

# Concepts and methods for studying interactions of roots and soil structure <sup>α</sup>

Meine van Noordwijk, Gerard Brouwer and Klaas Harmanny

DLO-Institute for Soil Fertility Research, P.O.Box 30003, 9750 RA Haren, The Netherlands

(Received November 27, 1991; accepted after revision July 8, 1992)

## ABSTRACT

Van Noordwijk, M., Brouwer, G. and Harmanny, K., 1993. Concepts and methods for studying interactions of roots and soil structure. In: L. Brussaard and M.J. Kooistra (Editors), Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships. *Geoderma*, 56: 351–375.

The central concept in present-day root ecological studies in agriculture is that reduced or improved root development may decrease or increase the efficiency of using water and nutrients and thus aggravate or reduce negative environmental impacts of agriculture. The normal root development of many agricultural crops is such that chemical fertility of the soil has to be so high that residues of  $\text{NO}_3$  and P in the soil remain at harvest which are close to or higher than those acceptable to meet environmental standards on quality of ground- and surface water. Poor soil structures, with a large fraction of the soil at a relatively large distance to the nearest macropore or crack, may affect root penetration and functioning, and thus lead to even higher residues of macronutrients at harvest. If roots are not able to penetrate the matrix of the aggregates a clustered root distribution in the cracks is the result. Negative effects on nutrient and water uptake efficiency of non-regular root distribution and incomplete root-soil contact can now be estimated from the Root Position Effectivity Ratio,  $R_{\text{per}}$ . Aeration of roots depends on the (maximum) distance between root tissue and the nearest "breathing section", where part of that root is in contact with air-filled macropores or cracks, on the air-filled porosity of the root and on protection against leakage of oxygen out of the root.

Methods are discussed for measuring: distance in pixel images, root distribution pattern on maps, synlocation of roots and other map features, root-soil contact, root position effectivity ratio, air-filled root porosity and continuity of air channels, and dynamics of root growth and decay.

## 1. INTRODUCTION

### 1.1. Roots as soil biota

Roots are a special category of "soil biota", as they are part of an organism which lives in two very different environments, the (upper layer of the) soil

*Correspondence to:* Dr. M. van Noordwijk, DLO-Institute for Soil Fertility Research, P.O.Box 30003, NL 9750 RA Haren, The Netherlands.

*Communication No. 47 of the Dutch Programme on Soil Ecology of Arable Farming Systems.*

and the (lower part of the) atmosphere. Three levels of complexity in root ecological studies can be distinguished (Van Noordwijk, 1993a): (A) studies of single roots interacting with their environment; (B) studies of root systems in a homogeneous environment, including root–shoot interactions; and (C) studies of root systems in a heterogeneous root environment, including root–shoot interactions. Penetration of dense layers of soil has often been studied at the single root level. When studied from a whole plant perspective (level B), negative effects of soil compaction can often be compensated by improved supply of water and nutrients (Schuurman, 1971). When studied in a heterogeneous environment (level C), penetration of dense soil layers may depend on conditions around other roots or other parts of the same root. Figure 1 shows that penetration of oat roots into a “very dense” soil layer (bulk density  $1.52 \text{ g/cm}^3$ ) was better when the topsoil was “dense”, than when it was “loose” (bulk densities  $1.38$  and  $1.24 \text{ g/cm}^3$ , respectively; Schuurman

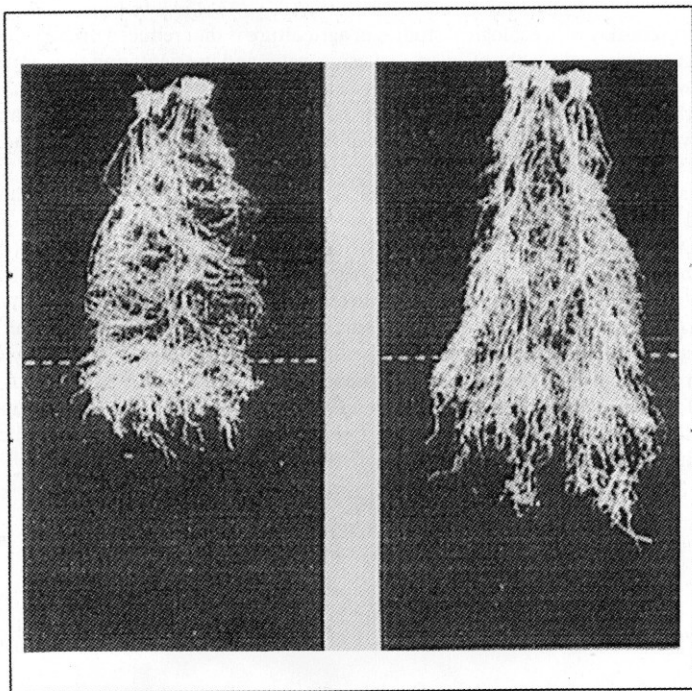


Fig. 1. Penetration of oat roots in a “very dense” sandy subsoil (bulk density  $1.52 \text{ g cm}^{-3}$ ) from a “loose” (b.d.  $1.24 \text{ g cm}^{-3}$ ; left) and “dense” (b.d.  $1.38 \text{ g cm}^{-3}$ ; right) topsoil in a tube experiment of Schuurman and De Boer (1974).

and De Boer, 1974). A method for studying root response to local variation in soil density in the field was described by Hairiah et al. (1991).

In the past, a “morphogenetic equilibrium” between roots and shoots was the central concept in root ecology. Any reduction in root growth, e.g., due to a poor soil structure, was assumed to reduce shoot growth. During the last three decades the interactions and feedback controls between shoot and root are interpreted as a “functional equilibrium”. Increased supply of water and nutrients can (largely) compensate for reduced root development in many situations (Van Noordwijk and De Willigen, 1987). The central concept now is that reduced root development may affect the efficiency of the use of water and nutrients and may enhance negative environmental impacts of agriculture (Van Noordwijk and De Willigen, 1991).

Figure 2 summarizes a possible causative chain (model) for effects of poor soil structure on environmental pollution. A causative chain can be represented as a series of links between “Patterns” and “Processes”. Patterns act as initial and boundary conditions for processes; processes lead to new patterns, at a higher level of complexity. Patterns are observed more easily than processes; processes can be better generalized and have a more universal value. A relation between soil structure (pattern) and environmental pollution (process) could be based on:

- (a) Poor soil structure negatively affects root development leading to a smaller or less effective root system, more susceptible to aeration stress and root death after heavy rainfall.
- (b) A smaller root system is less effective in the process of nutrient and water uptake and will leave a larger nutrient residue at harvest time; as the lower efficiency is predictable, the farmer will increase chemical soil fertility to compensate for yield effects, but increasing the residue as well.
- (c) Large nutrient residues at harvest time will lead to high nutrient concentrations in the leachate during winter rains, polluting the environment.

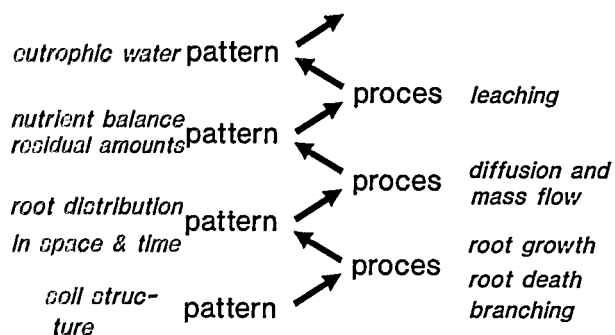


Fig. 2. Interrelationships between patterns and processes linking soil structure, root growth and functioning and environmental effects of agriculture.

## 1.2. Uptake efficiency

Agronomic nutrient or water use efficiency on the field scale depends on variability in supply and demand between individual plants on a field which is managed as if it were a homogeneous unit (Van Noordwijk and Wadman, 1992) and on three components of efficiency within the area exploited by a single plant:

- application efficiency: available supply per unit input; the available fraction is defined with respect to the time period relevant for the crop under consideration;
- uptake efficiency: uptake per unit available nutrient or water;
- utilization efficiency: crop production per unit uptake.

The uptake efficiency depends on the root system and will be the main focus of attention here. As analyzed by De Willigen and Van Noordwijk (1987), nutrient or water uptake efficiency depends on the required uptake rate per unit root (which is related to shoot/root ratio and growth rate of the plant), on the degree of synlocation of roots and resources (depending on resource mobility and soil water content) and on the degree of synchrony of resource supply and demand (depending on resource buffer capacity).

