Methods for Quantification of Root Distribution Pattern and Root Dynamics in the Field

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Summary

For a functional evaluation of root systems in the field, two aspects deserve special interest and require separate methods for their study:

A. Spatial root distribution: heterogeneity of local root density in relation to planting pattern, soil structure, and heterogeneity of nutrient supply. Recently such heterogeneities have become the subject of research as such and are no longer treated as merely a nuisance in establishing effects of experimental treatments on average root densities. On a detailed level the degree of actual soil-root contact has become of interest.

B. Root dynamics in time: root growth and decay in the field can hardly be studied by destructive means, due to the large spatial heterogeneities. They have to be quantified by repeated observations of the same roots, under conditions which approach field conditions as much as possible.

For both types of information additions to and improvements of classical techniques are given: relations between basic root parameters, sampling schemes, quantification of root anisotropy, quantification of pattern in root distribution, quantification of soil-root contact and the mini-rhizotron technique for quantifying root dynamics.

1. Introduction

As the root system in its size, distribution and dynamical pattern forms an important link between plant and soil, the current trend of devising more efficient fertilisation techniques requires thorough knowledge of roots *(Van Noordwijk & De Willigen [1986])*. Classical techniques for excavation and study of roots have been described extensively *(Schuurman & Goedewaagen [1971]; Böhm [1978])*; in this paper new additions to the methods will be discussed.

Root studies in the field usually have one of the following three objectives:
- study of overall root pattern as indicator of soil conditions,
- quantification of roots for an interpretation of the relative availability of water and nutrients in various soil layers throughout the season, and
- quantification of input of organic matter through the root system to the soil ecosystem.

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Here we will concentrate on the last two, quantitative objectives. Root research in the past mainly quantified root dry weight, which is still of interest for the third research objective. For the second objective, root length and root surface area are more interesting parameters. We will first consider the relations between these root parameters.

2. Relations between basic root parameters

2.1 Geometry of roots

Generally roots can be assumed to be cylindrical in shape, *i.e.* increase of diameter along the length of a root and deviations from a circular shape in cross section are negligible. For such a cylinder simple relationships exist between length, surface area and volume:

$$V_r = \pi \cdot R_o^2 \cdot L_r = A_r \cdot R_o/2 \ [cm^3] \qquad (1)$$

where:
V_r = root volume [cm^3]
A_r = root surface area [cm^2]
L_r = root length [cm]
R_o = root radius [cm]

Root volume is related to root fresh weight via the specific weight and root porosity; root dry weight is related to root fresh weight via the dry matter content:

$$D_r = M_{d,r} \cdot F_r = M_{d,r} \cdot (1\text{-}P_r) \cdot S_r \cdot V_r \ [g] \qquad (2)$$

where:
D_r = root dry weight [g]
F_r = root fresh weight [g]
$M_{d,r}$ = dry matter content of roots
P_r = air filled root porosity as fraction of V_r
S_r = specific weight of root tissue without air filled pores[g cm^{-3}]

A root system consists of a set of partly interconnected cylinders of various lengths and diameters. The relationships between root system values of basic dimensions such as length, surface area and volume are similar to those for single roots, except for the definition of the average root radius. If the root system consists of n classes of roots, each with root radius R_o (i) and root length per class L_r (i), we may define two types of average root radius, a linear average $\hat{R}_o$ and a quadratic average $\hat{\hat{R}}_o$:

$$\hat{R}_o = \left(\sum_{i=1}^{n} L_r(i) \cdot R_o(i)\right) / \sum_{i=1}^{n} L_r(i) \ [cm] \qquad (3)$$

$$\hat{R}_o = \sqrt{\sum_{i=1}^{n} L_r(i) \cdot R_o(i)^2 / \sum_{i=1}^{n} L_r(i)} \text{ [cm]} \qquad (4)$$

For the root system as a whole we find:

$$V_{rp} = \sum_{i=1}^{n} V_r(i) = \pi \cdot \hat{R}_o^2 \sum_{i=1}^{n} L_r(i) = \pi \cdot \hat{R}_o^2 \cdot L_{rp} \qquad [cm^3] \qquad (5)$$

$$A_{rp} = \sum_{i=1}^{n} A_r(i) = 2 \cdot \pi \cdot \hat{R}_o \sum_{i=1}^{n} L_r(i) = 2\pi \cdot \hat{R}_o \cdot L_{rp} \qquad [cm^2]$$

and consequently

$$V_{rp} = A_{rp} \cdot (\hat{R}_o/\hat{R}_o)^2 \cdot \hat{R}_o/2 \qquad [cm^3] \qquad (7)$$

The term $\hat{R}_o/\hat{R}_o$ has been neglected by several authors when relating root volume to root length via average root diameters. This term reflects the variation in root diameters in the root system, being 1.0 for homogeneous root systems and >1 for heterogeneous root systems. If root porosity and dry matter content vary independently from root diameter, equation (2) holds for the root system, using (linear) average values for all parameters. Combining (2) and (7) we find for the specific root surface area, A_{rp}/D_{rp}:

$$A_{rp}/D_{rp} = 1/(M_{d,r} \cdot (1-P_r) \cdot S_r \cdot (\hat{R}_o/2) \cdot (\hat{R}_o/\hat{R}_o)^2) \quad [cm^2/g]$$

For the specific root length, L_{rp}/D_{rp}, we find:

$$A_{rp}/D_{rp} = 1/(\pi \cdot M_{d,r} \cdot (1-P_r) \cdot S_r \cdot (\hat{R}_o^2 \cdot (\hat{R}_o/\hat{R}_o)^2) \quad [cm/g] \qquad (9)$$

Figure 1 shows commonly found values for the A_{rp}/D_{rp} and L_{rp}/D_{rp} ratio, as influenced by the parameters of equations (8) and (9). A plant apparently has four possibilities to obtain a larger surface area per unit dry weight: a low average root radius, a low dry matter content, a high root porosity and a homogeneous root system (in terms of root radius). The parameter S_r will not deviate much from 1.0.

