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5.2 ROOTS: LENGTH, BIOMASS, PRODUCTION AND MORTALITY

5.2.1 Introduction

In both crops and natural vegetation a substantial proportion of net primary production occurs below ground in the root system. This proportion varies with vegetation type, developmental stage, soil conditions and cultural practices. Shoot: root ratios on a dry matter basis are typically between 5:1 and 10:1 for annual crops at maximum standing biomass. For perennial crops and natural vegetation values vary between 1:2 and 5:1. Uptake of water and nutrients are related to root length or root surface area rather than root weight. Distribution and periodicity of root length is important to evaluate whether or not crop demand for nutrients and water coincides in space and time with the available supply (synchrony and synlocalization). Root death and root exudates are a major input of organic matter to the soil system and the extent, timing and location of root death are therefore important. By leaving a well distributed set of continuous channels of mostly easily decomposable organic matter, roots have a relevance for soil biota, including roots of subsequent crops, far beyond their often limited quantity of organic matter.

For these reasons root investigations are an important part of the TSBF programme. Unfortunately they present particular difficulties. Extraction of roots from soil is time consuming, labour intensive and still it is often incomplete.

For root research in the TSBF framework a hierarchical approach is recommended, with four stages of increasing complexity:

a) the study of overall rooting pattern of major components of the vegetation or cropping system (5.2.2),

b) quantification of root biomass at maximum standing crop (5.2.3),

c) estimate of total root production (5.2.4),

d) estimate of total root carbon input to the soil (5.2.5).

Root research requires destructive sampling of the soil, often causing considerable disturbance to the plots. Space should be allowed for this when designing field experiments. Information on the lateral spread of root systems is essential in deciding on 'guard' areas or borders in field experiments. Errors in interpreting results are easily made when no root information is available, as lateral spread of over 5 m (cassava) or up to 20 m (certain trees) is often more than expected.

Apart from the two classical descriptions of root methods by Schuurman and Goedewaagen (1971) and Böhm (1979), a number of recent reviews is available on methods to quantify root development and functioning in the field: Caldwell and Virginia (1991), Mackie-Dawson and Atkinson (1991), Taylor et al. (1991), Van Noordwijk (1987), van Noordwijk et al. (1992).

5.2.2 Study of overall root patter.

Three methods outlined below are based on the study of soil profiles from a soil pit. In soils without stones or woody roots a monolith sampler as described by Floris and van Noordwijk (1984) might have the advantage of less site disturbance, but generally soil pits are needed to have access to the root environment.

i) Root preparation on profile walls

A soil pit is dug close to the plant selected for study and by carefully removing soil close to the stem some main roots are identified; their course is followed by gradually removing the surrounding soil, using a pin. When all (major) roots within the first, say, 10 cm from the original profile wall have been exposed, a drawing is made e.g. on a 1:5 scale on graph paper (Fig. 1), using pins to mark grid points in the soil. Unfortunately most fine roots will break off during this procedure, and so only a qualitative picture is obtained. Still, the method allows width and depth of the root system and branching pattern to be recorded. The response of the root system to heterogeneities of the soil, to transitions between soil layers, to cracks (clay soils) and channels in the soil (made by soil fauna and/or previous roots) deserves special attention.

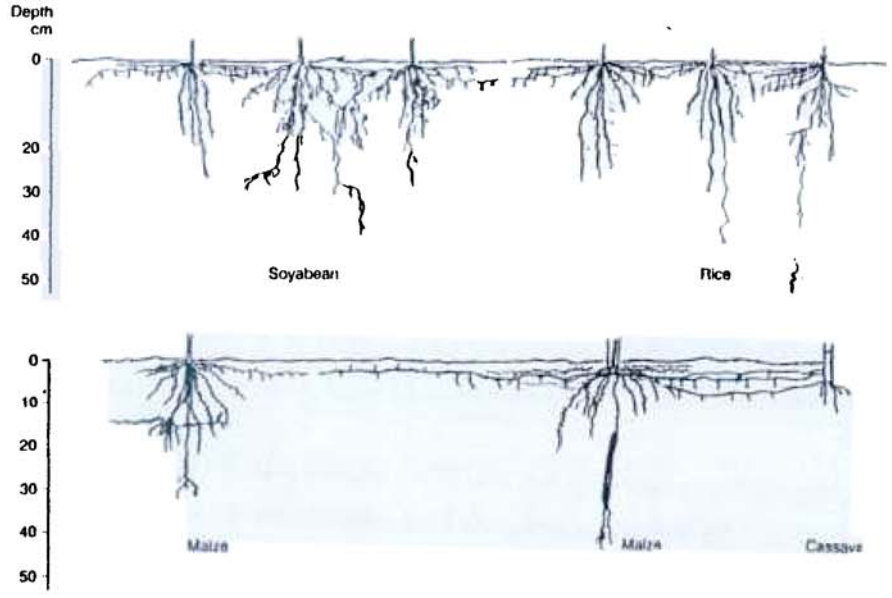

Figure 1. Example of root observations on profile wall, showing the response of maize roots to an acid subsoil (below 15 cm) and to the presence of old tree root channels (Van Noordwijk et al., 1991a).

In mixed cropping situations roots of the various components can usually be recognized after some training. By spending 1 to 2 days per major species the overall patterns of shallow and deep rooted species and lateral spread can be estimated: both are important when designing fertilizer or crop residue experiments or when designing mixed cropping systems. Results of this method are easily understood and can be used in discussions with e.g. farmers.

For shallow rooted crops lateral roots can also be observed from the stem base by digging a small trench, following the root. The presence of 'sinker' roots, vertically oriented branch roots from a horizontal branch root, is of special interest here. By excavating a small area around the stem a classification of roots by diameter and orientation can be made (Van Noordwijk et al., 1991b). This way replicated observations can be made and a 'typical' specimen can be selected for further observation.

ii) Root mapping on profile walls

Based on results of root pattern observations (described above) an estimate of total root density in the profile can be obtained by root mapping on polytene sheets. Profile walls are cleaned and straightened with a sharp knife, after having removed stones and thick roots which would hinder the process. Depending on soil texture, various steps can be taken to facilitate root observations:

- on sandy soils a water spray (knapsack sprayer) can be used to remove about 2 mm of soil to expose roots,
- on clay soils gently brushing the soil may help. Good results have also been obtained covering the profile wall overnight with a cotton cloth soaked in a 5% sodium hexametaphosphate solution to disperse the clay prior to spraying. In dry soils compressed air can also be used to blow away crumbs of soil; roots are remarkably resistant to such treatment.