Five levels of modelling nutrient and water uptake by crops can be distinguished (Van Noordwijk and Van der Geijn, 1993):

(O) models without roots, calibrated on nutrient uptake efficiency (NUPE) and water uptake efficiency (WUPE);

(A) models evaluating uptake efficiency of a given root system characterized by its root length density, degree of root-soil contact and root distribution pattern;

(B) as (A), but using curve fits on root dynamics per depth layer or radial zone around the plant, to describe root development and root decay during the growing season;

(C) as (A), but including a "functional equilibrium" type of shoot-root interaction during crop development;

(D) as (C) but including "local response" to heterogeneity of resources and stress factors.

For direct applications, model level O may be desirable for its simplicity; it may be sufficient, provided that models are adequately calibrated and are not used for extrapolation to new situations. Complexity increases stepwise for models A...D. Model level A is needed for more complex models.

An important reference point for model levels A...D is formed by the potential uptake rate of a single root in the centre of a cylinder of homogeneous soil, in full contact with the soil. The radius of this soil cylinder is derived from the root length density, assuming a regular root distribution and parallel roots. As diffusive transport is based on concentration gradients it can not supply all available nutrients to the root surface in a restricted period of time

and a certain residue will remain in the soil, especially in situations where crop demand determines uptake and hence crop growth is not restricted by the supply of the resource studied. If crop demand and nutrient supply are completely predictable for the farmer, this residue can—without affecting uptake rates—be reduced to a minimum value  $C_m$ , such that the potential uptake rate  $A_p$  just equals the required rate  $A_r$  at the end of the uptake period (De Willigen and Van Noordwijk, 1991):

$$C_m = C_{\text{lim}} + \frac{A_r G(\rho, \nu)}{\pi H D_\theta L_{rv} (\rho^2 - 1)} \approx C_{\text{lim}} + \frac{A_r D_r^2 G(\rho, \nu)}{4 H D_\theta} \quad (1)$$

where:

$C_m$  = minimum average concentration in soil solution at end of uptake period ( $\text{mg cm}^{-3}$ ),

$C_{\text{lim}}$  = concentration at root surface area required for physiological uptake process ( $\text{mg cm}^{-3}$ ),

$A_r$  = required uptake rate per unit soil surface ( $\text{mg cm}^{-2} \text{d}^{-1}$ )

$H$  = depth of soil layer (cm),

$D_\theta$  = effective diffusion coefficient for solutes, ( $\text{cm}^2 \text{d}^{-1}$ ), dependent on soil water content  $\theta$ ,

$L_{rv}$  = root length per unit volume of soil (root length density) ( $\text{cm cm}^{-3}$ ),

$R_1$  = radius of soil cylinder =  $(\pi L_{rv})^{-0.5}$ ,

$\rho$  = dimensionless cylinder size =  $2 R_1 / D_r = 2 (\pi L_{rv})^{-0.5} / D_r$ ,

$D_r$  = root diameter (cm),

$\nu$  = dimensionless flux of water.

$G(\rho, \nu)$  is the dimensionless function of root length density and transpiration rate:

$$G(\rho, \nu) = \frac{1}{2(\nu+1)} \left( \frac{1-\rho^2}{2} + \frac{\rho^2(\rho^{2\nu}-1)}{2\nu} + \frac{\rho^2(\rho^{2\nu}-1)(\nu+1)}{2\nu(\rho^{2\nu+2}-1)} \right) + \frac{(1-\rho^{2\nu+4})(\nu+1)}{(2\nu+4)(\rho^{2\nu+2}-1)} \quad (2)$$

The right-hand side of eq. (1) gives an approximation which is good, unless root length density or root diameter is very high. For a situation without mass flow ( $\nu=0$ )  $G(\rho)$  replaces  $G(\rho, \nu)$ :

$$G(\rho) = \frac{1}{2} \left( \frac{1-3\rho^2}{4} - \frac{\rho^4 \ln \rho}{\rho^2-1} \right) \quad (3)$$

This relatively simple cylindrical model can be used to deal with situations with a more complex geometry. A non-regular root distribution, even in combination with incomplete root-soil contact, in a layer with given nutrient concentration and soil water content can be represented by a series of cylinders, rather than by a single one. The potential uptake rate for such a situation is

obtained by the weighted average of the uptake for each cylinder. In the approximate version of eq. (1), total uptake of homogeneously distributed resources will be proportional to a sum of  $G$ -functions in stead of a single function  $G(\rho, \nu)$ . An "effective" root length density  $L_{rv*}$  can be found for which the  $G$  function equals this sum of  $G$ -functions (see section 3.5). The root position effectivity ratio,  $R_{per}$ , is defined such that  $R_{per} = L_{rv*}/L_{rv}$ , where  $L_{rv*}$  is the effective and  $L_{rv}$  the real root length density.  $L_{rv*}$  can be used for calculations of uptake rates, because the  $G$ -function appears in dynamic uptake models for all nutrients and water. By definition  $R_{per}$  will be between 0 and 1, as non-regular root distributions are less efficient in utilizing homogeneously distributed resources. For clustered resources the sum of the products of  $C_i$  and  $G(\rho, \nu)$ , should be considered and an "effective" root length density at the average concentration can be above obtained. In this situation  $R_{per}$  can be above 1.0 (synlocation, section 3.3). A method to compute  $R_{per}$  for observed root distributions will be presented in section 3.5.

### 1.3. Root development needed to meet environmental standards

The hypothesis that a better root development may help to reduce pollution needs quantification: how many roots are needed to reduce pollution to acceptable limits? Environmental standards are now proposed in The Netherlands and will (hopefully) soon be effectuated to restrict residues of macronutrients at harvest time to such a level that water leaching through arable soils has an acceptable quality. For nitrate the standard is based on an EC standard for drinking water of 50 mg/l (or 11.3 mg/l of N). Given a rainfall excess in winter of about 300 mm ( $3 \times 10^6$  l/ha) a maximum  $\text{NO}_3\text{-N}$  content in the rooted zone of 34 kg/ha can be derived. When allowance is made for some denitrification, a maximum residue of 45 kg/ha is now formulated as a future policy target and 70 kg/ha as immediately applicable standard (Neeteson, 1993). Because part of this residue will be due to spatial variability within a field and to mineralization after crop N uptake has diminished, the  $\text{NO}_3\text{-N}$  residue within the root zone of a reference plant should be less than 20 kg/ha (Van Noordwijk and Wadman, 1992). The root length density required to achieve this target depends according to eq. (1) on daily crop demand for N ( $A_r$ ) and soil water content ( $\theta$ ); as a first estimate effective root length densities in all zones containing  $\text{NO}_3$  above  $0.3 \text{ cm/cm}^3$  will be sufficient. As  $\text{NO}_3$  will not only occur in the plough layer, subsoil root development may be critical for meeting environmental standards for  $\text{NO}_3$ . For K, a groundwater standard of 12 mg/l can be met if the residue of available K is less than 360 kg/ha at harvest, when we assume linear adsorption of K and an apparent adsorption constant of  $10 \text{ ml/cm}^3$  (De Willigen and Van Noordwijk, 1987). Effective root length densities in the K-containing layers of at least  $1 \text{ cm/cm}^3$  are needed to achieve this target. For P a maximum concentration in surface

water of 0.15 mg/l can be met if leachates are equilibrated with soil with a  $P_w$  value of 12 to 17 mg  $P_2O_5$  per l depending on soil type (Van Noordwijk et al., 1990). Of the main arable crops only wheat—with a root length density in the plough layer of about 5 cm/cm<sup>3</sup>—will grow unrestrictedly at such a level of available P. For other crops, with a lower root length density, a higher available P supply is maintained in the plough layer, so a potential risk of pollution exists. For  $NO_3$  in subsoil and P in the plough layer we may conclude that the actual root development of arable crops is critical if environmental standards are to be met without inducing nutrient stress to the crop. Reductions in root development and in effectivity of the root system for uptake, which may be the results of a poor soil structure, can therefore be unacceptable.

#### 1.4. "Good" soil structure

The concept of a "good" or "poor" soil structure should be defined before we can investigate its relations with root development. By putting a spade in the ground and observing the soil on a broken surface, large differences in soil structure can be noted (Preuschen, 1983). By comparison with photographs of reference structures, a rating from 1 (very bad) to 10 (excellent) was developed in The Netherlands and was used extensively to evaluate effects of soil tillage and other soil management practices (Boekel, 1982). Although valuable as such, a better quantification is needed to link soil structure to other processes, such as transport of water, nutrients and gasses and root development. Boone (1988) developed a functional interpretation scheme for soil compaction, based on the width of the interval in soil water content between "too wet" for aeration and "too dry" for root growth. Lipiec et al. (1991) used a "degree of compactness" by taking the ratio of the actual bulk density and the maximum bulk density obtained by a static pressure of 200 kPa. For both a silty loam and a loamy sand a value of 90% appeared to be critical for spring barley. If maximum penetration resistance is 3 MPa and 10% air-filled porosity of the soil is needed for aeration, water tension may range between -800 and -10 kPa on the silty loam and between -200 and -10 kPa on the loamy sand, at 90% compactness. Critical values on both sides of the range depend on crop species and other growth conditions.