Equation (9) shows that the average root radius alone does not give sufficient information to estimate the ratio of root length and root dry weight. Moreover, the condition used for deriving equation (9) that root porosity and dry matter content vary independently of root diameter will not usually be fulfilled. Root length and dry weight have to be measured on individual (sub)samples to get reliable results.

Specific root length for various crops and situations usually is in the range 100-300m/g, for roots with an average root diameter of 0.2-0.3mm.

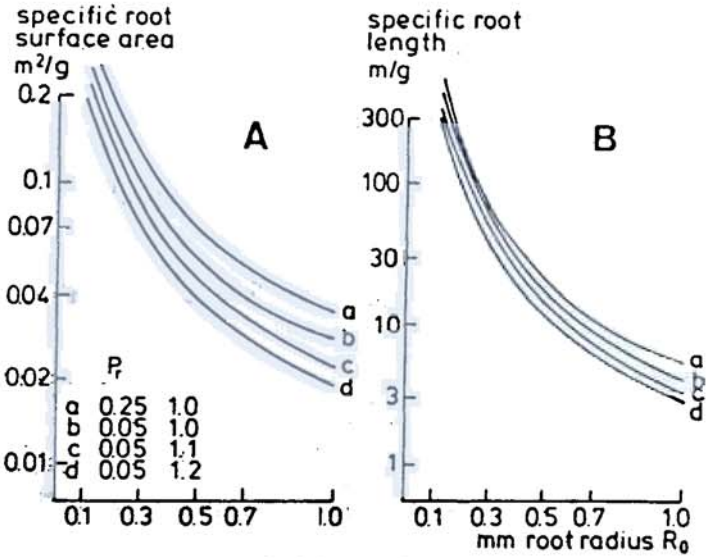

Fig. 1 Ratio of root dry weight and root surface area (A) or root length (B) as function of average root diameter (equations [8] and [9]); parameters used $M_{d,r} = 0.075$, $P_r = 0.05$ or 0.25, $S_r = 1.0$; the lines for $P_r = 0.25$ can also be interpreted as $M_{d,r} = 0.059$, $P_r = 0.05$.

2.2 Methods

Usually root length and diameters are determined on subsamples, using dry weight as a reference for calculating total root length and surface area. Root length is measured on a grid by counting root-line intersections following *Tennant [1975]*, choosing the grid size in such a way that about 400 intersections are found per subsample (this reduces the experimental error to less than 5%). Root diameter is measured on 20-30 randomly chosen roots per subsample. For calculations of average root diameter data of a number of subsamples have to be pooled, depending on the coefficient of variation; for a c.v. of 0.3, 40 readings are required to reduce this experimental error to less than 10%, for a c.v. of 0.5 100 readings and for a c.v. of 0.7 200 readings, in practice the c.v. of root diameters in root samples excluding tap and main roots is found somewhere in this range. By measuring root diameter at every 10th intersection of a root system with a line grid, both types of average root radius defined in (3) and (4) can be calculated:

$$\bar{R}_o = \sum_{i=1}^{n} R_o/n \qquad (10)$$

$$\bar{R}_o^2 = \sum_{n=1}^{n} R_o\,(i)^2/n = \mathrm{var}(R_o) + \bar{R}_o^2 \qquad (11)$$

with $Var(R_o)$ as the variance in the usual statistical definition. The latter equation implies that the factor $(\bar{R}_o/R_o)^2$ in equations (8) and (9) is given by:

$$\left(\frac{\bar{R}_o}{R_o}\right)^2 = CV(R_o)^2 + 1 \qquad (12)$$

with $CV(R_o)$ as the coefficient of variation (standard deviation divided by the mean).

When data on specific root length (or surface area) of subsamples are to be used for calculation of total root length in a sample as a whole, losses of dry weight due to sample handling have to be known. Losses of dry weight of roots during washing and handling of roots have been evaluated recently *(Brouwer & van Noordwijk [1978]; Floris & van Noordwijk [1979]; Floris & De Jager [1981]; Grzebisz et al.*, in prep.). As Figure 2 shows a rapid loss of dry weight initially after sampling is followed by a stabilisation of root dry weight at 60-80% of the original value, depending on crop and washing procedure used; sodium-pyrophosphate for clay soils and HCl for seperating roots from rockwool result in relatively large losses. The first step in dry matter loss may be due to root respiration, although respiration inhibitors did not stop dry matter loss of cucumber roots.

