling as conducted in the past should not be used to quantify rhizodeposition and papers published using this approach should be treated with skepticism. This use of isotopes, however, could be beneficial if experiments are done with care and attention to isotope dilution.

## 5. What Don't We Know About Root Exudation?

It should be clear that we actually have very little reliable data on the quantitative nature of rhizosphere C flow. There is a need to target research towards areas which will increase our fundamental understanding of root C flow rather than undertake more applied studies using inappropriate methodology. We need to investigate the mechanistic basis of the exudation flux in more plant species. Although we have presented evidence for the passive nature of this flux in maize (Jones and Darrah, 1996), this is an isolated study which should be extrapolated only with caution to other plant species. The relative extent to which passive exudation is controlled by either the concentration gradient across the soil-root interface or the permeability coefficient of the plasma membrane is also worthy of investigation in order to identify the key regulatory points. This is particularly relevant to associative $N_2$ fixation where it has been speculated that some microbial species are capable of stimulating exudation either directly (by altering membrane integrity) or indirectly (by lowering the external concentration and increasing the concentration gradient). The potential of chemicals released by associative $N_2$ fixers to modulate the physical properties of the plasma membrane (e.g.

lipid fluidity) or their biochemical function (e.g., $H^+$-ATPase activity) needs to be clarified.

The spatial regulation of exudation also requires further investigation. Although all the evidence points towards exudation being greatest at root tips and lateral root breakage points, the relative importance of total C loss from these root zones in comparison to the rest of the root system remains unknown. Further, there is little quantitative information of the role of root hairs in root exudation and whether the increase in surface area enhances rhizodeposition. In addition, at a finer spatial scale more work is needed to address whether epidermal cell junctions, where microbes in the rhizoplane appear to typically reside, are indeed enhanced points of exudation or whether they just provide suitable physically protected and hence stable areas for colonization. With respect to temporal regulation, it has been shown that phytosiderophore exudation is highly regulated not only by plant Fe status but also by time of day-maximum exudation is just after sunrise (Marschner, 1995). In comparison with this group of compounds, however, we understand little about how diurnal rhythms affect the exudation patterns of the dominant low molecular weight cell solutes (i.e., sugars, amino acids, organic acids), yet we know that plant source-sink relationships are highly diurnally regulated (Dennis and Turpin, 1990).

To understand the role of endophytic bacteria, we need to know the concentration of organic and inorganic solutes in different root apoplastic zones. Specifically, more work is needed to assess the availability of N solutes such as amino acids and how these might influence the $N_2$ fixation potential of associative $N_2$ fixers. A possible approach could be the development of reporter gene technology for endophytic bacterial strains which are modified to express lux or degenerative gfp when N starved (Jaeger et al., 1999; Jensen et al., 1998). This would allow visualization and semi-quantitative analysis of apoplastic solute concentrations as already demonstrated for rhizoplane and phylloplane (Farrar et al., 2003).

To understand the root's influx mechanisms for recapturing exudates from soil, more work is needed to characterize the mechanistic basis of this process to assess whether this phenomenon is ubiquitous in plants. We also need to quantify the kinetics of root C uptake in comparison to those of $N_2$ fixing bacteria. Care must be taken in the pre-culture of these organisms to avoid anomalous results, since growth of rhizobacteria on one C substrate will significantly affect their potential to take up and assimilate another (Jones et al., 1996). Although we have a good genetic and physiological understanding of the transporters involved in the uptake of sugars and amino acids, their expression and function in a rhizosphere context has rarely been considered. There is therefore a need to use the molecular tools already developed in more ecologically relevant scenarios.

Lastly there is a need for mathematical models which consider a plant in three dimensions and which incorporate developmental changes where plant transport processes are capable of being regulated by source and sink pool sizes. Whilst the accuracy of a model is dependent upon the quality of the input parameters, we must accept that for the foreseeable future this may be our best hope of assessing some of the critical control points of rhizosphere carbon flow.