Apart from the problem of finding the relevant parameters, the applicability of such schemes to aggregated soils may be questioned on conceptual grounds, as important aspects of "structure" are not represented adequately by bulk density. Modern soil micromorphological techniques can be used to quantify void continuity and to relate transport properties (processes) to observable patterns (Kooistra, 1990). Ismail (1975) developed a classification scheme for void pattern distribution by quantitative image analysis techniques on thin sections, based on area and diameter of voids. Ringrose-Voase

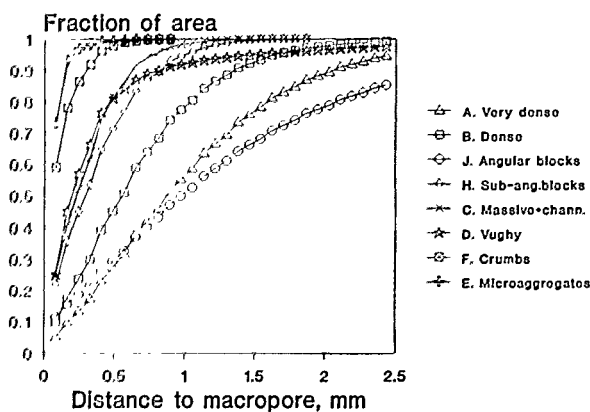


Fig. 3. Cumulative frequency distribution of nearest neighbour distances for points in the soil matrix to the nearest macropore or gap for 8 different soil structures, based on digitized images of polished thin section faces of Ringrose-Voase (1987).

(1987) gave a similar scheme for quantitative description of soil macrostructure by image analysis of polished thin section faces. In soils with fine aggregates, all area is close to the nearest macropore or gap; in soils with large blocks a larger fraction of the area on a soil surface will have a relatively large distance to the nearest macropore or gap. In Fig. 3 the frequency distribution of soil-macropore distances is quantified for eight soil structures identified by Ringrose-Voase (1987). If small, round pores—which may be actually filled by roots—would be neglected, distances would be much larger. In the Dutch visual rating system, these structures range from poor (3) to very good (8) (Boekel, pers. commun.). A method used to measure distances in such images is presented in section 3.1. Such a distance classification can be used for modelling oxygen diffusion in soil (Rappoldt, 1991), as an alternative to actual separation of aggregate sizes by sieving. The transition from two-dimensional observations to three-dimensional reality needs some special attention. Ringrose-Voase (1987) discussed this transition on the basis of a shape classification of macropores and cracks.

## 2. INTERACTIONS BETWEEN ROOTS AND SOIL STRUCTURE

### 2.1. Effects of roots on soil structure

By extracting water roots may enhance drying-wetting cycles in the soil and thus modify soil structure. Roots may even influence zones of the soil without or at least before penetrating them. As penetration resistance increases when a soil dries out, water uptake may reduce or prevent subsequent root penetra-



tion of that or a neighbouring layer of the soil in soils with sufficient capillary rise (Bengough, pers. commun.).

Roots may enhance or reduce inter-aggregate binding through exudates, decaying tissues and root-associated fungal networks. The long-term effect of the presence of roots in a layer will normally be to enhance aggregate stability, but reductions of aggregate stability by root exudates are possible in the short term. Physical movements of the root tip (see below) might add to the loosening effect. Loosening of aggregates would—similar to the effects of mechanical disturbance or soil tillage (Nordmeyer and Richter, 1985)—increase the accessibility of soil organic matter to bacteria and/or accessibility of bacteria to the next steps in the food chain and thus stimulate N-mineralization. Structural deaggregation by root activities and enhanced N-mineralization may provide an alternative explanation for some of the evidence for N<sub>2</sub> fixation in the rhizosphere. Griffiths and Robinson (1992) showed that the (observed) stimulatory effects of plants on the net mineralization of soil organic nitrogen can not be adequately explained by the C in exudates as energy source for microbial activity; but the presence of roots might improve the availability of other energy sources. An exudate front diffusing ahead of a relatively slow growing root may induce a microbial (or soil structural) response in the soil before the root tip has arrived (Darrah, 1991). McCully (1987) described that young maize roots are closely associated with the soil matrix and appear as “sheathed” roots when extracted from soil, while older roots are “bare” and have less contact with the soil. It seems that the close association between a root and its soil sheath can not be obtained without a disruption of existing aggregates and a subsequent re-stabilization of soil material around the root. Jaillard (1987) observed a reorientation of clay in the near rhizosphere by micromorphological techniques. We formulate a new hypothesis here that effects of exudates and/or root movements on destabilizing soil structure may be responsible for enhanced N-mineralization and for re-orientation of soil aggregates.

## *2.2. Effects of soil structure on roots*

Soil structure may influence root development and thus resource accessibility for the plant as a whole. Four aspects will be considered here: (1) effects on root length density; (2) effects on root distribution pattern (section 3.2); (3) effects on root–soil contact (section 3.4); and (4) effects on aeration (section 3.6) and hence on dynamics of growth and decay of the root (section 3.7).

Much attention has been given to the ability of roots to penetrate a homogeneous compacted layer of soil. In the last decade the response of roots to aggregate surfaces, encountered after passing through a gap or void, has also been studied (Hewitt and Dexter, 1984). Much less attention was given to

---

spiral movements of the root tip under such circumstances (Spurny, 1966), described as "circumnutation" in old plant physiological literature (Darwin, 1880). Barlow et al. (1991) studied geotropic response of roots at the cellular level and found evidence for spiralling movements as well. Circumnutation of root tips may be important as it allows a root tip—with the possible use of mucigel as a glue—to grow along aggregate faces and establish partial root-soil contact when a root enters a gap or macropore (Kooistra et al., 1992). For many agricultural crops the rate of elongation may decrease to about one half by rather small mechanical stresses (20 to 50 kPa) (Lucas, 1987). If such retardation would happen on one side of the root only in roots growing on an aggregate surface in a gap or macropore, it would form an efficient mechanism to follow this surface, even where it bends away.

Roots penetrating the soil matrix will have complete contact with the soil matrix and micropores, which will be waterfilled when the soil is at field capacity. Complete root-soil contact facilitates uptake of water and nutrients, but aeration of the root may be difficult. A certain degree of air-filled porosity of the cortex is necessary to allow oxygen to diffuse to all cells of the root in this situation. The porosity required depends on the distance to the nearest "breathing section" on the root, where gas exchange with a macropore is possible. Cellular structure outside the cortex (epi- and exodermis) may influence entry of oxygen in the "breathing section", but also loss of oxygen out of the root tissue in the remaining part of the root. Models of longitudinal transport of oxygen in roots (De Willigen and Van Noordwijk, 1989) so far assume this radial transport resistance to be uniform, but critical measurements to support or refute this assumption are lacking. While a root tip is penetrating into an aggregate, aeration is problematic, because cortical air channels do not extend to the tip where metabolic activity is highest and oxygen has to diffuse across almost the complete diameter of an aggregate (Fig. 4). After

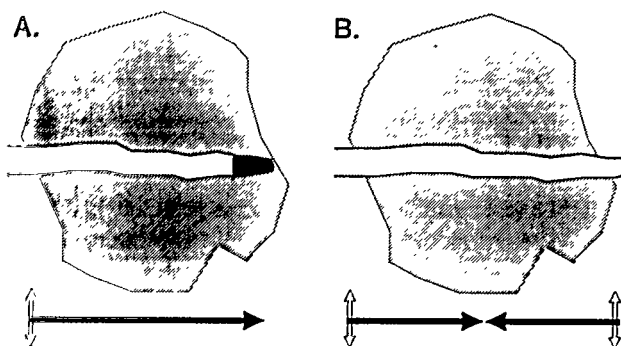


Fig. 4. While a root is penetrating an aggregate the internal oxygen transport must bridge almost the complete diameter of the aggregate (A); once the root tip has emerged into a new macropore or crack where oxygen is available, the transport distance is reduced to the aggregate radius (B).

the root has entered a new macropore only the radius of the aggregate has to be bridged, from two sides. Additionally, oxygen demand per cm of root length for maintenance and uptake will be less than that required for root growth (Lambers, 1987). Once formed, a root may thus survive a certain reduction in oxygen supply when the soil becomes wetter. When the soil becomes waterlogged, however, the oxygen stored in air-filled pores may be depleted within a day (Boone and Veen, 1992). Physiological mechanisms to survive temporary aeration stress may be essential (Drew, 1990), unless internal oxygen transport from aboveground tissues is possible. Plant species differ considerably in air-filled root porosity and also in the ability to increase such porosity when aeration is insufficient. Cortical air spaces are normally continuous within a single root axis, but barriers may exist between main axes and branch roots. A method to study air-filled porosity and continuity of air spaces is described in section 3.6.