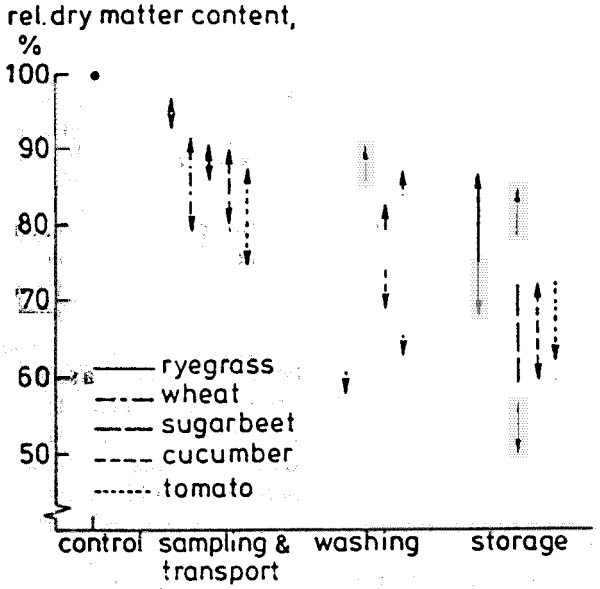

Fig. 2 Dry matter loss during washing and handling of root samples, simulating various standard procedures on roots obtained from a solution culture: ryegrass *(Floris & De Jager [1981])*, wheat *(Van Noordwijk & Floris [1979])*, tomato *(Brouwer & Van Noordwijk [1978])*, cucumber (unpublished, treatments include a respiration inhibitor (0.1 mM KCN + 25 mM salicyl-hydroxamate at pH 5)), and sugar beet *(Grzebis et al.*, in prep.).

Stabilisation of dry matter after the initial losses allows L_r/D_r ratios for subsamples to be used for the remaining part of the roots. Values for $M_{d,r}$ usually reported for field studies probably are 20-40% lower than those of intact roots.

An unsolved problem in root research is the establishment of a reliable criterion for distinguishing between live and dead roots. Conventionally visual and «manual» criteria (colour and elasticity) are used when cleansing roots. TTC (tetrazoliumchloride) can sometimes be used on rapidly washed roots, as its colour reaction depends on H-donation by metabolically active roots under anaerobic conditions *(Schuurman & Goedewaagen [1971])*. Other staining and microscopic techniques are also used *(Holden [1975]; McCully & Canny [1985]). Dyer & Brown [1983]* described a technique for observing fluorescence of roots in the cell-elongation stage. For a physiological interpretation of possibilities for uptake, the TTC method may give the most reliable criterion, despite problems of observing a colour change on dark-coloured older roots.

Root porosity can be measured by comparing the specific weight of roots as such and after grinding, when all pores may be expected to be water-filled *(Jensen et al. [1969])*. Results of this technique are in agreement with visual inspection of microscope slides *(Van Noordwijk & Brouwer,* in prep.). Root porosity is important for internal aeration of roots when the external oxygen supply is insufficient.

3. Spatial distribution of roots

3.1 Distribution patterns on various scales

Root parameters may be expressed per plant, per unit soil volume or per unit cropped soil area. The first way is most relevant for studying root/shoot relationships, the second for studying relative depletion of nutrients and water present in the soil and the third for studies on a crop level, for instance of dry matter input into the soil ecosystems by roots.

These three bases of comparison may be distinguished by a second subscript: L_{rp} [cm], L_{rv} [cm cm^{-3}] and L_{ra} [cm cm^{-2}], respectively (similarly for A_r etcetera). L_{rv} can be called «root (length) density». The dimensionless L_{aa} has previously been defined as *Root Area Index*, in analogy to the Leaf Area Index *(Barley [1970])*. A theoretical framework for a functional interpretation of L_{rv} values for depletion of N, K, P and water is now available *(Van Noordwijk [1983]; De Willigen & Van Noordwijk,* in prep.).

When considering root systems under closed crop canopies roots of neighbouring plants usually are intermingled and an individual plant may not be a convenient base for expressing root parameters. The size of the root system of an «average» plant corresponds to the amount of roots under a «unit soil area», U_a, as defined in Figure 3A.