## 6. The Fate of Root Exudates in Soil

Once in the soil, and if not recaptured by the root, exudate C has a number of fates (Fig. 1). Two processes typically dominate exudate fate, uptake by the soil microbial biomass and (in the case of charged compounds) sorption to the soil's solid phase (Jones and Edwards, 1998). Leaching of C out of the rhizosphere and abiotic mineralization are of minimal importance except under exceptional circumstances (Jones et al., 1996). In the case of microbial assimilation, uptake from the soil is extremely rapid and C availability is the main factor limiting microbial growth rather than uptake (Jones, 1998). Further discussion of these processes can be found in Jones et al. (2003).

## 7. Associative $N_2$ Fixation in the Rhizosphere – Theoretical Approach

*Description of the model*

We will make a theoretical evaluation of the potential amount of $N_2$ that can be fixed by associative nitrogen fixing bacteria residing in the rhizosphere. Due to the uncertainties accompanying the physiological state of endophytic bacteria we will concentrate on the potential for $N_2$ fixing bacteria that colonize the rhizoplane and ectorhizosphere. The model is shown in Fig. 6 and the parameter values are shown in Table 1. Here we assume that soluble low molecular weight root exudates are the primary drivers of associative $N_2$ fixation in soil. Although C also enters the soil in the form of root excreted mucilage and as a result of root turnover we assume that this complex form of C will be of little use for associative $N_2$ fixing bacteria. The amount of $N_2$ fixation is essentially driven by the amount of root exudate carbon captured by the microorganisms in the rhizosphere. This process is dependent upon a number of plant variables including the amount of exudation per unit root (E), the efficiency of the root exudate re-capture mechanism (I) and the amount of root in a given volume of soil (R). The model firstly assumes that the exuded carbon is a mixture of sugars, amino acids and organic acids at a ratio of 10:3:1 (Table 1). Although this implicitly ignores a range of other exudates, these are

Table 1. Summary of the associative $N_2$ fixation model parameters. See text for further details of the parameters

| Symbol | Definition | Units | Value | Reference |
|---|---|---|---|---|
| $E_S$ | Rate of root sugar exudation | μmol $cm^{-1}$ root $y^{-1}$ | 29.2 | Jones and Darrah (1996) |
| $E_A$ | Rate of root amino acid exudation | μmol $cm^{-1}$ root $y^{-1}$ | 9.7 | Jones and Darrah (1994) |
| $E_O$ | Rate of root organic acid exudation | μmol $cm^{-1}$ root $y^{-1}$ | 2.9 | Jones (1998) |
| $I_S$ | Proportion of sugars recaptured by root | μmol $\mu mol^{-1}$ | 0.5 | P.R. Darrah, unpublished |
| $I_A$ | Proportion of amino acid recaptured by root | μmol $\mu mol^{-1}$ | 0.5 | P.R. Darrah, unpublished |
| M | Amount of exudates taken up by microbial biomass | μmol $ha^{-1}$ $y^{-1}$ | – | |
| N | Proportion of exudates captured by the $N_2$ fixers | μmol $\mu mol^{-1}$ | 0.01 | |
| $Y_S$ | Proportion of sugars used for energy production | μmol $\mu mol^{-1}$ | 0.2 | D.L. Jones, unpublished |
| $Y_A$ | Proportion of amino acids used for energy production | μmol $\mu mol^{-1}$ | 0.3 | Jones (1998) |
| $Y_O$ | Proportion of organic acids used for energy production | μmol $\mu mol^{-1}$ | 0.8 | Jones et al. (1996) |
| $C_S$ | Amount of N fixed per unit of sugar metabolised for $N_2$ fixation | μmol $N_2$ $\mu mol^{-1}$ | 0.25 | Tate (2000) |
| $C_A$ | Amount of N fixed per unit of amino acid metabolised for $N_2$ fixation | μmol $N_2$ $\mu mol^{-1}$ | 0.075 | D.L. Jones, unpublished |
| $C_O$ | Amount of N fixed per unit of organic acid metabolised for $N_2$ fixation | μmol $N_2$ $\mu mol^{-1}$ | 0.125 | D.L. Jones, unpublished |
| $F_S$ | $N_2$ fixation from sugars | kg N $ha^{-1}$ $y^{-1}$ | – | |
| $F_A$ | $N_2$ fixation from amino acids | kg N $ha^{-1}$ $y^{-1}$ | – | |
| $F_O$ | $N_2$ fixation from organic acids | kg N $ha^{-1}$ $y^{-1}$ | – | |
| NF | Total annual nitrogen fixation | kg N $ha^{-1}$ $y^{-1}$ | – | |
| R | Soil rooting density | cm root $ha^{-1}$ | $0.75 \times 10^9$<br>$3 \times 10^9$<br>$12 \times 10^9$ | D.L. Jones, unpublished |