After preparation the profile is covered with a transparent polytene sheet and carefully searched for roots; a predrawn 10 x 10 cm grid on the polytene helps to work systematically. All roots are marked with dots on the sheet; differently coloured pens can be used for different size classes or plant species. Roots are only recorded where they intercept the plane of observations; branch roots outside that plane, exposed by preparation of the profile wall, are neglected. Major features in soil structure and horizonation should also be marked (Fig. 2a). Root intensity (number of interceptions, N, per unit map area) can now be estimated for each soil horizon, as a function of distance to the plant. A more detailed analysis of root pattern (regular, random or clustered) within each horizon is possible by computer analysis of the root maps. Statistical tests of spatial correlation between roots and other map features (e.g. cracks, tree roots, termite channels) can be performed on the basis of root intensity in zones with increasing distance to the features of interest (Van Noordwijk et al., 1992). The relation between root distribution and the pattern of cracks and macropores can be studied by infiltrating a methylen blue solution (0.05 g/l) into the soil before digging an observation pit.

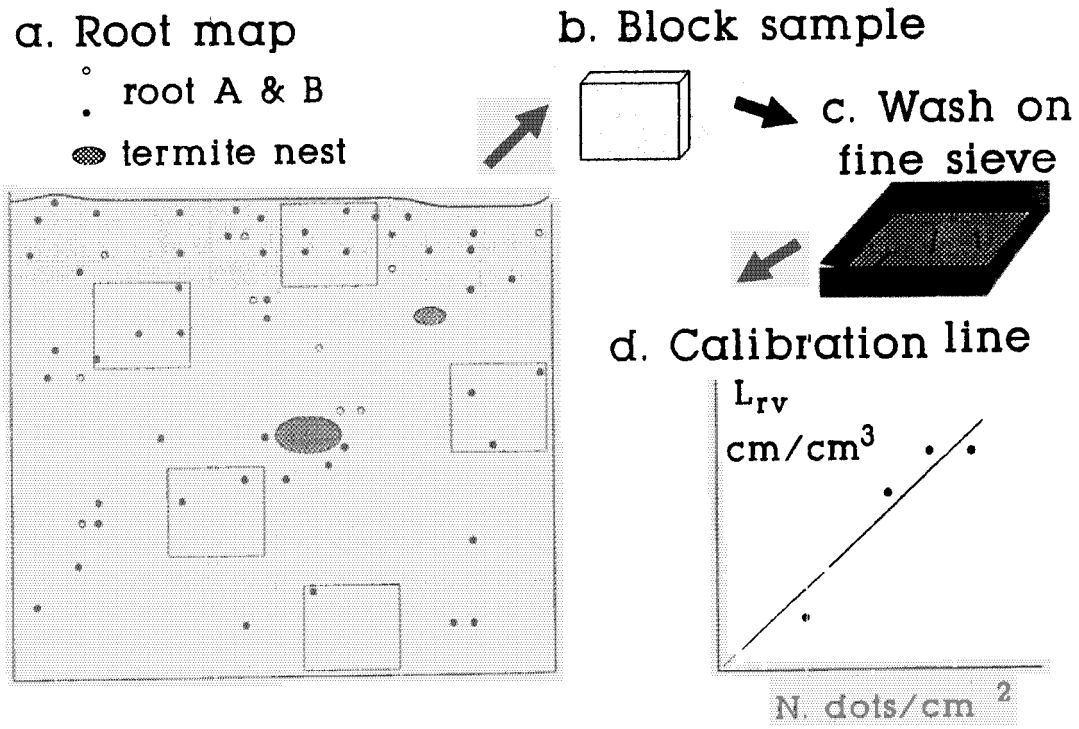

Fig. 2 Root mapping on polytene sheets; roots A and B might refer to different diameter classes or species.

Counts of N per horizon indicate the relative decrease of root intensity with depth. Root length density, L_{rv}, (cm root length per cm^3 of soil) can be estimated for each horizon:

$$L_{rv} = 2 \times F \times N$$

where the calibration factor F is equal to 1.0 for roots with random orientation, if all roots are seen. The factor F depends on root orientation (between 0.5 and infinity) and improved estimates can be obtained if root intensities in horizontally oriented planes is recorded as well (Van Noordwijk, 1987). Experimental calibration factors may be considerably (e.g. three-fold) larger than theoretical ones.

Problems with the method are: (i) roots of different plants are hard to distinguish, (ii) distinction of live and dead roots is not easy and (iii) a considerable fraction of fine roots may be overlooked. To obtain (semi)quantitative results it is necessary to calibrate the maps by taking small blocks of soil (e.g. 20 x 10

x 1 cm volume) from various layers on the root map (Fig. 2 b,c,d), wash them over a fine sieve (0.3 mm mesh) and determine root length (see below).

iii) Pinboard monolith sampling

Monolith samples can be obtained with pinboards ("fakir beds"), made by inserting U-shaped pins (made from stainless steel) in plywood (Fig. 3; further details are given by Schuurman and Goedewaagen, 1971, and Bohm, 1979). The size of the pinboard is determined by the crop (based on previous observations, such as i) and practical considerations (samples of 100 x 60 x 10 cm of soil will weigh about 100 kg). By washing the soil away the roots become exposed and can be observed. If a coarse mesh screen (e.g. material used for TSBF litter bags) is put on the pins before the board is pushed into the soil (perpendicular to the crop row), this screen can help to keep the roots in their original location while washing the sample.

Washing the sample can be facilitated by soaking overnight in water, deep freezing (for clay soils), soaking in oxalic acid (for soils with free calcium carbonate) or soaking in hexametaphosphate, preferably under vacuum. Whatever the pretreatment used, gentle washing must follow.