Apart from the two classical descriptions of root methods by Schuurman and Goedewaagen (1971) and Böhm (1979), several recent reviews are available on methods to quantify root development and functioning in the field: Anderson and Ingram (1989), Caldwell and Virginia (1991), Jaillard (1987), Mackie-Dawson and Atkinson (1991), Taylor et al. (1991), Van Noordwijk (1987). We will concentrate here on some new methods to study the interaction of roots and soil structure, relevant to the concepts discussed above.

### 3. METHODS

#### *3.1. Distance measurements in pixel images*

As diffusion depends on gradients in concentration, model presentations should try to conserve the frequency distribution of distances between "source" and "sink" in the transport process (Van Noordwijk, 1987; Rappoldt, 1991; Tardieu, 1991). The traditional way of measuring "nearest neighbour" distances (Diggle, 1983) is to take a large number of randomly located points, measure the (Euclidean) distance to all objects of interest and choose the smallest distance. The process can be speeded up by using algorithms to restrict the number of potential neighbours for which distances have to be measured (Diggle, 1983). An alternative method to measure the frequency distribution of nearest neighbour distances is based on the fact that a circular disk of radius  $r$  contains all points with a distance to the circle centre less than or equal to  $r$ . By drawing circles of radius  $r$  around all objects of interest and measuring the area within the union of all these circles, the area fraction within distance  $r$  can be measured. By repeating this procedure for increasing values of  $r$ , until the whole area of the sample is included, the complete frequency distribution of nearest neighbour distances can be measured.

Computer image analysis is normally based on digitized arrays of square pixels. Although real circles cannot be presented, they can be closely approximated; measurements of area within the union of circles around objects of interest, is easily done by counting the number of pixels within that union. Several image analysis programs contain "distance transforms", which classify pixels by the radius of an approximated circle needed to reach them from the nearest object of interest. If such distance transforms are not available, and when the presence of barriers complicates distance measurements, a sequence of dilation or expansion steps can be used. Two basic forms for expansion exist, a 4-connective one where each pixel has four and a 8-connective one where it has eight neighbours. Alternating these two forms in a 2:1 ratio, in a 4-8-4 sequence, an octagon (Fig. 5A) is created which has a surface area close to that of a circle with a radius  $n$ , for each step in the sequence. By

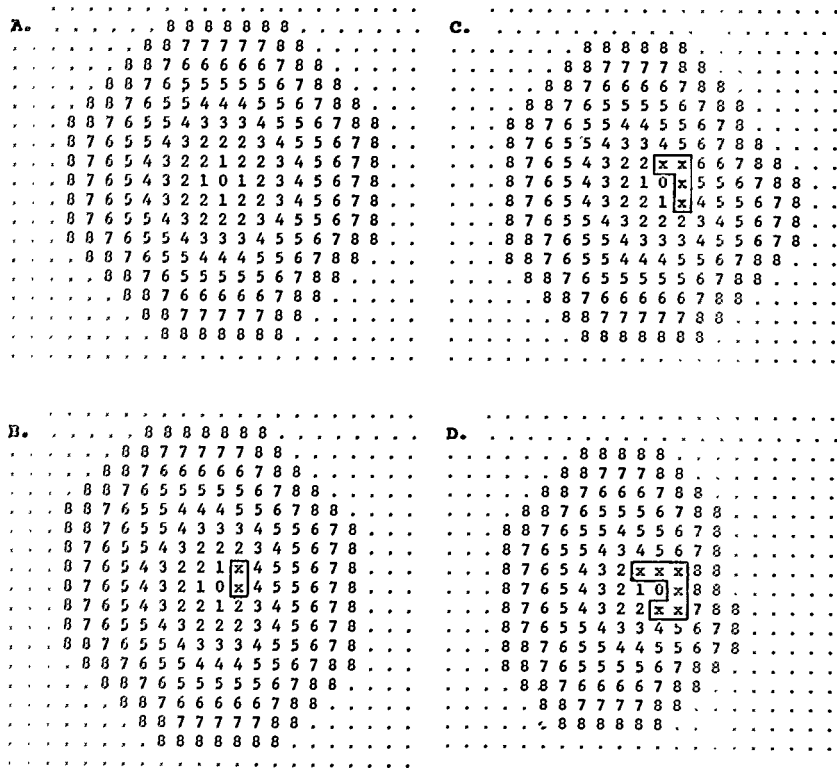


Fig. 5. Classification of area according to distance to the nearest root for the first 8 expansion steps around a root located at 0, for a root with complete (A) or incomplete root-soil contact (RSC) (B-D). (B)=75, (C)=50 and (D)=25%. Incomplete root-soil contact is represented by a number of x-pixels not included in the expansion. Distances were derived from a sequence of 4/8-4-connective steps (Kooistra et al., 1992), that means step 2, 5 and 8 are 8-connective.

skipping one 4-connective step after every 29 steps, the circle approximation can be improved (Kooistra et al., 1992). Figure 3 was derived using such an algorithm. Barriers can be introduced by—each step—subtracting the image of the barriers from the expanded image before measuring the area.

### 3.2. Root distribution pattern on soil maps

Root distribution can be observed on horizontally and vertically oriented planes in the soil. The relation between root length density,  $L_{rv}$ , and the number of root intersections per unit surface area,  $N$ , can be described as:  $L_{rv} = C_0 X N$ , where  $C_0$  is a (theoretical) calibration factor depending on root orientation and  $X$  an additional empirical calibration factor due to sampling errors. If roots are randomly oriented  $C_0$  is 2.0. For non-random orientation of roots  $C_0$  is close to 2.0 if  $N$  is the average point density in three mutually perpendicular planes of observation in the middle of the soil volume, but will vary between 1.0 and infinity if  $N$  is based on only one plane of observation (Van Noordwijk, 1987). For plane(s) of observation outside the volume of soil, a further correction factor is needed to estimate the number of intersections in the middle of the volume of soil; this correction is usually neglected but may be relevant for large volumes of soil or in situations with a pronounced trend in root length density. The empirical calibration factor  $X$  is probably due to sampling errors. When calibrating washed root length density against point density on thin sections Van Noordwijk et al. (1992) found  $X$  to be 1.0. If point densities are based on the core break method, an empirical calibration factor  $X$  of about 3.0 is not uncommon (Drew and Saker, 1980; own unpublished results). When root observations are based on profile walls similar values for  $X$  may be found, depending on the method used for preparing the profile walls. Such high calibration factors probably indicate that a major part of the finest branch roots are not recognized and cast doubt on the validity of this method of observing root distribution.

Several methods exist for preparing a profile walls for observation (Böhm, 1979). Removing soil with a brush, fork or pin, spraying the soil with a hand-sprayer, covering the soil overnight with cloth soaked in sodiumpyrophosphate to disperse clay or blowing soil away with an air current may all help, under certain conditions, to improve visibility of roots. A drawback of such methods, however, is that the plane of observation becomes less clearly defined. Certain observation procedures actually record root length in an exposed volume of soil. For studying the relation of roots with soil structure observations in a single plane should be preferred. Spraying the soil with water sometimes leads to smearing of the soil and roots may actually be covered by soil. In our laboratory we currently only smooth profile walls with a sharp blade and do not remove soil otherwise. Further development of techniques based on autofluorescence of roots and/or translocation of fluorescent dyes

applied aboveground might improve observations in future. Work on fluorescent dyes in our laboratory has not been successful so far.

Root patterns differ from regularity for two types of reasons: an inherent pattern, because branch roots originate from main axes and are originally close to them, and an imposed pattern, because soil characteristics such as structure restrict or stimulate root development locally. The first aspect is reflected in a model of the three-dimensional architecture of a maize root system (Pages et al., 1989). This model, and that of Diggle (1988) is based on branching rules as observed on washed root systems grown in soils without structural obstacles. Although at first sight root systems may appear to have a fractal nature, on more detailed analysis branching rules and root orientation change with branching order. The model of Pages et al. (1989) can generate root maps and numerical values of root coordinates for any plane of observation. Analysis of patterns on such maps, which reflect the inherent branching pattern, can be compared with those observed in real soils. Differences in pattern may then be attributed to soil characteristics, such as soil structure.

### *3.3. Synlocation of roots and resources*

Non-regular root distribution in structured soils usually means that nutrients, water and/or oxygen are also non-homogeneously distributed. A measure of spatial correlation between roots and resources (synlocation) is needed to quantify these effects.

Staricka et al. (1991) measured the frequency distribution of residue concentrations per unit soil volume for different soil tillage systems. Local concentrations of several times the average concentration occur on a  $\text{cm}^3$  scale with each type of tillage implement. As such residue concentrations can be observed macroscopically, they can be included in root maps. Synlocation of roots and residue can be quantified by measuring the area of, and the number of dots coinciding with, the residue and a series of expanded images (see section 3.1). Point density is thus estimated as a function of distance to the residue and can be analyzed statistically (Van Noordwijk et al., 1993a). Any object which can be observed macroscopically can be included in the map and analyzed similarly.