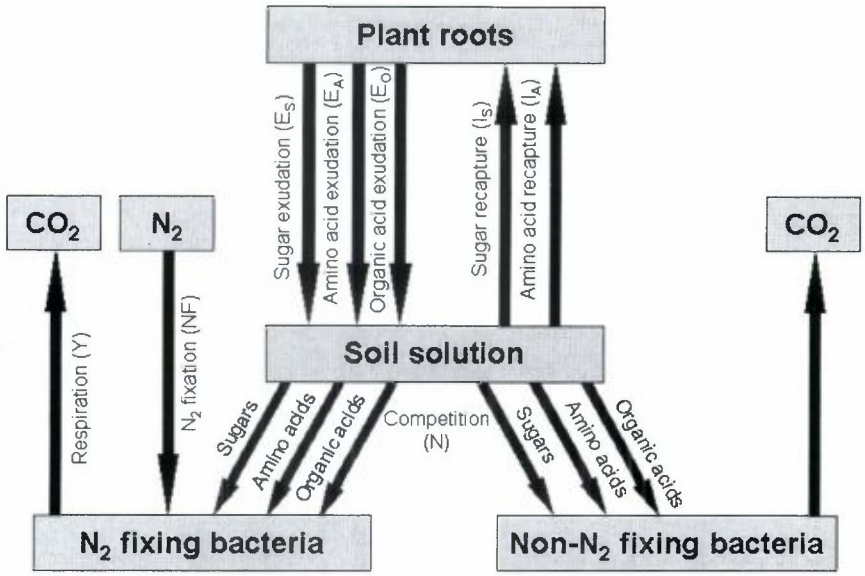


Figure 6. Schematic representation of the mathematical model used to calculate the amount of associative $N_2$ fixation in the rhizosphere that can be supported by the root exudation of carbon.

probably exuded in small quantities and have a low potential to fuel $N_2$ fixation (e.g. flavonoids, sterols, phenolics; Farrar and Jones, 2000). Secondly, we assume that the internal use of this carbon by the soil microorganisms (new biomass production versus respiration) is compound-specific (Table 1). As the amount of exudate mineralization in the rhizosphere by abiotic processes is minimal and losses by leaching are small, then the amount of exudate carbon captured by the soil microbial biomass (M; μmol $ha^{-1}$ $y^{-1}$) per unit of root can be described by the equation

$$M = R \times ((E_S - (E_S \times I_S)) + (E_A - (E_A \times I_A)) + E_O)$$

For organic acids, there is no reported root re-uptake system and therefore no influx component is included. As there is no evidence to suggest that associative $N_2$ fixing bacteria possess transport systems with different kinetic properties from those of non-$N_2$ fixing bacteria, the model assumes that the uptake of exudate C by $N_2$ fixing bacteria is simply dependent upon their relative abundance in the soil in comparison to non-$N_2$ fixing microorganisms. This partitioning of exudate C between $N_2$ fixers and non-$N_2$ fixers is therefore described by a constant (N). Once taken up by the soil microorganisms, the