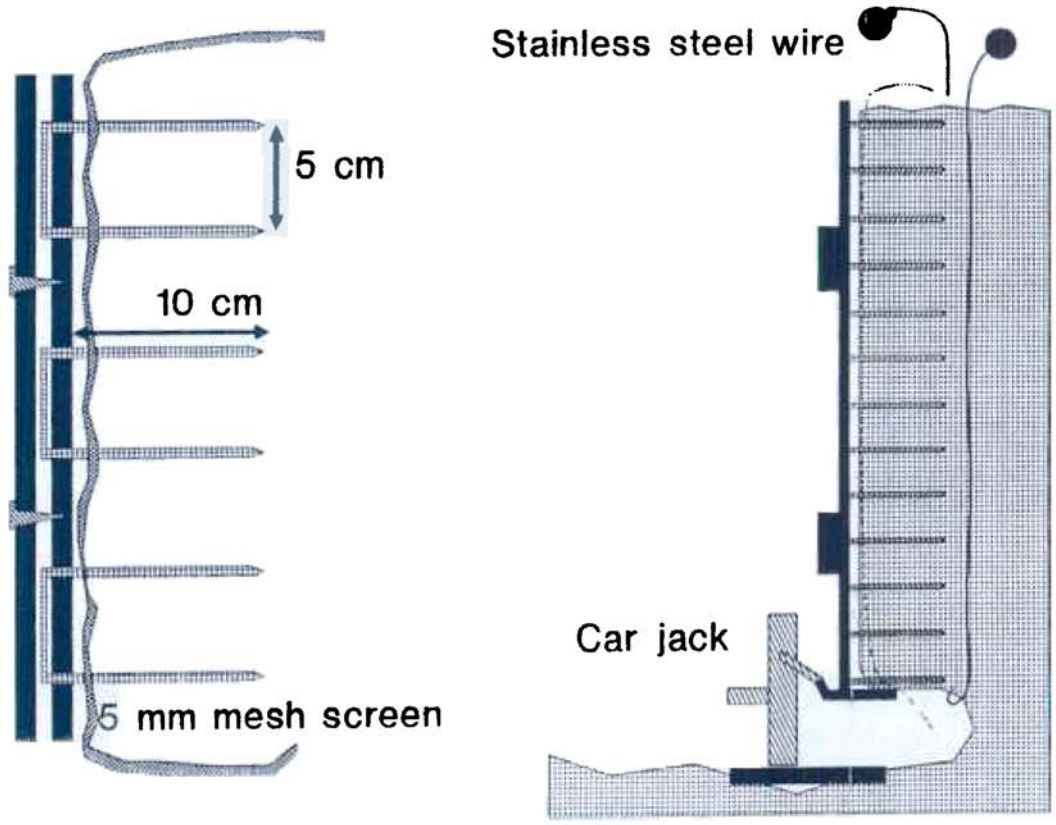

Fig. 3. Pinboard construction and sampling in the field; after pushing the pinboard into a plane profile wall, the board is supported on a car jack and soil below and to the sides of the board is cut away; extending pieces of the mesh screen can be used to tie the soil block to the board; finally the back side of the sample is cut by 'sawing' movements with a stainless steel wire.

After washing away the soil, the root system can be lifted on the mesh screen, photographed (on a black cloth as background) and/or cut according to soil layers (indicated e.g. by a string between the pins while washing the sample), depth zones and/or distance to the plant to obtain root biomass and/or root length (see below for root length). To estimate total biomass per plant root weight density per zone and depth has to be integrated over the relevant volume.

Although the pinboard method is mc. ime consuming than methods (i) and (ii) above, it gives good information per unit effort spent. Major weaknesses are that roots may break or be displaced during washing. The distinction between live and dead roots is easier via pinboard sampling then in methods where the root system is not sampled in its entirety.

5.2.3 Quantification of root biomass and length

The previously described methods may, with adequate calibration, be used for estimates of root weight and length. More accurate estimates can be obtained from well replicated core (auger) samples, washed on a fine meshed sieve. To obtain reliable estimates of root biomass several points should be noted:

- samples should be taken from representative volumes of soil; in row crops special sampling schemes (Van Noordwijk et al., 1985) may be needed,
- samples should be taken around the expected maximum standing root biomass; a late season sampling may result in a high proportion of dead roots,
- the methods for sampling, storing samples and washing will unavoidably lead to some loss of dry weight and nutrients; relevant correction factors may be obtained by simulating all procedures on roots grown in nutrient solution. Losses can be in the range 20 - 50% for dry weight, and thus correction factors of 1.25 - 2.0 should be applied to the final data (Van Noordwijk, 1987; Grzebisz et al., 1989). Nutrient contents are generally only reliable if sample handling is completed within one day.

Design of corer

The corer remove a known volume of soil from a known depth in the profile, without the need for digging a soil pit and destroying part of an experimental field. A core of 50 - 80 mm diameter is satisfactory. The corer can be inserted either manually or mechanically. Manual coring is difficult at depths greater than 50 cm and in clay or stony soil. In dry sandy soil a smaller core diameter may be needed to reduce losses of soil when extracting the core. A suitable hand corer consists of a 15 cm steel tube with a serrated cutting edge mounted on a 1 m pipe, with a plunger to remove the core (Fig. 4; Bohm, 1979). Marks on the pipe indicate 10 cm depth increments. With the corer soil is extracted in successive 10 cm increments.

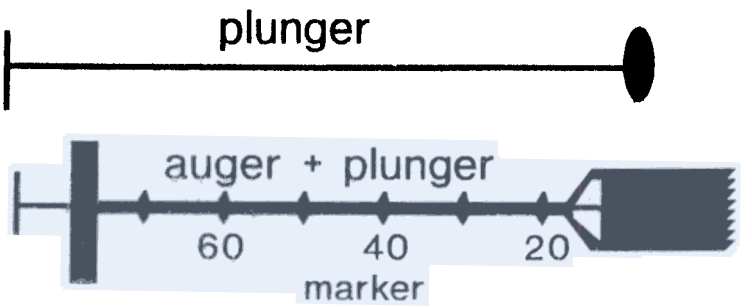

Fig. 4. Design of a hand corer.

Alternatively a sharpened steel tube of appropriate length can be driven into the soil. In this case it is important to have a tripod and chain hoist to extract the tube, a file to resharpen the cutting edge, and longitudinally split liners of thin metal within the tube so that the intact soil core can be pulled out. A powered motor breaker can be used to drive the corer into the soil (Welbank and Williams, 1968), but compaction of the core will occur and should be allowed for when cutting subsamples from the core.

In very stony soil, or where there are many woody tree roots, coring may not be possible. Regular, known volumes of soil (monoliths) can be taken from the face of a pit and treated in the same way as cores.

Sample depth

Ideally the profile should be sampled to the limits of rooting depth. At depth, however, rooting intensity is low and spatial variability high. Based on initial profile wall observations a meaningful lower limit can be set. In some cases a linear relationship of the log of root mass versus depth (a negative exponential root distribution) may help to extrapolate root densities in the soil beyond sampling depth. All soils must be sampled to a minimum depth of 30 cm.

Where horizon development occurs, subdivide the cores at horizon boundaries of known depth in the first instance and within horizons in 10 cm increments. In cultivated soil or where there are no clear horizons subdivide the mineral soil in 10 cm increments. Surface organic horizons should be treated separately.

Sampling intensity

Even in the most homogeneous soils a considerable spatial variability of root density will occur. For auger samples of about 385 cm^3 (10 cm height, 7 cm diameter) a coefficient of variation in root weight of at least 40% may be expected (Van Noordwijk et al., 1985a); on heterogeneous soils the coefficient of variation may be much higher. This variability implies that large number of replicate

Assessment of root mass

Washed root samples can be stored in sealed polytene bags for a short time in a refrigerator, but deep-freeze storage is preferable. Thymol can be added as a bactericide, but should be handled carefully; classical storage media (ethanol etc) tend to make roots brittle.

Carry out biomass estimation on each size class of all samples. Dry the roots and weigh. Next the dried samples should be combusted for 5 hr in a muffle furnace at 550°C and the residue weighed. Results should be expressed as ash-free oven-dry mass per unit volume of soil.