### *3.4. Root-soil contact*

Roots penetrating the soil matrix will initially have complete contact with the soil matrix plus micropores. Roots growing along aggregate surfaces or in macropores with a diameter larger than that of the root, will have incomplete contact with the soil. Root-soil contact can be directly observed on thin sections of the soil, provided that sample preparation, and especially the dehydration steps involved, did not lead to shrinkage of the root. Tests of the pro-

cedure for a pot experiment were given by Van Noordwijk et al. (1992), results for this pot experiment by Kooistra et al. (1992) and for a field trial by Van Noordwijk et al. (1993b). Jaillard (1987) combined a micromorphological technique with detailed chemical analysis of the rhizosphere.

### 3.5. Root position effectivity ratio, $R_{per}$

De Willigen and Van Noordwijk (1987) studied the effects of two types of complications in the geometry of the root-soil system: irregular distribution of roots and incomplete root-soil contact. The solutions given for both aspects separately left a question about combined effects. As both aspects are based on shifts in the transport distances involved, it may be possible to approach them in a similar way and thus include combined effects.

An analytical solution (Veen et al., 1992) showed that the nutrient residue was approximately proportional to the degree of root-soil contact. This solution was based on a sink in the centre of a circle. In a regular distribution of roots, a circle is a reasonable approximation, as it closely resembles the hexagons of a "watershed" division in which all transport is supposed to go in the direction of the nearest sink. We may expect, however, that incomplete root-soil contact will affect the watershed division, and that the root will be eccentric in its water-shed area (Fig. 5). In analyzing the effects of non-regular distribution De Willigen and Van Noordwijk (1987) found that an eccentric position in a soil cylinder of a root with complete root-soil contact reduces the uptake potential. For a root with incomplete root-soil contact, however, an eccentric position in the watershed area, with less soil on the "back" side where no root-soil contact exists, may improve the uptake potential. The increase in nutrient residue by incomplete root-soil contact may be less if the water-shed division is based on actual nearest neighbour distances. With the algorithm described in section 3.1 the frequency distribution of soil-to-root distances can be derived in the presence of air-filled barriers around a root, allowing only partial root-soil contact. Figure 5 shows the first eight steps of such an expansion for a root-soil contact of 100, 75, 50 and 25%, respectively. With decreasing root-soil contact, the root becomes progressively more eccentric in its watershed area, and the analytical solution by De Willigen and Van Noordwijk (1987) may overestimate the effect of incomplete contact.

For the derivation of  $R_{per}$ , the frequency distribution of soil-to-root distances should be determined (see Appendix; Van Noordwijk, 1992b). The next step is the construction of a set of circle annuli which conserve the frequency distribution of distances for an average root and to re-interpret this set of circle annuli as a set of circle sectors (Fig. 6; Appendix, points 4 and 5). Rappoldt (1991) described an algebraic solution for deriving sector frequencies from the frequency distribution of distances, if all distance incre-

---

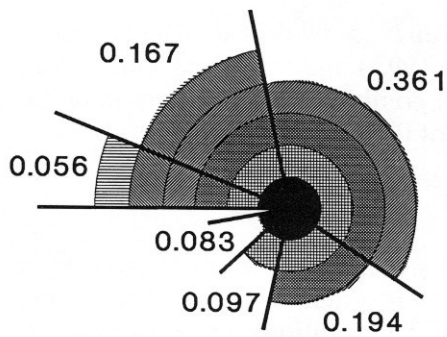


Fig. 6. Annulus fraction presentation of the frequency distribution of distances between (randomly chosen points in) the soil and the nearest root surface, for an average root in a realistic root distribution pattern (each type of shading indicates a different class of distances). Division of this shape into circle sectors allows application of cylindrical uptake models (Appendix; Van Noordwijk, 1993b).

ments (including the first) are equal. If the first increment is half as large, as used for an expansion in a pixel image, a modified algebraic solution is needed, or the iterative procedure used here. All sector frequencies are positive (or zero) when distances are measured on a map without barriers or interruptions. If gaps around the root are included in the distance measurements, some of the sector frequencies are negative.

For each of the sectors the cylindrical model can be applied to predict uptake. For the root system as a whole, the potential uptake rate can be predicted from the weighted sum of the uptake from each cylinder class, subtracting results for any negative frequencies. This approach assumes the average concentration in the soil solution, as well as soil water content to be constant. A partly unresolved question is to which extent and how rapidly physiological interactions occur between uptake rates of roots, if some roots can take up more than others. If supply is limiting uptake (the weighted sum is less than current demand), no such interaction is to be expected and by adding the potential uptake rate for each sector we obtain the potential uptake rate for the root system. Within a single soil layer with a uniform soil water content and average concentration in soil solution, for each root distribution with root length density  $L_{rv}$  a uniformly distributed, equivalent root length density  $L_{rv*}$  can be found which would leave the same residue in the soil (Appendix, step 6 and 7).  $R_{per}$  is now defined as  $L_{rv*}/L_r$  (Van Noordwijk, 1993b).

Most values of  $R_{per}$  observed so far on root maps from different soil structures are in the range 0.6–0.9. Figure 7 shows  $R_{per}$  for root distributions simulated by a layered Poisson process (Diggle, 1983), as defined by three parameters:  $N$  (number of dots per  $520 \times 520$  pixel frame), Relint (average number of roots per group; intensity of the clustering) and Reldis (maximum



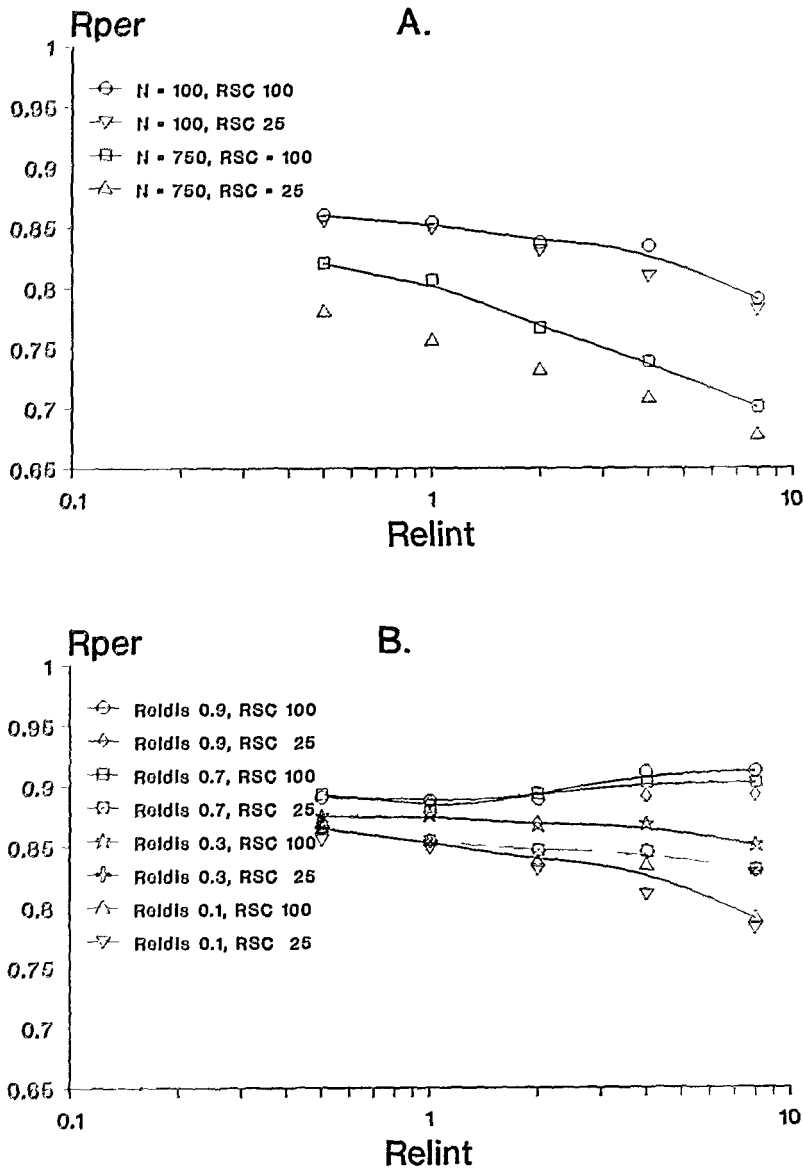


Fig. 7. Root position effectivity ratio,  $R_{per}$ , for root maps made in a layered Poisson process, with on average Relint branch roots per cluster and with distances to the main axes of Reldis times the average distance between neighbouring main roots; thick lines for complete, normal lines for 25% root soil contact. (A) Results for a different number of roots per field of view (520 x 520 pixels), with Reldis=0.1. (B) Results for various values of Reldis for N=100.