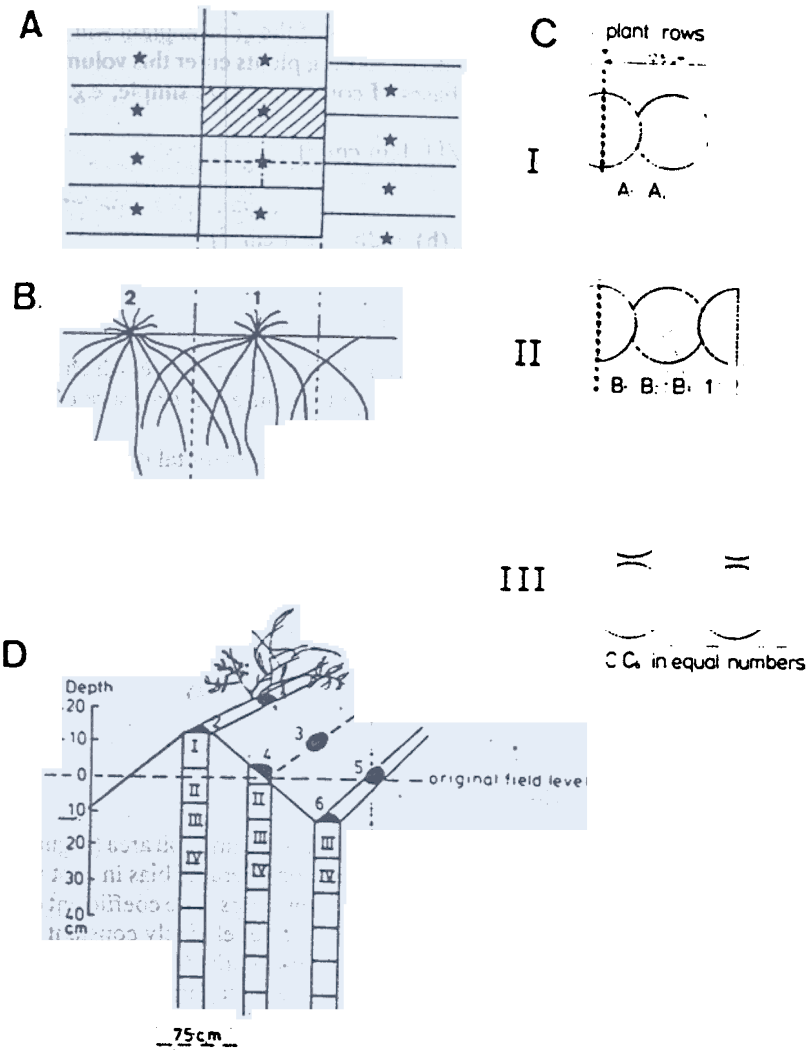

Fig. 3 A. Top view of the «unit soil area», Ua, for row crops; plants are indicated by an asterisk, Ua by the shaded area. B. Side view of the root system under the unit soil area. C. Sampling schemes for auger sampling: I. for row crops with narrow spacing *(e.g.* 12.5 cm), equal number of samples A1 and A2, II. for row crops with wide spacing *(e.g.* 25 cm), B1, B2 and B3 in 1:2:1 ratio, III. for crops with wider spacing in the row *(e.g.* sugar beet, C1, C2, C3, C4, C5, C6 in equal numbers), D. for ridged crops such as potato, D1, D2, D3, D4, D5, D6 in equal numbers *(Van Noordwijk et al. [1985a])*.

The unit soil area equals the reciprocal of plant density. Figure 3B shows that it may be expected that an equal number of roots of the central plant will be found outside the unit soil area, as roots of neighbouring plants enter this volume of soil. Relationships between the various bases of comparison are simple, *e.g.*

$$L_{ra} = L_{rp}/U_a \text{ [cm cm}^{-2}\text{]} \tag{13}$$

$$L_{ra} = \int_0^{Zr} L_{rv}(h) \cdot dh \quad \text{[cm cm}^{-2}\text{]} \tag{14}$$

where h = depth and Z_r = depth of rooted zone.

The unit soil area may be divided into four quarters of equal size, which form the smallest representative area of the field, except for different exposure of the soil surface to the sun.

Root density parameters vary in both vertical and horizontal direction. Part of this variation may be correlated with depth h or with radial distance r to the plant. L_{rv} (h, r, t) gives the average root length per unit volume of soil at depth h, at radial distance r from the plant and at time t.

Especially in the top layers of the soil a considerable part of the variation can be attributed to radial distance to the plant, r; this fact has practical consequences for sampling schemes. Depending on the adequacy of the L_{rv} (h, r) description used, a certain amount of variation around this average value will remain, which can be described as «pattern» in the root distribution (chapter 3.4)

3.2 Washed root samples

A careful consideration of sampling schemes to cover the unit soil area (Figure 3 C) may help to describe L_r (h, r) and may avoid the considerable bias in root weight which is inherent to the conventional scheme for row crops. The coefficient of variation in root weight in auger samples was found to be relatively constant with a value of 30-50% for different sampling occasions and depths *(Van Noordwijk et al. [1985a])*. Such a variation necessitates a large number of replicates, *e.g.* at least 10 to recognize 35% differences between two means and 25 for 20% differences, respectively.

Roots can be washed free from soil using samples either obtained by a soil auger (easily replicated, small samples) or obtained as soil monoliths, usually washed on pinboards (showing the complete shape of a root system). A monolith sampler which minimizes damage to experimental plots has been described by *Floris & Van Noordwijk [1984]*. Washed roots have to be separated from debris and dead roots by hand, which still is the rate-limiting step in root research.

For measuring mycorrhizal hyphae and root hairs root samples have to be washed with greater care than otherwise. Small samples of soil are used from which

roots are hand-picked and gently washed without trying to remove all soil particles. Mycorrhizal hyphae can be stained with trypan blue in lactophenol *(Philips & Hayman [1970])* and examined under a microscope. In addition to the conventional estimation of the percentage of root length which shows infection, hyphal length can be estimated from very gently washed samples. An intersection technique for recording both hyphal and root length in the sample is used, with root length as a basis for calculations for the root system.

3.3 Counts of roots intersecting a plane

An important aspect of variation in root distribution, related to root orientation, is described by the degree of anisotropy. An anisotropy factor A_n' can be defined, according to *Lang & Melhuish [1970]* from the number of roots N_a, N_b and N_c intersecting three mutually perpendicular planes A, B and C, respectively:

$$A_n' = \sqrt{(N_a - N_m)^2 + (N_b - N_m)^2 + (N_c - N_m)^2 / N_m^2} \qquad (15)$$

where

$$N_m = (N_a + N_b + N_c) / 3 \qquad (16)$$

and N_a, N_b and N_c are the number of roots seen per unit sample area. The definition given by *Lang & Melhuish* implies that A_n' falls in the range 0-2.45. For completely parallel root systems A_n' equals $\sqrt{6} = 2.45$. A normalized anisotropy factor A_n can be defined as:

$$A_n = A_n' / \sqrt{6} \qquad (17)$$

If root densities in two dimensions are equal, we may write $N_b = N_c = p \cdot N_a$ and consequently

$$A_n = |1 - p| / (2p + 1) \qquad (18)$$

Root-plane intersections can be counted in various ways. The two main sampling approaches used are: counts on auger samples which are broken for inspection *(Schuurman & Goedewaagen [1971])*, or counts on smoothed profile walls on which roots are made visible by removing some soil by spraying *(Böhm [1978])*. Roots can be counted in grids directly or after mapping on polythene sheets, either in a vertical or in the horizontal plane. The core-break method provides data of easily replicated, small samples, the profile wall method shows spatial arrangement of roots, for instance in relation to soil structure. As a third, less practicable method, blocks of soil hardened by resins can be inspected *(Melhuish [1968])*.