Root length measurements

Root length is a relevant parameter for water and nutrient uptake. The specific root length (length per unit dry weight) of roots depends on diameter, variability of diameters, dry matter content (per unit fresh weight) and air-filled porosity (per unit volume) (van Noordwijk, 1987). Within a species or situation variability of the specific root length may be rather small (e.g. with a coefficient of variation of 10-15% while root weight per unit soil volume has a coefficient of variation of about 40%), so it is reasonable to measure the specific root length only for some subsamples. Normal values are from about 10 m/g for fine tree roots, 50-200 for fine roots of dicotyledonous crops and 200-600 for cereals and grasses (Van Noordwijk and Brouwer, 1991).

Root length can be estimated by counting the number of intersections between roots and sample lines. This method is based on Buffon's needle problem, described in 1777, where the chance that a needle randomly thrown on a tiled floor would intersect one or more of the edges of the tiles was formulated as a function of the length of the needle and the size of the tile. Application of the method for measuring root length is based on Newman (1966) and Tennant (1975). Roots are spread out with random orientation in a thin layer of water on a glass plate (about 25 x 25 cm), water is removed and a grid (photocopied on an acetate folio for overhead projection) is put over or underneath the sample. Line by line all intersections of roots and grid lines (taking the upper or left boundary of the line as criterion in case of doubt) are added (Fig. 5). Results for horizontal (H) and vertical (V) grid lines are added to the number N. If the grid size is D (mm), root length L (mm) is derived as:

$$L = \pi N D / 4$$

If D is set equal to $40/\pi$, i.e. 12.7 mm, L = 10 N (mm). By adding results for H and V lines the method is insensitive to preferential oreintation of roots on the plate, but spreading the roots must be done without regard of the position of the grid lines. To improve contrast roots can be stained beforehand, e.g. with saffranin red (1 g/l).

that of Smucker et al. (1982), now commercially available, uses less water and accepts smaller samples but requires both water pressure and compressed air. After elutriation roots must still be sorted by hand and this may take several hours. Neither apparatus handles organic soils. With both hand and machine washing, loss of fine roots occurs and a periodic check of washing water and residues should be made to quantify such losses; such losses should be kept less than 5% of root weight (which may imply that they are still over 10% of root length); this loss is distinct from that due to respiration and loss of cell contents from remaining root tissue.

Presoaking overnight in 5% sodium hexametaphosphate expedites the process of washing roots from clay soils, but the chemical discolours the roots, particularly in soils with high organic matter content and may disrupt the tissue, making subsequent identification of live roots more difficult. Such pretreatment will also interfere with chemical analyses and should therefore be avoided if possible. Any lengthy washing procedure may alter the element content of root tissue and a subsample hand sorted with a minimum of water and processed on the day of sampling must be used for analysis.

Classifying the roots

Fine roots are the most important part of the root system for water and nutrient uptake, as they form the largest part of total root length or root surface area. For woody perennial vegetation there is a fairly obvious distinction between the more or less permanent, secondarily thickened roots and the ephemeral, unthickened roots. This functional distinction usually falls somewhere between 1 and 3 mm root diameter. Roots above 10 mm diameter are not adequately sampled by coring. For herbaceous perennial and short lived vegetation no such clear distinction exists. For TSBF studies these roots should be separated into <2 mm and >2 mm classes. In mixed vegetation separation of roots of different species may be difficult and not necessary for TSBF research.

It is desirable to separate root samples into living and dead categories. This is particularly important in crop situations where old, dead roots from the previous crops may still be present. Living roots can be distinguished by their lighter colour, turgid appearance and flexible rather than friable nature when manipulated. Some preliminary anatomical investigation may help in establishing criteria for making decisions. Incubation of excised roots in soil in modified litter bags can be used to establish visual clues to the root decay process. Cross checking between operators and working block by block in stead of treatment by treatment help to reduce experimental error. Congo red staining has been used to differentiate wheat roots with intact epidermis from ones without (Ward et al., 1978). Stain in 1% aqueous solution of Congo Red for 3 min, rinse, blot dry, then saturate for 3 min in 98% ethanol before a final rinse. Living roots stain dark pink to bright red. The criteria for making the living/dead distinction must be clearly stated. Where adequate criteria cannot be developed, asses total root mass only.

ter. At least 20 readings per sample are required (assuming three samples per treatment per layer).

Mycorrhizas

It is desirable to have some measure of the type and intensity of mycorrhizal infection in each treatment. At peak root biomass take approximately 60 cm of fresh clean root at random from each depth and stain to visualize fungal hyphae. The method of Phillips and Hayman (1970) can be followed with samll adaptations: short (ca 2 cm) lengths of roots (stored in alcohol-acetic acid mixture after washing) are heated for about 1 hr at 90°C in 10% KOH (or left for 2-3 days at ambient temperature), washed in water, if necessary (dark tissue) bleached in alkaline peroxide (3% NH_4OH in 3% H_2O_2, prepare daily), washed in water, acidified for 3 min in 1% HCl, stained for 5 min at ambient tempearture in 0.05% trypan blue (Merck Art No. 11732) in lactic acid and destained in clear lactic acid. In the original description lactophenol (1:1:1:1 mixture of phenol, lactic acid, glycerol and water) was used in stead of lactic acid. As phenol is toxic it should not be used, unless staining results are unsatisfactory. A modified procedure was described by Kormanik and McCraw (1982). For roots with a high lignin content the KOH treatment may have to be intensified. After staining the samples are inspected under a (dissecting) microscope. Vesicular arbuscular mycorrhiza (VAM) infection is characterized by the formation of unseptated hyphae outside the root and inter- and/or intracellular hyphae in the cortical cell layers of the root (Sieverding, 1991). The percentage of root sections which has mycorrhizal structures is assessed. Ectomycorrhizas are characterized by a fungal sheath and/or Hartig net; the percentage of root tips with such structures (which can often be recognized macroscopically and without staining) is assessed.

See also Appendix B - The Rhizosphere

Sampling frequency

The amount of living and dead root in the ecosystem fluctuates as a balance of new root growth and root death and decay. Given the large number of replicate samples required to obtain a reasonable (let alone accurate) estimate of root density at a given moment, a regular sampling programme to monitor changes during the growing season easily becomes unmanageable. For TSBF site characterization a minimum of two samples per year is required.