TABLE 1

Root densities and root position effectivity ratio ( $R_{\text{per}}$ ) for horizontally oriented thin-section samples from three depths at two fields (12B and 16A) of the Lovinkhoeve soil ecology experiment, June 1990 under winter wheat (Van Noordwijk et al., 1993b)

Depth (m)	$N/\text{cm}^2$		$R_{\text{per}}(100)$		$R_{\text{per}}(25)$	
	12B	16A	12B	16A	12B	16A
0.15	4.90	4.25	0.79	0.80	0.73	0.75
0.25	2.85	1.86	0.82	0.82	0.78	0.77
0.40	0.81	0.28	0.88	0.89	0.85	0.87

$R_{\text{per}}(100)$  and  $R_{\text{per}}(25)$  were calculated assuming complete or 25% root-soil contact, respectively, for all roots, with random orientation of the root-soil contact zone.

distance between branch roots and main axis, relative to the average distance between neighbouring main roots; grain size of the clusters). For a truly random pattern  $R_{\text{per}}$  values are 0.85–0.90 for realistic values of  $N$ . For clustered patterns  $R_{\text{per}}$  values in the range 0.7–0.85 are found, depending on  $N$  and Relint (Fig. 7A). If Relint is above 0.5 an increase in Relint has little effect on  $R_{\text{per}}$  (Fig. 7B).

Table 1 gives some results of  $R_{\text{per}}$  measurements on the root pattern observed on thin sections of the Lovinkhoeve soil ecology project. Observations were made on the thin sections described by Van Noordwijk et al. (1993b) to avoid sampling errors involved in larger profile wall maps. The  $R_{\text{per}}$  values found (assuming complete root-soil contact) were in the range 0.8–0.9, close to that expected for random patterns. In Table 1 and Fig. 7 results are included for 25% root-soil contact, as well. The effect of partial root-soil contact on  $R_{\text{per}}$  are smaller than expected and further checks of the method are needed.

### 3.6. Air-filled root porosity

Wetland plants normally have well developed aerenchyma in their roots, which may be connected to aerial parts of the plant (Crawford, 1989). Many non-wetland plants can also form air channels in their cortex, under conditions of poor aeration (Drew, 1990). Such air channels are normally not connective with shoot aerenchyma. Erdman et al. (1988) described that no shoot to root continuity of air channels exists in wheat, but that the coleoptile sheath allows oxygen to reach a point close to the basis of nodal roots. The main function of cortical air spaces may be to bridge sites of better and poorer aeration along a single root axis. The distance which can be bridged depends on respiration rate of root tissue, air-filled root porosity and on the conductance for oxygen of the exo- and epidermis root (Luxmoore et al., 1970; De Willi-

gen and Van Noordwijk, 1989). Reliable methods for measuring air-filled root porosity are available (Jensen et al., 1969; Van Noordwijk and Brouwer, 1989) and the adaptability of roots to poor aeration as a function of age and physiological state can be investigated (Laan et al., 1989). The conductance of tissues between cortical air spaces and the outside world is more difficult to quantify as yet. A related question is the connectivity of air channels between main axes and branch roots. Anatomical evidence suggests that a branch root, originating from the stele of a main axis, normally has a fully developed epidermis inside the cortex of the main axis and may even be covered by cell walls of collapsed cortical cells. Semi-quantitative evidence on the effects of such a barrier on oxygen transport can be obtained by using redox dyes (Crawford, 1989; Laan et al., 1989).

Figure 8 shows a method to visualize oxygen transport in roots. A plant is fixed to a plate and the root system is spread out between pins under water (Fig. 8A); oxygen is removed from the root surface by applying nitrogen gas. A water vessel (aquarium) is prepared with a redox dye, e.g., Cristal-Violet  $C_{22}H_{30}ClN_3$  using  $10 \text{ mg l}^{-1}$ , applied from a concentrated stock solution prepared in ethanol. The water is de-oxygenated and the dye is brought into the

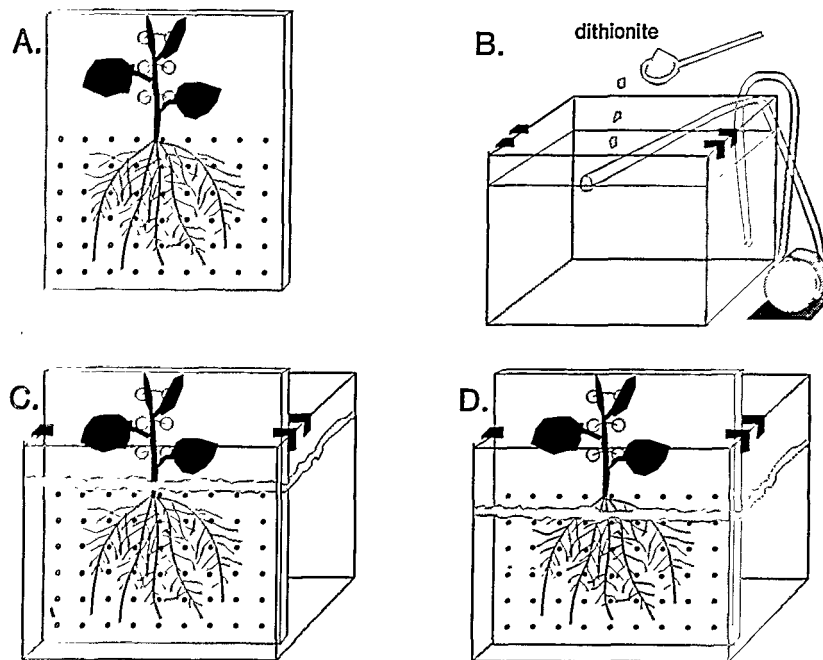


Fig. 8. Redox dye method for studying oxygen leakage out of roots as indication of functioning air channels; further explanation in text.

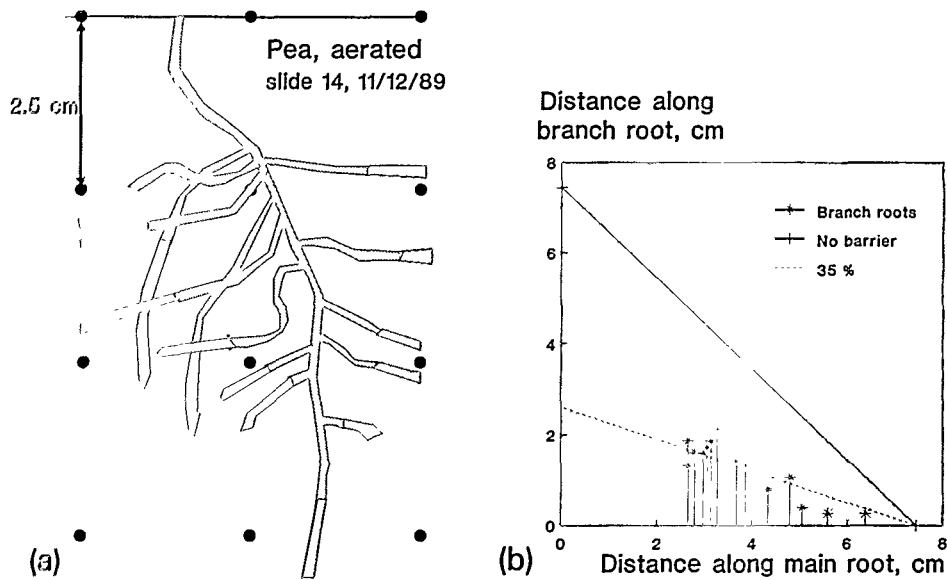


Fig. 9. (a) Blue pattern on a branched pea root in the redox dye test shown in Fig. 8. (b) Distance between water surface and furthest blue color on roots (explanation see text).

colorless reduced stage by adding sodiumdithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) and mixing the solution with an aquarium pump, without disturbing the surface (Fig. 3B). The plate with the root system is now placed into the solution; the amount of water in the vessel can be chosen such that either the shoot base is just under the water level ("high tide", oxygen in the roots has to come from the shoot) or that the basal part of the roots is exposed to air ("low tide", Fig. 3D). The water level is closed off using plastic grains as a mulch, to reduce oxygen enrichment of the water. A blue color will develop on the root surface where oxygen is leaking out of the epidermis. The pattern of blue coloring can be recorded on photographs or drawings. Maximum color intensity is reached in about 2 h, but the extend of the blue zone is not time dependent.