The number of roots seen per unit area of the sample can be related to the length of roots in a volume of soil by:

$$L_{rv} = 2 \cdot X \cdot N_m \qquad (19)$$

where $X = 1$ for $A_n = 0$.

For root distributions which are not anisotropic equation (19) holds approximately when the average number of intersections for the three mutually perpendicular planes is used for N_m. *Marriot [1972]* has corrected earlier calculations by *Lang & Melhuish [1970]* on the effect of A_n on X in such a case. For two extreme types of root distribution a different relation is found, as shown in Figure 4A. As a curve-fit a quadratic relationship is adequate for both cases:

$$X = 0.5\ A_n^2 + 1$$

$$X = 0.8\ A_n^2 + 1$$

for the «linear» and the «planar» situation, respectively, with (1, 0, 0) and (1, 1, 0) roots in the three planes in the extreme case. In the usual application of the core-break method roots are counted only on a horizontal plane. Further correction is required as N_m may deviate from N_a. For roots with a preferential vertical orientation we may use $N_b = N_c < N_a$, *i.e.* $p < 1$. From (18), (19) and (20) it follows that

$$L_{rv} = N_a \cdot (3p^2 + 2p + 1) \quad (2p + 1)$$

and for roots with preferential horizontal orientation $N_b = N_c > N_a$, *i.e.* $p > 1$, from (18), (19) and (21):

$$L_{rv} = N_a \cdot (16p^2 + 8p + 6) \quad (10p + 5)$$

For $p = 1$ these equations reduce to $L_{rv} = 2\ N_a$; for $p = 0$ it follows that $L_{rv} = N_a$ and for large p (23) can be approximated by $L_{rv} = N_a\ (1.6p + 0.8)$. Figure 4B shows L_{rv}/N_a as a function of p.

When root counts are made in one plane only and no knowledge of p is available, as is usual in both the core-break method and the profile wall method, calibration is necessary by correlating N_a and L_{rv}. Values for L_{rv}/N_a found in this way may differ from theoretical values because of errors in counting all roots, for instance overlooking roots or counting dead remains of roots which are distinguished as such in washed samples.

Calibration factors L_{rv}/N_a usually vary with sample position, sample depth and time, as we may expect from the strong influence of factor p. Core-break methods thus can only give a rough indication of root distribution in the field. Theoretically A_n would not influence the relationship between N and L_{rv} when roots would be counted on half-spheres (De Wit, pers. comm.). This has not been practised yet.

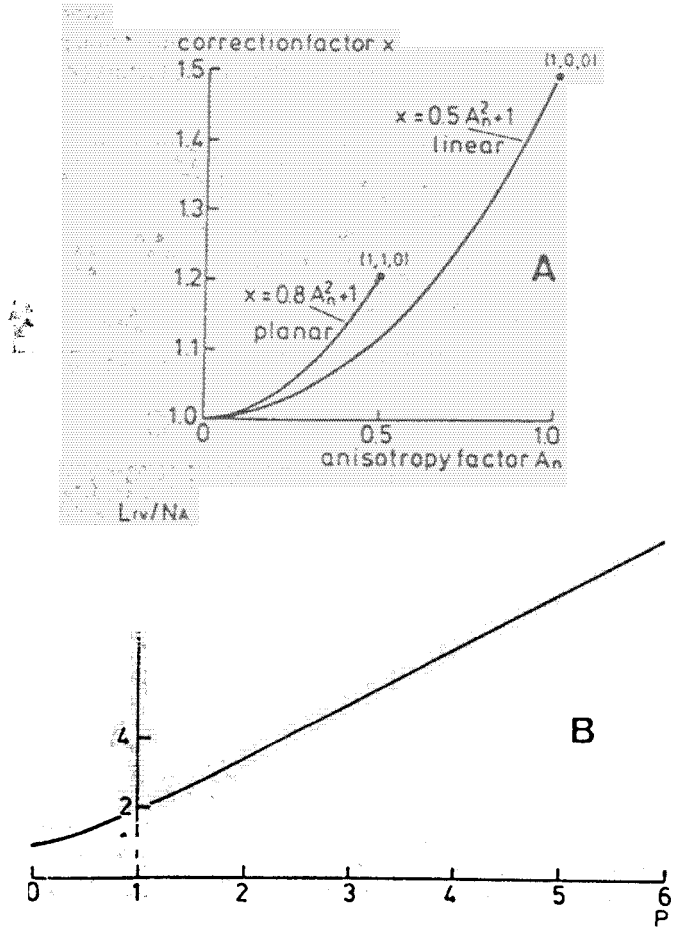

Fig. 4 A. Correction factor X in equation (19) as a function of anisotropy factor A_n, modified from calculations by *Marriot [1972]* for two extreme types of deviations from anisotropy («planar», roots in two dimensional orientation, «linear» roots in one dimensional orientation); B. L_r/N_a as a function of p according to equations (22) and (23).