For perennial vegetation some prior knowledge of the phenology of the root system will allow sampling to coincide with the likely peaks and troughs in root biomass ; root growth often alternates with periods in which reproductive growth is a major sink for carbohydrates. Where no prior knowledge exists samples should coincide in herbaceous vegetation with maximum and minimum above ground biomass, and in forest with the months of minimum and maximum rainfall. For annual crops maximum root development often coincides with flowering (transition from vegetative to generative

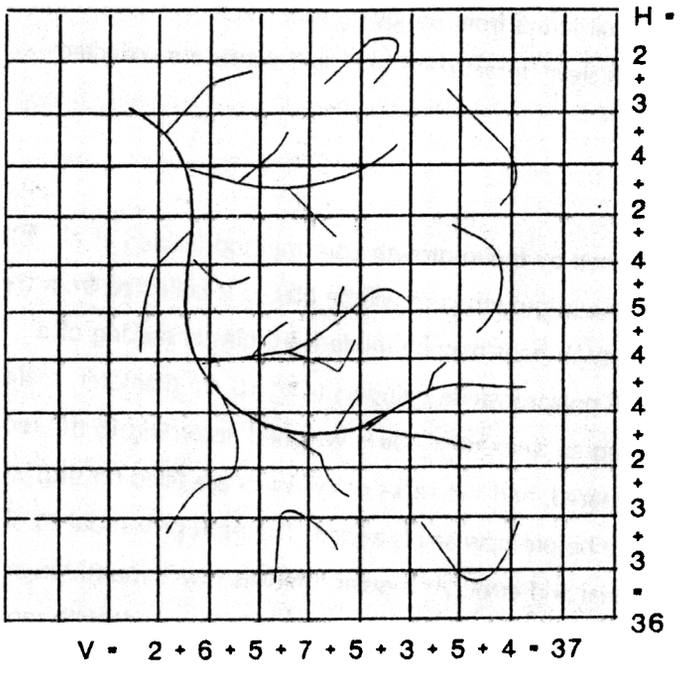

Fig. 5 Line intercept method for determining root length by counting the number of interceptions between roots and horizontal (H) plus vertical (V) lines of a grid.

To optimise working efficiency at an acceptable random error level the grid size should be chosen to obtain about 400 intercepts (200 H + 200 V) per sample. This method can be applied manually (using a magnifying lens for fine roots) or can be automated in various ways (Rowse and Phillips, 1974; Richards et al., 1979; Wilhelm et al., 1982). Each variant of the method should be calibrated by cutting a known length of cloth to small pieces. Commercial equipment is available (Comair Root Length Scanner, Commonwealth Aircraft Corporation Ltd, 304 Lorimer St, Port Melbourne, Victoria 32307, Australia). Recently computer image analysis methods are used as well, based on a video camera or line scanner. Only small fields of view can be analysed with sufficient resolution and root samples should be spread out more carefully than when human eyes are used as image analyser, so the gain may be less than expected. For TSBF purposes manual versions of the method are recommended.

Measurements of the frequency distribution of root diameters can be made on the sample spread out on a grid, by measuring on every Xth interception, using a binocular microscope with ocular microme-

Root decay (both on a root length and a root weight basis) can be studied in a modified litterbag method, incubating known amounts of excised roots (e.g. collected by sieving soil at harvest time) in a ceramic pot filled with sieved, root-free soil and placing the pots in the field (possibly at two depths). A screen cover on top of the pot may prevent soil fauna from removing root tissue. At regular intervals pots are retrieved and washed on a fine mesh sieve. Intact roots and root debris are collected separately.

Ingrowth cores

Seasonal patterns of root growth can be studied by the in-growth core method (Fabiao et al., 1985; Steen, 1984). Soil from the depth at which the in-growth core will be placed is collected from the site, air dried and roots removed by sieving. In-growth bags may be made from plastic sacking of a minimum of 4 mm mesh, or from more rigid polypropylene cylinders (4 to 10 cm diameter, sealed at the bottom and top with mesh). For each bag an amount of soil is weighed according to the required bulk density of the soil. Auger holes are prepared, and the sacks or cylinders are filled (in situ) with soil, compacting cm by cm, loosening the surface before new soil is added. The right procedure to obtain the required bulk density can be found by trial and error. At regular intervals (say 1 month) ingrowth bags (minimum 3) are retrieved and washed over a fine mesh sieve. Data allow periods with rapid root growth to be identified.

Because of the altered physical, chemical and biological conditions in the sieved and repacked soil, growth of the roots into the bag may not represent growth in the bulk soil, particularly on compacted or clay-rich soils. Periods of active root growth in different layers, however, should be accurately reflected.

The technique can also be used to record root response to localised fertiliser application or other heterogeneities in the soil (Cuevas and Medina, 1988; Hairiah et al., 1991). Fertiliser application should be made by mixing the soil with a fertiliser solution prior to filling the bags.

Minirhizotrons

Simultaneous processes of root growth and root death or decay can only be quantified if the fate of individual roots can be followed. The most simple method uses a glass sheet against a soil profile wall, tracing roots on transparent polytene sheets. By using different colours of marking pen both new root growth and disappearance of roots can be quantified. An intermediate (but tedious) technique uses glass tubes inserted into the soil and regularly inspected (on grid lines in the glass) for roots using a mirror and a torch. More sophisticated versions use a fiber-optic system with a camera (Fig. 6) and flash light or a video system (Taylor, 1987).

stage). Sampling at harvest time may lead to a considerable amount of dead roots. Sampling before planting a new crop is relevant for estimating the rate of decay of dead roots from previous crops.

5.2.4 Estimation of total root production

To estimate total root production the biomass estimates obtained (corrected for sampling schemes and dry matter losses) should be corrected for root turnover between sampling dates. Due to the large variability of results from destructive sampling it is not possible, in practice, to obtain turnover estimates from frequent sampling schemes.

The frequent sampling method derives from the following scheme with two pools (live and dead roots) and three rates (production, mortality and disappearance):

Production --> Live root mass --> Mortality --> Dead root mass --> Disappearance

R_p L_t R_m D_t R_d

The following equations hold:

$L_{t+1} = L_t + \int R_p - \int R_m$

$D_{t+1} = D_t + \int R_m - \int R_d$

If live and dead roots cannot be separated:

$(L+D)_{t+1} = (L+D)_t + \int R_p - \int R_d$

Thus:

$\int R_p \geq (L+D)_{t+1} - (L+D)_t$

Sampling errors in determing L_t and D_t play a dominant role, however. In part of the literature estimates of root production are based on statistically significant increments of root mass. Statistical significance, however, not only depends on the size of the difference but also on the sampling intensity. Although this method has been used in past decades, and the literature contains many estimates based on this method, a methodological study by Singh et al. (1984) showed that considerable over and under estimates can occur. For a full discussion of the biological and statistical aspects of the topic, including frequency of sampling in a range of systems, consult McClaugherty et al. (1982), Fairley and Alexander (1985), Goltz et al. (1984), Lauenroth et al. (1986), Hansson and Andren (1986), Singh et al. (1984) and Vogt et al. (1986). More reliable methods for estimating root turnover are based on separate study of root growth and root decay processes.