Figure 9a shows the dye pattern observed on a branched pea root. In Fig. 9b the distance between the transition blue-white and the atmosphere is given, separated in a distance along the main root (X-axis) and branch root (Y-axis). Points would fall on a  $-45^\circ$  line if no barrier existed between main and branch root and transport in the branch root was as effective as that in the main root. The pattern observed suggests that a partial barrier between main and branch root does exist. Observations on other crops, e.g. rice, suggests that in large parts of older roots the root exodermis is impermeable for oxygen, increasing the roots effectivity as air channel, but decreasing the possibility of oxygen uptake in favorable zones as well.

3.7. Dynamics of root growth and decay

Minirhizotrons offer a flexible method of studying the dynamics of root growth and decay under field conditions (Lussenhop et al., 1992). By recording the fate of individual roots or at least on the number of roots which died and the number which appeared new in each observation interval (Fig. 10), information can be obtained which is much more reliable than that of sequential destructive sampling. For studies of the effects of soil structure on dynamics of root growth and decay, however, one has to be careful that the observation plane does not introduce artefacts. Incomplete contact between the minirhizotron wall and the soil may allow roots to grow in a gap, with little relation with the surrounding soil. Condensation of water on the minirhizotron wall, caused by differences in temperature between soil and air inside the minirhizotron, is a clear symptom that a gap exists between minirhizotron and soil. Gijssman et al. (1991) described an inflatable system which avoids such a gap. A comparison between fixed-wall and inflatable minirhizotrons may allow a test of the hypothesis that roots growing in macropores (cracks) are more susceptible to herbivory and may live shorter than roots which created a new channel in the soil matrix.

4. EPILOGUE

A combination of the methods described here may allow a better under-

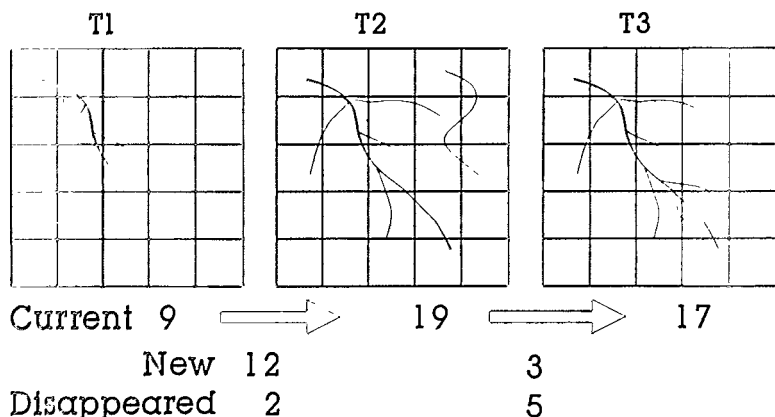


Fig. 10. Analysis of root growth and death from sequential root images, e.g., obtained by minirhizotron technique. For each interval the number of new intersections between roots and lines of a grid is counted, as well as the number which has disappeared. Actual root intensity, root growth and root disappearance can then be expressed as fraction of the cumulative number of new root intersections for the whole season. If at one date a destructive root sampling yielded reliable estimates of actual root length density, absolute root production estimates can be obtained.

standing of processes by which soil structure affects root growth and functioning and of differences between crops in susceptibility for poor soil structures. Still, for practical applications no real alternatives exist as yet for the synthetic view on soil structure of spade tests and visual rating systems (Taylor et al., 1991).

#### APPENDIX. STEPS IN DERIVING THE ROOT POSITION EFFECTIVITY RATIO, $R_{per}$

(1) Digitize a map of the root distribution in a horizontal or vertical plane and derive a list of the  $X, Y$  coordinates of roots.

(2) Introduce "barriers" in the root map, e.g., representing incomplete root-soil contact.

(3) Derive the frequency distribution of distances from points in the soil to the nearest neighbour root, e.g. by stepwise dilation of root points, allowing for the root barriers, and measurement of the area (number of pixels) after each step; a relative frequency distribution is obtained by dividing all values by the number of roots on the map.

(4) Derive an "annulus fraction"  $f_{a,i}$  representation of these nearest-neighbour distances by dividing the fraction of soil in each distance interval  $R_i$  to  $R_{i+1}$  by the area of a circle annulus,  $\pi(R_{i+1}^2 - R_i^2)$ ; the result can be presented graphically as in Fig. 6.

(5) Determine the circle frequencies,  $f_{c,i}$  by "cutting the pie", i.e., for  $j=0$  to  $n$  calculate:

$$f_{i,n-j} = f_{a,n-j} - \sum_{i=n-j}^n f_{c,i}$$

(6) Derive the  $G$ -sum  $\sum f_{c,i} G(\rho_i)$ , where the  $G$ -function is based on  $R_i$  (eq. 3).

(7) Find (by iteration)  $\rho^*$  for which  $G(\rho^*) = \sum f_{c,i} G(\rho_i)$  and the corresponding  $L_{rv}^*$ .

(8)  $R_{per} = L_{rv}^* / L_{rv}$ .

A programme for steps 2 to 8 has been developed for a Quantimet 570 image analyzer, and is available on request.

#### REFERENCES

- Anderson, J.M. and Ingram, J.S.I., 1989. Tropical Soil Biology and Fertility: a Handbook of Methods. CAB International, Wallingford.
- Barlow, P.W., Brain, P., Butler, R. and Parker, J.S., 1991. Modelling the growth of gravireacting roots. *Aspects Appl. Biol.*, 26: 221-226.
- Boekel, P., 1982. Soil structure in modern agriculture. Proc. 9th Conf. Int. Soil Tillage Res. Org., Osijek, Yugoslavia, pp. 402-408.
- Böhm, W., 1979. *Methods of Studying Root Systems*. Springer, Berlin, 188 pp.
- Boone, F.R., 1988. Weather and other environmental factors influencing crop response to tillage and traffic. *Soil Tillage Res.*, 11: 283-324.
- Boone, F.R. and Veen, B.W., 1992. Mechanisms of crop responses to soil compaction. In: B.D.

- Soane and C. van Ouwerkerk (Editors), *Soil Compaction in Crop Production*. Elsevier, Amsterdam (in press).
- Caldwell, M.M. and Virginia, R.A., 1991. Root systems. In: R.W. Pearcy, J. Ehleringer, H.A. Mooney and P.W. Rundel (Editors), *Plant Physiological Ecology: Field Methods and Instrumentation*. Chapman and Hall, London, pp. 367–398.
- Crawford, R.M.M., 1989. *Studies in Plant Survival, Ecological Case Histories of Plant Adaptation to Adversity*. Blackwell, Oxford, 296 pp.
- Darrah, P.R., 1991. Models of the rhizosphere. II. A quasi three-dimensional simulation of the microbial population dynamics around a growing root releasing soluble exudates. *Plant Soil*, 133: 147–158.
- Darwin, Ch., 1880. *The Power of Movement in Plants*. John Murray, London.
- De Willigen, P. and Van Noordwijk, M., 1987. Roots, plant production and nutrient use efficiency. Doctoral Thesis, Agricultural University, Wageningen, 282 pp.
- De Willigen, P. and Van Noordwijk, M., 1989. Model calculations on the relative importance of internal longitudinal diffusion for aeration of roots of non-wetland plants. *Plant Soil*, 113: 111–119.
- De Willigen, P. and Van Noordwijk, M., 1991. Modelling nutrient uptake: from single roots to complete root systems. In: F.W.T. Penning de Vries, H.H. van Laar and M.J. Kropff (Editors), *Simulations and Systems Analysis for Rice Production (SARP)*. Pudoc, Wageningen, pp. 277–295.
- Diggle, P.J., 1983. *Statistical Analysis of Spatial Point Patterns*. Academic Press, London, 148 pp.
- Diggle, A.J., 1988. ROOTMAP—a model in three-dimensional coordinates of the growth and structure of fibrous root systems. *Plant Soil*, 105: 169–178.
- Drew, M.C., 1990. Sensing soil oxygen. *Plant, Cell Environ.*, 13: 681–693.
- Drew, M.C. and Saker, L.R., 1980. Assessment of a rapid method using soil cores for estimating the amount and distribution of crop roots in the field. *Plant Soil*, 55: 297–305.
- Erdman, B., Wiedenroth, E.M. and Ostareck, D., 1988. Anatomy of the root–shoot junction in wheat seedlings with respect to internal oxygen transport and root growth retardation by external oxygen shortage. *Ann. Bot.*, 62: 521–529.
- Gijzen, A.J., Floris, J., Van Noordwijk, M. and Brouwer, G., 1991. An inflatable minirhizotron system for root observations with improved soil/tube contact. *Plant Soil*, 134: 261–269.
- Griffiths, B. and Robinson, D., 1992. Root-induced nitrogen mineralisation: A nitrogen balance model. *Plant Soil*, 139: 253–263.
- Haimah, K., Van Noordwijk, M. and Setijono, S., 1991. Tolerance to acid soil conditions of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringiana*. I. Root development. *Plant Soil*, 134: 95–105.
- Hewitt, J.S. and Dexter, A.R., 1984. The behaviour of roots encountering cracks in soil. II. Development of a predictive model. *Plant Soil*, 79: 11–28.
- Ismail, S.N.A., 1975. Micromorphometric soil-porosity characterization by means of electro-optical image analysis (Quantimet 720). Soil survey papers No. 9. Soil Survey Institute, Wageningen.
- Jaillard, B., 1987. Techniques for studying the ionic environment at the soil/root interface. 20th Colloq. Int. Potash Inst., Baden bei Wien, pp. 247–261.
- Jensen, C.R., Luxmoore, R.J., Gundy, S.D. and Stolzy, L.H., 1969. Root air space measurements by a pycnometer method. *Agron. J.*, 61: 474–475.
- Koortstra, M.J., 1990. Applications of micromorphology to agronomic and environmental sciences. Trans. 14th Int. Congr. of Soil Science, Kyoto, VII: 226–331.
- Koortstra, M.J., Schoonderbeek, D., Boone, F.R., Veen B.W. and Van Noordwijk, M., 1992. Root–soil contact of maize, as measured by a thin-section technique. II. Effects of soil compaction. *Plant Soil*, 139: 119–129.