3.4 Quantifying root pattern by nearest neighbour distances

When considering L_{rv} on a small scale (small volumes of soil) part of the variation is due to the fact that roots occur as discrete events, branch roots originating on main roots. Root distribution on this scale deviates from randomness either in the direction of regularity or in the direction of clustering. Definitions of such patterns are given in plant ecology *(Pielou [1969]*, Figure 5A). The «pattern» can be quantified

by measuring «nearest neighbour distances», *i.e.* by classifying all soil according to the distance to the nearest root (Figures 5B and 5C). Root distribution pattern can be influenced by soil factors (*e.g.* structure) as well as plant factors (*e.g.* branching).

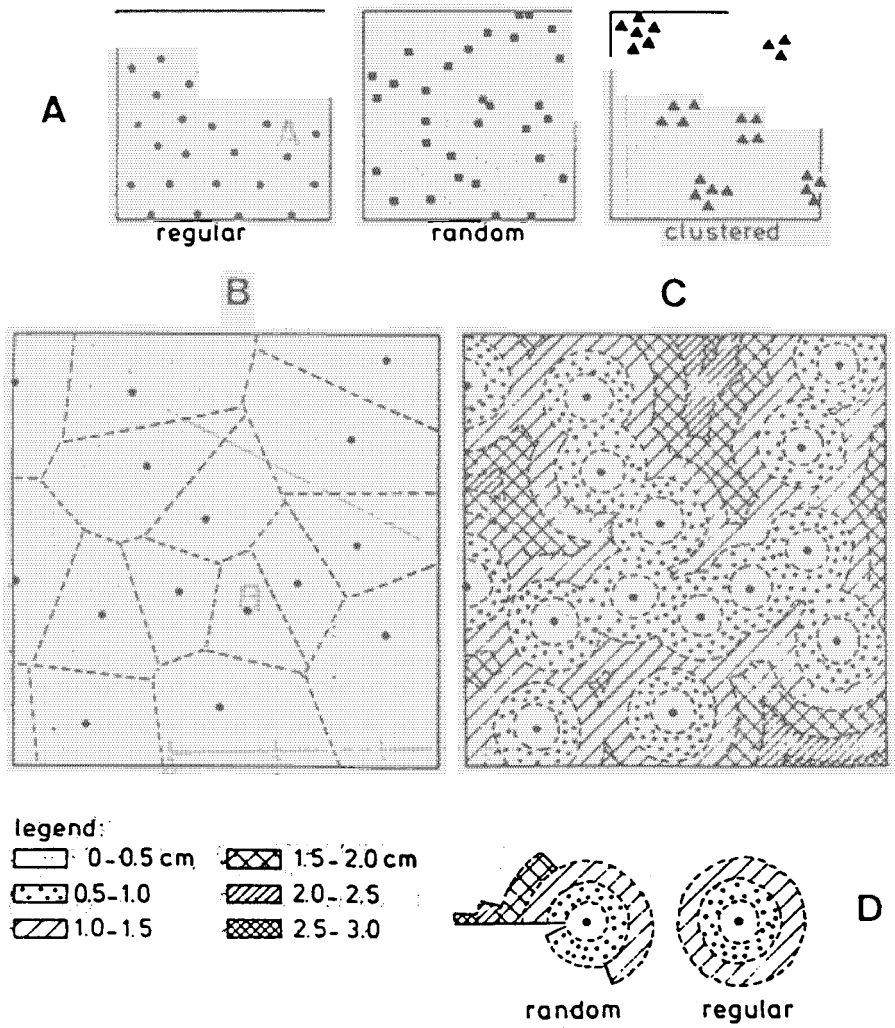

Fig. 5 A. Three basic types of spatial distribution: regular, random and contiguous (clustered); B. Division of area to nearest roots (Dirichlet tesselation); C. Classification of area on a root map according to the distance to the nearest root; D. Situation around an ‹average root›, for a regular and random root distribution.

On the basis of a comparison of point-root and root-root distances (Figure 6A), statistical tests of randomness are possible *(Diggle [1983])*.

The description of nearest neighbour distances on root maps is not only a technique for tests of randomness, it may also provide insight into the frequency distribution of real diffusion distances involved in nutrient and water depletion by a root system. In the three-dimensional reality, however, diffusion distances will be shorter than in our two-dimensional maps. The difference may be quantified as follows.

For the two-dimensional maps the frequency distribution of point-root distances in case of random distribution of roots, can be derived from a Poisson distribution as *(Pielou [1969]; Marriot [1972]:*

$$P\,[\delta < D_2] = 1 - \exp\,(-\lambda\, D_2^2) \qquad (24)$$

where:
D_2 = two-dimensional distance
δ = distance of a point on the map to the nearest root
λ = number of roots per circle of unit radius.

For randomly oriented roots equation (19) shows:

$$L_{rv} = 2\,\lambda\,/\,\pi \qquad (25)$$

For three-dimensional distances of points to randomly oriented and spaced lines *Ogston (1958)* and *Barley (1970)* derived that:

$$P\,[\delta < D_3] = 1 - \pi\, L_{rv}\, R_o^2 - \exp\,(-\pi L_{rv}\,(D_3^2 + 4/3 \cdot \psi \cdot D_3^3) \qquad (26)$$

where:
D_3 = three-dimensional distance
ψ = number of root tips per unit root length

The second term in equation (26) is a correction for the volume occupied by the roots, which normally is negligible. As Figure 6B shows, ψ in equation (26) is of considerable importance. Its role follows from the possibilities of end-point contact for a half sphere around the root tip, added to the tangential contact for cylinders around the root.