Root decay

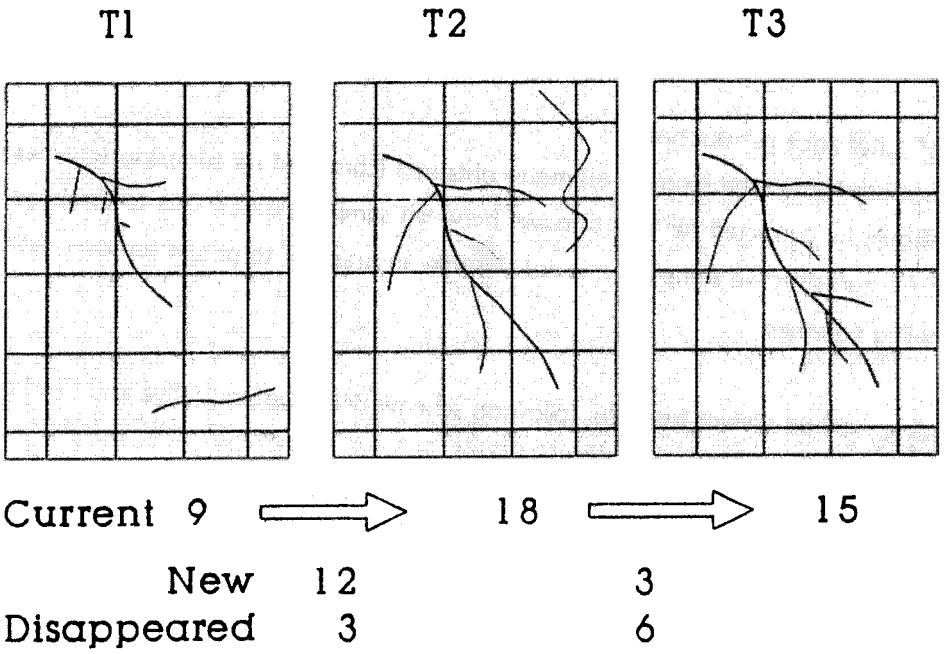

Fig. 7 Analysis of sequential images to derive root turnover.

5.2.5 Estimate of total carbon input to the soil

In addition to structural root tissue, carbon inputs to the soil include sloughed root cap cells, mucilage produced at the root tip, decayed root hairs and other cellular material, soluble carbohydrates, amino acids and other exudates, and CO_2 from root respiration. These root-rhizosphere transitions form a continuum, the study of which usually is based on ^{14}C labelling and sophisticated laboratory equipments, outside the scope of TSBF studies.

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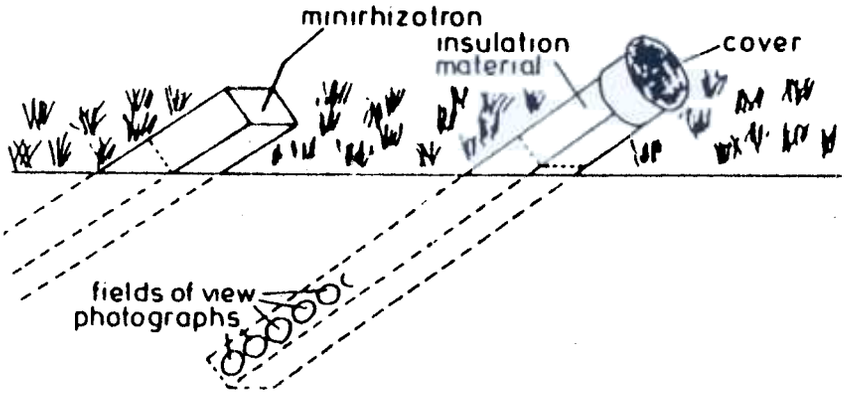

Fig. 6 Minirhizotron system to observe dynamics of root growth and decay under field conditions; at each time of observation a series of images with increasing depth is taken; these images can be analysed as shown in fig. 7.

If a series of images has been obtained analysis can proceed as in Fig. 7: for each depth in the soil images are compared step by step (T1 with T2, T2 with T3 etc.) and the number of new root intersections with a grid is scored plus the number of intersections which has disappeared since the last observation. By cumulating all new root intersections that actual root intensity on the observation plane can be expressed as a fraction of the total annual production. Curve fits (e.g. logistic) of root growth and root decay per layer can be obtained. If at one point in time a by destructive sampling a reasonable estimate of standing root mass was obtained, these relative figures can be used to estimate annual root production. The assumption need not be made that root length on the observation surface has a known or constant relation to root length density in the soil. Calibration lines do in fact differ between soil horizons, crops, soil types etc. (Taylor, 1987). The main assumption needed for estimates of total root production are that the relative pattern of root growth and decay on the observation surface represents that in the soil. When glass or perspex (rigid) walls are used for the observation structure (mini-rhizotron), gaps between this surface and the soil may be unavoidable and roots grow and die in a 'gap' environment. Gijsman et al. (1991) described an inflatable minirhizotron system which reduces the problem of gap formation.

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Appendix D ROOTS: LENGTH, BIOMASS, PRODUCTION AND MORTALITY

Meine van Noordwijk

INTRODUCTION

In both crops and natural vegetation a substantial proportion of net primary production occurs below ground in the root system. This proportion varies with vegetation type, developmental stage, soil conditions and cultural practices. Shoot:root ratios on a dry matter basis are typically between 5:1 and 10:1 for annual crops at maximum standing biomass. For perennial crops and natural vegetation, values vary between 1:2 and 5:1. Uptake of water and nutrients are related to root length or root surface area rather than root weight. Distribution and periodicity of root length is important to evaluate whether or not crop demand for nutrients and water coincides in space and time with the available supply (synchrony and synlocalization). Root death and root exudates are a major input of organic matter to the soil system and the extent, timing and location of root death are therefore important. By leaving a well distributed set of continuous channels of mostly easily decomposable organic matter, roots have a relevance for soil biota, including roots of subsequent crops, far beyond their often limited quantity of organic matter.

For these reasons root investigations are an important part of the TSBF programme. Unfortunately they present particular difficulties; extraction of roots from soil is time consuming, labour intensive and still it is often incomplete.

D.2 QUANTIFICATION OF ROOT BIOMASS AND LENGTH

The methods described in Section 3.3 of this Handbook may, with adequate calibration, be used for estimates of root weight and length. More accurate estimates can be obtained from well replicated core (auger) samples, washed on a fine meshed sieve. To obtain reliable estimates of root biomass several points should be noted:

- samples should be taken from representative volumes of soil; in row crops special sampling schemes (Van Noordwijk *et al.*, 1985) may be needed;
- samples should be taken around the expected maximum standing root biomass; a late season sampling may result in a high proportion of dead roots;
- the methods for sampling, storing samples and washing will unavoidably lead to some loss of dry weight and nutrients; relevant correction factors may be obtained by simulating all procedures on roots grown in nutrient solution. Losses can be in the range 20 - 50% for dry weight, and thus correction factors of 1.25 - 2.0 should be applied to the final data (Van Noordwijk, 1987; Grzebisz *et al.*, 1989). Nutrient contents are generally only reliable if sample handling is completed within one day.