amount of C used for $N_2$ fixation versus other cellular processes (maintenance and biomass production) is described by a partition coefficient (Y) which is dependent upon the type of exudate and its ATP yield (Table 1). The amount of exudate C used for bacterial energy production is defined by the parameter Y while the amount of exudate C used for growth and cell maintenance is defined as (1 – Y). Although there are values for C use efficiency in symbiotic $N_2$ fixing bacteria growing in substrate-rich liquid culture (Finan et al., 1981; SanFrancisco et al., 1985), there are no values for these organisms under soil conditions. However, we interpret published values for whole soil microbial communities to mean that both $N_2$ fixers and non-$N_2$ fixers partition their carbon in the same way. We acknowledge that $N_2$ fixers will probably place the carbon normally used by non-fixing bacteria for acquiring N from the soil into $N_2$ fixation (i.e. that normally used in ion transport and protease production), however, we hypothesize that this deviation in energy resources is probably small in comparison to the overall C balance of a soil microorganism. For each carbon compound we assume a $N_2$ fixation efficiency ($C_S$, $C_A$, $C_O$) of 1 µmol of $N_2$ per µmol of glucose, 0.5 µmol of $N_2$ per µmol of organic acid, and 0.4 µmol of $N_2$ per µmol of amino acid. These values for energy production from root exudate C to fuel associative $N_2$ fixation are based upon limited published data and represent what we consider to be maximum conversion rates (Tate, 2000). In reality, the production of energy for $N_2$ fixation from these substrates may be much lower. Therefore the maximum amount of root exudate driven $N_2$ fixation ($F_S$, $F_O$, $F_A$) can be described by the series of equations

$$F_S = ((E_S - (E_S \times I_S)) \times R) \times N \times Y_S \times C_S$$
$$F_A = ((E_A - (E_A (I_A)) \times R) \times N \times Y_A \times C_A$$
$$F_O = (E_O \times R) \times N \times Y_O \times C_O$$

whilst the total amount of N fixed per hectare (NF) can be described by the equation

$$NF = F_S + F_A + F_O$$

The model assumes that there are no spatial limitations in the availability of carbon resources and that all the C released will ultimately be captured by either the root or the soil bacteria. Solid phase sorption reactions can significantly reduce organic acid availability. We assume that this sorption process is reversible and that the mineral phase simply acts as a carbon buffer so that all the organic acids will eventually become available to either the root or soil microorganisms. The exudation parameters have been chosen from a variety of independent sources and reflect measured rates of exudation for specific compounds from maize roots (Jones and Darrah, 1993, 1994; Pellet et al.,

1995; Schonwitz and Ziegler, 1982). Indeed we have parameterised for maize due to the large amount of data available for it. The values for root density are derived from a maize crop with low, medium and high rooting density which are directly related to the age of the plant. We assume a recapture efficiency of 50% for sugar and amino acid exudate. Our studies on wheat seedlings indicate an amino acid root recapture efficiency of between 5 to 10% (Owen and Jones, 2001), which is probably an underestimate due to the geometry of the experimental system. Using the mathematical model of Darrah (1991) with an influx component included, recapture efficiency of a maize root for glucose was estimated to be around 50% (DL Jones and PR Darrah, unpublished) whilst experiments in non-sterile hydroponic culture for wheat seedlings also indicate a recapture efficiency of 50% (DL Jones, unpublished).