For $\psi = 0$ we may compare equation (26) to equation (24) and relate D_2 to D_3:

$$D_3 = D_2\,/\,\sqrt{2} = 0.71 \cdot D_2 \qquad (27)$$

This result strictly depends on random orientation of the roots with regard to the plane in which two-dimensional distances are measured. If D_2 is measured in a plane perpendicular to a parallel root system D_3 will equal D_2. In no case will D_3 be larger than D_2 measured in any plane.

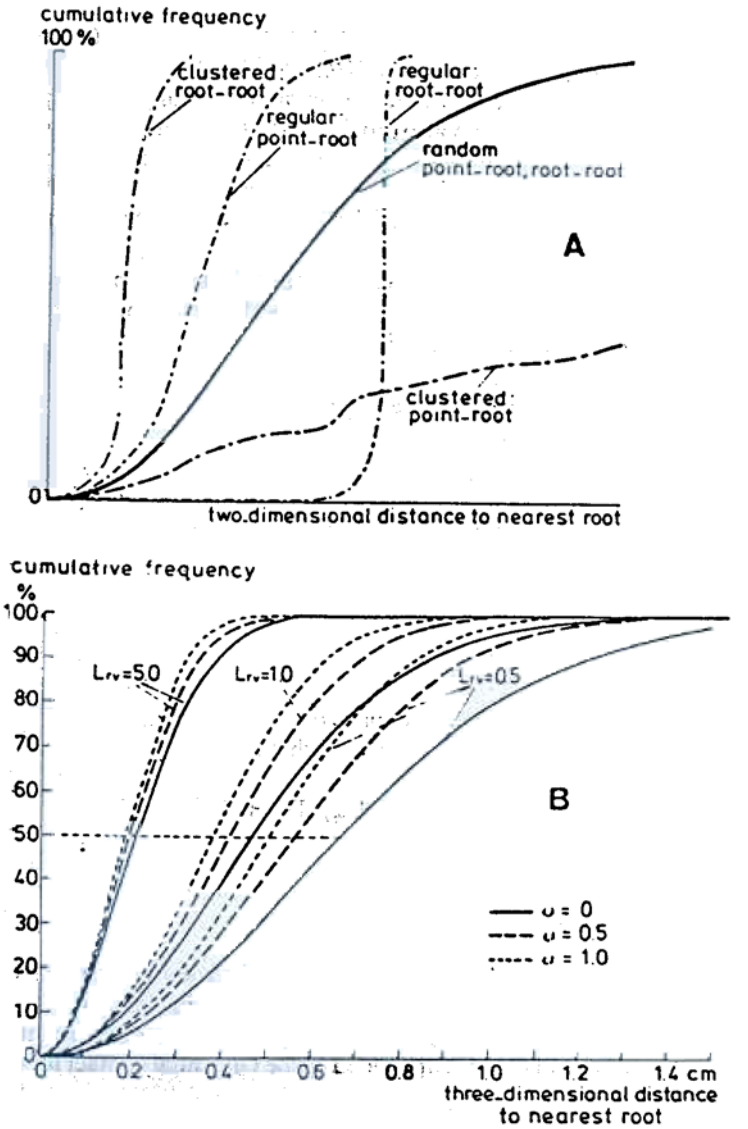

Fig. 6 A. Cumulative frequency of two-dimensional nearest neighbour distances for random points to roots (p − r) and roots to roots (r − r) for the three types of distribution shown in figure 5A; B. Cumulative frequency of three-dimensional distances of random points in the soil to the nearest root, for three root length densities and for three values of φ, the number of root tips per unit root length, according to equation (26).

Deviations from the regular root pattern usually assumed in theoretical models, have a considerable effect on relative depletion potential of a root system, especially for nutrients of lower mobility in the soil such as potassium and phosphate *(De Willigen & Van Noordwijk [1987])*.

3.5 Soil-root contact

Uptake of water and nutrients can only take place by direct contact between a root and the solid + water phase of the soil *(De Willigen [1984])*. The complement of soil-root contact is formed by root-air contact; for roots of low porosity the degree of root-air contact is important for aeration *(De Willigen & Van Noordwijk [1984])*. Root-soil contact can be quantified from thin sections of the soil, as used in soil micro-morphology, but no method for routine analysis is available as yet.

Root-soil contact will vary along the length of a single root and for a root system a frequency distribution of % contact has to be known.

For establishment of soil-root contact and for a direct role in uptake processes, root hairs can be relevant. The average length of root hairs L_h [cm], average radius R_h [cm] and root hair density per cm of root H_{rl} [cm^{-1}] may be necessary to describe root hairs in detail.

In some cases a «root hair cylinder» of radius $R_o + L_h$ is a useful concept.

4. Root dynamics

4.1 Dynamics of fine roots

A root may be expected to increase linearly in length of its main axis and exponentially when all branch roots are considered. When considering the increase of «root depth» in time linear functions will be adequate, while exponential functions may be required for describing root length density as a function of depth.

The root system of a plant is in a dynamic balance between root growth and root decay or root death. The total length of a root system is mainly determined by the length of fine branch roots. These fine branch roots may die after some time, while the main axis on which they are formed survives, at least to carry on its transport function.

Comparable to definitions for populations, a birth rate, death rate and average life expectancy can be defined for fine roots. If the potential for water or nutrient uptake is considered to be age-dependent, cohorts of roots of the same age can be described.