D.2.1 Corer design

The corer removes a known volume of soil from a known depth in the profile, without the need for digging a soil pit and destroying part of an experimental field. A core of 50 - 80 mm diameter is satisfactory, and the corer can be inserted either manually or mechanically. Manual coring is difficult at depths greater than 50 cm and in clay or stony soil. In dry sandy soil a smaller core diameter may be needed to reduce losses of soil when extracting the core. A suitable hand corer consists of a 15 cm steel tube with a serrated cutting edge mounted on a 1 m pipe, with a plunger to remove the core (Figure D.1; Bohm, 1979). Marks on the pipe indicate 10 cm depth increments. Soil is extracted with the corer in successive 10 cm increments.

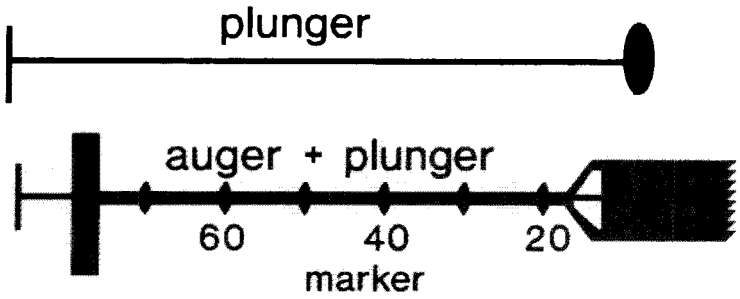

Figure D.1 Design of a hand corer

Alternatively a sharpened steel tube of appropriate length can be driven into the soil. In this case it is important to have a tripod and chain hoist to extract the tube, a file to resharpen the cutting edge, and longitudinally split liners of thin metal within the tube so that the intact soil core can be pulled out. A powered motor breaker can be used to drive the corer into the soil (Welbank and Williams, 1968), but compaction of the core will occur and should be allowed for when cutting subsamples from the core.

In very stony soil, or where there are many woody tree roots, coring may not be possible. Regular, known volumes of soil (monoliths) can be taken from the face of a pit and treated in the same way as cores.

D.2.2 Sample depth

Ideally the profile should be sampled to the limits of rooting depth. At depth, however, rooting intensity is low and spatial variability high. Based on initial profile wall observations a meaningful lower limit can be set. In some cases a linear relationship of the log of root mass versus depth (a negative exponential root distribution) may help to extrapolate root densities in the soil beyond sampling depth. All soils must be sampled to a minimum depth of 30 cm.

Where horizon development occurs, subdivide the cores at horizon boundaries of known depth in the first instance and within horizons in 10 cm increments. In cultivated soil or

where there are no clear horizons subdivide the mineral soil in 10 cm increments. Surface organic horizons should be treated separately.

D.2.3 Sampling intensity

Even in the most homogeneous soils a considerable spatial variability of root density will occur. For auger samples of about 385 cm^3 (10 cm height, 7 cm diameter) a coefficient of variation in root weight of at least 40% may be expected (Van Noordwijk *et al.*, 1985a); on heterogeneous soils the coefficient of variation may be much higher. This variability implies that large number of replicate samples are needed if precise estimates of root weight are needed.

It is advisable to obtain reliable information at one or two, well chosen situations, rather than non-reliable data on many. If 25 replicates would be analysed the standard error of the mean would be five times smaller than the standard deviation of individual samples, and thus the 95% confidence interval of the mean would be plus or minus 20%, even in the most homogeneous soil. Within each replicate plot of a treatment take no fewer than 3 cores. Within each replicate the samples at each depth increment can be pooled for further treatment. In natural vegetation where no sample stratification strategy is obvious, take the cores on random coordinates. Where patterns are likely to occur (e.g. row crops, alley cropping) the first stratification should be on an area basis (within rows vs. between rows), then sample randomly within the strata. Beware of high root mass directly underneath the plants. Root data are seldom normally distributed and an appropriate transformation ($(n+1)^{0.5}$ or log n) is often required before assessing the significance of differences between treatments or sampling intervals.

D.2.4 Root extraction

The best approach is to wash roots from the cores immediately upon return from the field. Core samples can be stored in sealed polythylene bags in a refrigerator for a few days or deep freeze until processed. If deep freeze facilities are not available, samples can be stored air-dried and re-wetted before washing. Losses of dry weight due to the methods used for storage should be checked.

Soil texture, structure, degree of compaction and organic matter content greatly influence the precision and time required to extract roots from cores. The simplest method involves gently washing a presoaked sample over a large diameter 0.3 - 0.5 mm mesh sieve. The work can be simplified by washing over a combination of sieves: one with 1.1 and one with 0.3 mm mesh. The first sieve will contain mostly roots, the second mostly debris. The material removed from the sieve(s) can then be mixed in water and the suspended material decanted (live roots have a specific density of about 1.0 g/cm^3). This residue is then hand sorted in shallow dishes under water to remove fragments of organic matter and dead roots; normally it is better to pick live roots from the sample and leave debris behind in the dish. Good light conditions, a calliper with 0.1 mm accuracy and a pair of (watchmaker) forceps are necessary; a stereo dissecting microscope (from x 4 to x 20) may be helpful. Operator fatigue is a problem and it may take about 6 hours to sort one core (to 1 m depth) from maize on a sandy loam, a similar core from forest with a high organic matter content or just one sample of the top layer of permanent grassland.

A number of root washing machines have been designed (Bohm, 1979). The most successful employ the process of elutriation, i.e. washing roots and organic debris free of soil and separating them by flotation, onto a 0.5 mm mesh sieve leaving behind the heavier mineral particles. The apparatus of Cahoon and Morton (1981) requires adequate water pressure and uses a lot of water; that of Smucker *et al.* (1982), now commercially available, uses less water and accepts smaller samples but requires both water pressure and compressed air. After elutriation roots must still be sorted by hand and this may take several hours. Neither apparatus handles organic soils. With both hand and machine washing, loss of fine roots occurs and a periodic check of washing water and residues should be made to quantify such losses; such losses should be kept less than 5% of root weight (which may imply that they are still over 10% of root length); this loss is distinct from that due to respiration and loss of cell contents from remaining root tissue.

Presoaking overnight in 5% sodium hexametaphosphate expedites the process of washing roots from clay soils, but the chemical discolours the roots, particularly in soils with high organic matter content and may disrupt the tissue, making subsequent identification of live roots more difficult. Such pretreatment will also interfere with chemical analyses and should therefore be avoided if possible. Any lengthy washing procedure may alter the element content of root tissue and a subsample hand sorted with a minimum of water and processed on the day of sampling must be used for analysis.