- Laan, P., Smolders, A., Blom, C.W.P.M. and Armstrong, W., 1989. The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flood tolerance of *Rumex* species. *Acta Bot. Neerl.*, 38: 131–145.
- Lammers, H., 1987. Growth, respiration, exudation and symbiotic associations; the fate of carbon translocated to the roots. In: Gregory, P.J., Lake, J.V. and Rose, D.A. (Editors), *Root Development and Function*. Cambridge University Press, Cambridge, pp. 125–145.
- Lipiec, J., Hakansson, I., Tarkiewicz, S. and Kossowski, J., 1991. Soil physical properties and growth of spring barley as related to the degree of compactness of two soils. *Soil Tillage Res.*, 19: 307–317.
- Lucas, W.J., 1987. Functional aspects of cells in root apices. In: P.J. Gregory, J.V. Lake and R.A. Rose (Editors), *Root Development and Function*. Cambridge University Press, Cambridge, pp. 27–52.
- Lussenhop, J., Fogel, R. and Pregitzer, K., 1993. Observing soil biota in situ. In: L. Brussaard and M. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. *Geoderma*, 56: 25–36, Session I, this volume.
- Luxmoore, R.J., Stolzy L.H. and Letey, J., 1970. Oxygen diffusion in the soil plant system: I. A model. *Agron. J.*, 62: 317–322.
- Mackie-Dawson, L.A. and Atkinson, D., 1991. Methodology for the study of roots in field experiments and the interpretation of results. In: D. Atkinson (Editor), *Plant Root Growth, an Ecological Perspective*. Blackwell, London, pp. 25–47.
- McCully, M.E., 1987. Selected aspects of the structure and development of field-grown roots with special reference to maize. In: P.J. Gregory, J.V. Lake and R.A. Rose (Editors), *Root Development and Function*. Cambridge University Press, Cambridge, pp. 53–70.
- Necteson, J.J., 1993. The risk of nitrate leaching after application of nitrogen fertilizers to agricultural crops. In: D.C. Adriano (Editor), *Advances in Environmental Science, Groundwater Series*. Springer, New York (in press).
- Nordmeyer, H. and Richter, J., 1985. Incubation experiments on nitrogen mineralization in loess and sandy soils. *Plant Soil*, 83: 433–445.
- Pages, L., Jordan, M.O. and Picard, D., 1989. A simulation model of the three-dimensional architecture of the maize root system. *Plant Soil*, 119: 147–154.
- Pruschen, G., 1983. Die Spatendiagnose und ihre Auswertung. In: L. Kutschera (Editor), *Root Ecology and its practical Applications*. Bundesanstalt Gumpenstein, Irdning, pp. 355–368.
- Rappoldt, C., 1991. The application of diffusion models to aggregated soil. *Soil Sci.*, 150: 645–661.
- Ringrose-Voase, A.J., 1987. A scheme for the quantitative description of soil macrostructure by image analysis. *J. Soil Sci.*, 38: 343–356.
- Schuurman, J., 1971. Effect of supplemental fertilization of oats with restricted root development. *Z. Acker- Pflanzenbau*, 133: 315–320.
- Schuurman, J.J. and De Boer, J.J.H., 1974. The response of root growth, uptake and shoot growth of oats on soil compaction. *Landbouwk. Tijdschr.*, 86: 256–265 (in Dutch).
- Schuurman, J.J. and Goedewaagen, M.A.J., 1971. Methods for the examination of root systems and roots. PUDOC, Wageningen, 2nd ed, 86 pp.
- Spurny, M., 1966. Spiral feedback oscillations of growing hypocotyl with radicle in *Pisum sativum*, L. *Biol. Plant.*, 8: 381–392.
- Staricka, J.A., Allmaras, R.R. and Nelson, W.W., 1991. Spatial variation of crop residue incorporated by tillage. *Soil Sci. Soc. Am. J.*, 55: 1668–1674.
- Tardieu, F., 1991. Spatial arrangement of maize roots in the field. In: B.L. McMichael and H. Persson (Editors), *Plant Roots and their Environment*. Elsevier, Amsterdam, pp. 506–514.
- Taylor, H.M., Upchurch, D.R., Brown, J.M. and Rogers, H.H., 1991. Some methods of root investigations. In: B.L. McMichael and H. Persson (Editors), *Plant Roots and their Environment*. Elsevier, Amsterdam, pp. 553–564.



- Van Noordwijk, M., 1987. Methods for quantification of root distribution and root dynamics in the field. 20th Colloq. Int. Potash Inst., Baden bei Wien, pp. 263–281.
- Van Noordwijk, M., 1993a. Three levels of complexity in root ecological studies. In: L. Kutschera (Editor), *Root Ecology and its Practical Application* (in press).
- Van Noordwijk, M., 1993b. Root position effectivity ratio,  $R_{per}$ , a simple measure of the effects of non-homogeneous root distribution on uptake of homogeneous resources. In: L. Kutschera (Editor), *Root Ecology and its Practical Application* (in press).
- Van Noordwijk, M. and Brouwer, G., 1989. Quantification of air-filled root porosity: A comparison of two methods. *Plant Soil*, 111: 255–258.
- Van Noordwijk, M. and De Willigen, P., 1987. Agricultural concepts of roots: from morphogenetic to functional equilibrium. *Neth. J. Agric. Sci.*, 35: 487–496.
- Van Noordwijk, M. and De Willigen, P., 1991. Root functions in agricultural systems. In: B.L. McMichael and H. Persson (Editors), *Plant Roots and their Environment*. Elsevier, Amsterdam, pp. 381–395.
- Van Noordwijk, M. and Van der Geijn, S.C., 1992. Root, shoot and soil parameters required for process-oriented models of crop growth limited by water or nutrients. In: A. Smucker (Editor), *New Methods of Root Research*. ASA Spec. Publ. (in press).
- Van Noordwijk, M. and Wadman, W., 1992. Effects of spatial variability of nitrogen supply on environmentally acceptable nitrogen fertilizer application rates to arable crops. *Neth. J. Agric. Sci.* (in press).
- Van Noordwijk, M., De Willigen, P., Ehlert, P.A.I. and Chardon, W.J., 1990. A simple model for P uptake by crops as a possible basis for P fertilizer recommendations. *Neth. J. Agric. Sci.*, 38: 317–332.
- Van Noordwijk, M., Kooistra, M.J., Boone, F.R., Veen, B.W. and Schoonderbeek, D., 1992. Root–soil contact of maize, as measured by a thin-section technique. I. Validity of the method. *Plant Soil*, 139: 109–118.
- Van Noordwijk, M., De Ruiter, P.C., Zwart, K.B., Bloem, J., Moore, J.C., Van Faassen, H.G. and Burgers, S., 1993a. Synlocation of biological activity, roots, cracks and recent organic inputs in a sugar beet field. In: L. Brussaard and M. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. *Geoderma*, 56: 265–276, Session I, this volume.
- Van Noordwijk, M., Schoonderbeek, D. and Kooistra, M.J., 1993b. Root–soil contact of field grown winter wheat. In: L. Brussaard and M. Kooistra (Editors), *Int. Workshop on Methods of Research on Soil Structure/Soil Biota Interrelationships*. *Geoderma*, 56: 277–286, Session I, this volume.
- Veen, B.W., Noordwijk, M. van, Willigen, P. de, Boone, F.R. and Kooistra, M.J., 1992. Root–soil contact of maize, as measured by a thin-section technique. III. Effects on shoot growth, nitrate and water uptake efficiency. *Plant Soil*, 139: 131–138.