4.2 Sequential root observations of root dynamics

When destructive methods are used for frequent observations, the heterogeneity of root distribution leads to very high coefficients of variation for the estimates of relatively small differences in total root density between two observation dates. Several authors have neglected this variation and have attributed every positive difference between estimated root density at time T + 1 and that at time T to root growth, and every negative value to root decay. Especially for frequent sampling programmes true root dynamics can be vastly overestimated by this technique *(Singh et al. [1984])*. Another problem of this method is that simultaneous root growth and decay in the same soil layer – if it would occur – cannot be measured.

As alternatives for frequent destructive measurements, various techniques exist to observe root growth and decay on individual roots; such techniques suffer from the problem that the observations can only be made under non-natural conditions. Large-scale rhizotrons *(Huck & Taylor [1982])* are expensive and observations are limited to one soil type or artificially filled soil columns. As a less expensive and more flexible alternative the «mini-rhizotron» technique is now available for recording root growth along a glass or lexane wall under field conditions *(Sander & Brown [1978]; Vos & Groenwold [1983]; Van Noordwijk et al. [1985b])*. If observation tubes are introduced into the soil at sowing or planting time, root development and root decay can be observed for the whole growing period under conditions of little disturbance. Calibration of root lengths seen on the rhizotron wall (by evaluating photographic negatives on a grid for counting intersections) to L_v from washed samples gives variable results (unpublished results, *J. Vos*, pers. comm.), necessitating calibration with washed samples if information on the exact root distribution is required.

By visual inspection of a series of photographs from the same mini-rhizotron at the same depth in soil, the length of new roots on each observation date can be quantified as well as the length of the roots which have disappeared since the previous observation. As root decay and root death may be gradual processes a visual criterion for presence or absence has to be set and observations preferably have to be made by one person. At any time t in the growing season the following equations holds:

$$R(t) = CTR(t) - CDR(t) \tag{28}$$

where:
R(t) = standing root intensity in the observation plane at time t [cm cm^{-2}]
CTR(t) = cumulative total of roots observed till time t [cm cm^{-2}]
CDR(t) = cumulative total of roots decayed till time t [cm cm^{-2}]

For annual roots CTR(t) is equal to CNR(t), the cumulative total of new roots observed till time t. By the end of the growing season at t = e we obtain data for CNR(e), CTR(e), CDR(e) and R(e). From these data we can define two ratios which may summarize the root dynamics during the growing season as a whole:

$$TRL = CDR(e) / CTR(e)$$

and:

$$RRR = CNR(e) \quad R(e) \qquad 30)$$

where:

TRL = turnover of root length during a growing season [−]
RRR = root replacement ratio during a growing season [−].

The latter quantity is especially relevant for perennial crops as it gives information on the average longevity of individual roots. If RRR = 1 we may conclude that the average longevity of a root is 1 year, provided that R(e) is constant from year to year. The frequency distribution of individual root longevities cannot be estimated this way.

To avoid the comparatively large voids between the standard lexane rhizotron wall and the soil *(Van Noordwijk et al [1985b])* we also use metal frames in which an inflatable rubber tube (made from a motorcycle inner tyre) can be introduced and kept under constant pressure. The pressure used is such that roots grow unimpeded between the tube and the soil. At regular intervals the observation equipment (fibre-optics plus camera) are inserted in the lexane tubes and in the metal frame (after removing the deflated tyre) for photographing roots at every depth in the soil. For each photograph total root intensity is measured with the line-intersect method with due correction for the magnification factor. Individual roots are compared on subsequent photographs with a counting grid as overlay in exactly the same position by checking each intersection between a root and a line. In this way the length of new roots since the previous observation and the length of roots which had disappeared can be measured.

In future computer-aided image analysis may reduce the large amount of time involved now.

If the criterion of visibility used in analysing photographs is similar to that used in cleaning washed root samples, the results obtained can be used for estimating net root production for a whole growing season based on destructive sampling with an auger, monolith or pinboard method on one date and a series of photographs covering the whole growing season. Net root production over one growing season can be estimated from:

$$NRP = DRP(m) \times CDL \times CTR(e) \quad R(m) \qquad (31)$$

where:

NRP	= net root production over the whole growing season [kg/ha]
DRP(m)	= root dry weight per unit soil area at the time of maximum standing root mass [kg/ha]
CSS	= correction factor for the sampling scheme used [−]
CDL	= correction factor for losses of dry weight in sampling and washing procedures [−]

CTR(e) / R(m) = cumulative total of roots seen per growing season (cm cm^{-2}) divided by root intensity (cm cm^{-2}) at the time of the DRP(m) sampling [−].

The correction factor CSS equals 1.0 if an appropriate sampling scheme is used. For data obtained by other schemes correction factors can sometimes be estimated *(Van Noordwijk et al [1985a])*. The correction factor CDL can be estimated from seperate experiments (Figure 3). If relative root dynamics are different for various layers in the soil a correction may be required as the ratio between root intensity seen per cm^2 of observation wall and root length density measured in washed samples is not necessarily constant for crops, seasons and soil layers. A point of concern is whether or not estimates of root turnover are based on unbiased samples as regards root diameter, as we may expect that fine branch roots have a different (probably higher) turnover than thicker main roots.

5. Conclusion

A recent trend in root research is to try to understand root functions on a more detailed level by studying the synchronisation of root activity and the presence of mobile soil resources and the ‹synlocalisation› of active roots, micro-sites of oxygen supply, less mobile nutrient sources and soil organisms. To solve such research problems, root research using methods as outlined here, has to be coordinated with small-scale studies of soil chemistry, soil physics and soil biology.

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