*Results of the root exudate-$N_2$ fixation model*

The model predicts that the amount of $N_2$ fixation which could be directly attributable to root exudation ranges from 0.2 to 4 kg N $ha^{-1}$ (Table 2). As expected, the theoretical predictions were highly dependent upon the model's input parameters and in particular highlighted the importance of competition between the fixing and non-fixing microbial biomass. The model assumed a conservative value for the amount of competition between the root and soil microbial community for C released by the root into the rhizosphere (50% of the exudates lost by the root are subsequently recaptured). However, reductions in this parameter value will have little significant effect on the $N_2$ fixation potential in comparison to changes in competition within the soil microbial community itself. In particular the degree of exudate capture by the $N_2$ fixers versus the non-$N_2$ fixing microorganisms in the soil appears to a critical control point in the amount of $N_2$ fixed within the rhizosphere (Fig. 7). In our model and the results presented in Table 2 we assumed that the number of non-$N_2$ fixers in the rhizosphere was $10^8$ cfu $g^{-1}$ soil and the number of $N_2$ fixers was $10^6$ cfu $g^{-1}$ soil. One of the main problems with the model prediction is that the size of the naturally occurring $N_2$ fixer populations can vary from $10^1$ to $10^6$ cfu $g^{-1}$ soil depending upon soil type and environmental conditions (Tate, 2000). However, we feel that an efficiency of 1% for capture of exudate by $N_2$ fixers is fairly optimistic for natural ecosystems. In situations where the natural $N_2$ fixer population is augmented with enriched microbial cultures (e.g. *Azospirillum*, *Azotobacter*) the exudate capture efficiency may be up to 10%, due to rapid microbial die-off (Fig. 7). The $N_2$ fixation values predicted by the model are in good accordance with experimentally derived values of rhizosphere $N_2$ fixation which range from 0.03 to 20 kg N $ha^{-1}$ $y^{-1}$ (Bremer et al., 1995; Paul and Clark, 1996, Fig. 7).

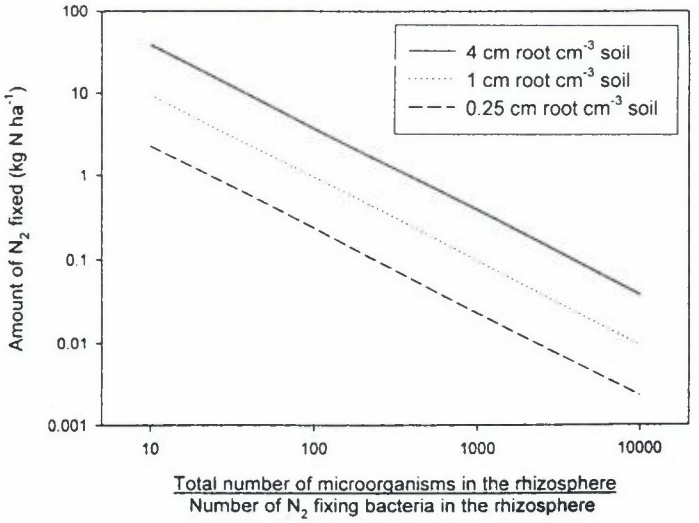


Figure 7. Calculated amount of associative $N_2$ fixation that could be theoretically driven by carbon exuded from maize roots. The $N_2$ fixation predictions are for three different soil-root densities (0.25 to 4 cm root $cm^{-3}$ soil) and calculated as a function of the proportion of $N_2$ fixing organisms in the soil relative to the number of non-$N_2$ fixing organisms (which are also competing for the exuded C). See text for further details of the model.

Table 2. Predicted amount of associative $N_2$ fixation that can occur in response to the exudation of carbon compounds from plant roots. The amount of $N_2$ fixation from the three dominant root exudates types (sugars, amino acids and organic acids) was calculated at three different soil root densities (0.25, 1 and 4 cm of root per $cm^3$ of soil which is equivalent to $0.75 \times 10^9$, $3 \times 10^9$ and $12 \times 10^9$ cm root $ha^{-1}$).

| Soil root density | Associative $N_2$ fixation (kg N $ha^{-1}$ $y^{-1}$) Root exudate type | | | Total |
|---|---|---|---|---|
| | Sugars | Amino acids | Organic acids | |
| 0.25 | 0.15 | 0.02 | 0.06 | 0.23 |
| 1.0 | 0.61 | 0.09 | 0.25 | 0.95 |
| 4.0 | 2.45 | 0.37 | 0.98 | 3.80 |

Our model provides a framework for more detailed studies. In particular, future models should be constructed with a spatial component to allow fixation

gradients across the rhizosphere to be calculated. In addition, a model which incorporates the subsequent release of $N_2$ fixed in the rhizosphere back to the plant would be desirable.

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