D.2.5 Classifying the roots

Fine roots are the most important part of the root system for water and nutrient uptake, as they form the largest part of total root length or root surface area. For woody perennial vegetation there is a fairly obvious distinction between the more or less permanent, secondarily thickened roots and the ephemeral, unthickened roots. This functional distinction usually falls somewhere between 1 and 3 mm root diameter. Roots above 10 mm diameter are not adequately sampled by coring. For herbaceous perennial and short lived vegetation no such clear distinction exists. For TSBF studies these roots should be separated into <2 mm and >2 mm classes. In mixed vegetation, separation of roots of different species may be difficult and is not necessary for TSBF research.

It is desirable to separate root samples into living and dead categories. This is particularly important in crop situations where old, dead roots from the previous crops may still be present. Living roots can be distinguished by their lighter colour, turgid appearance and flexible rather than friable nature when manipulated. Some preliminary anatomical investigation may help in establishing criteria for making decisions. Incubation of excised roots in soil in modified litter bags can be used to establish visual clues to the root decay process. Cross checking between operators and working block by block instead of treatment by treatment help to reduce experimental error.

Congo red staining has been used to differentiate wheat roots with intact epidermis from ones without (Ward *et al.*, 1978). Stain in 1% aqueous solution of Congo Red for 3 min, rinse, blot dry, then saturate for 3 min in 98% ethanol before a final rinse. Living roots stain dark pink to bright red. The criteria for making the living/dead distinction must be clearly stated. Where adequate criteria cannot be developed, assess total root mass only.

D.2.6 Assessment of root mass

Washed root samples can be stored in sealed polyethylene bags for a short time in a refrigerator, but deep-freeze storage is preferable. Thymol can be added as a bactericide, but should be handled carefully; classical storage media (ethanol, etc.) tend to make roots brittle.

Carry out biomass estimation on each size class of all samples. Oven-dry the roots and weigh. Next the dried samples should be combusted for 5 hr in a muffle furnace at 550°C and the residue weighed. Results should be expressed as ash-free oven-dry mass per unit volume of soil.

D.2.7 Root length measurements

Root length is a relevant parameter for water and nutrient uptake. The specific root length (length per unit dry weight) of roots depends on diameter, variability of diameters, dry matter content (per unit fresh weight) and air-filled porosity (per unit volume) (van Noordwijk, 1987). Within a species or situation variability of the specific root length may be rather small (e.g. with a coefficient of variation of 10-15% while root weight per unit soil volume has a coefficient of variation of about 40%), so it is reasonable to measure the specific root length only for some subsamples. Normal values are from about 10 m/g for fine tree roots, 50-200 m/g for fine roots of dicotyledonous crops and 200-600 m/g for cereals and grasses (Van Noordwijk and Brouwer, 1991).

Root length can be estimated by counting the number of intersections between roots and sample lines. This method is based on Buffon's needle problem, described in 1777, where the chance that a needle randomly thrown on a tiled floor would intersect one or more of the edges of the tiles was formulated as a function of the length of the needle and the size of the tile. Application of the method for measuring root length is based on Newman (1966) and Tennant (1975). Roots are spread out with random orientation in a thin layer of water on a glass plate (about 25 x 25 cm), water is removed and a grid (photocopied on an acetate folio for overhead projection) is put over or underneath the sample. Line by line all intersections of roots and grid lines (taking the upper or left boundary of the line as criterion in case of doubt) are added (Figure D.2). Results for horizontal (H) and vertical (V) grid lines are added to the number N. If the grid size is D (mm), root length L (mm) is derived as:

$$L = \pi N D / 4$$

If D is set equal to $40/\pi$, i.e. 12.7 mm, L = 10 N (mm). By adding results for H and V lines the method is insensitive to preferential oreintation of roots on the plate, but spreading the roots must be done without regard of the position of the grid lines. To improve contrast roots can be stained beforehand, e.g. with saffranin red (1 g/litre).

To optimise working efficiency at an acceptable random error level the grid size should be chosen to obtain about 400 intercepts (200 H + 200 V) per sample. This method can be applied manually (using a magnifying lens for fine roots) or can be automated in various ways (Rowse and Phillips, 1974; Richards *et al.*, 1979; Wilhelm *et al.*, 1982). Each variant of the method should be calibrated by cutting a known length of cloth to small pieces. Commercial

equipment is available (Comair Root Length Scanner, Commonwealth Aircraft Corporation Ltd, 304 Lorimer St, Port Melbourne, Victoria 32307, Australia). Recently computer image analysis methods are used as well, based on a video camera or line scanner. Only small fields of view can be analysed with sufficient resolution and root samples should be spread out more carefully than when human eyes are used as image analyser, so the gain may be less than expected. For TSBF purposes manual versions of the method are recommended.

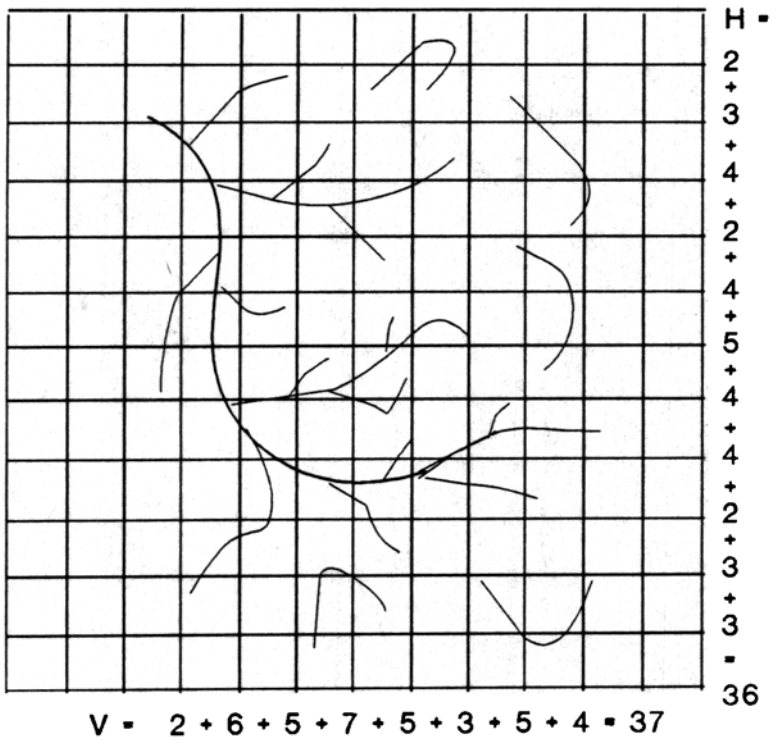

Figure D.2 Line intercept method for determining root length by counting the number of interceptions between roots and horizontal (H) plus vertical (V) lines of a grid.

Measurements of the frequency distribution of root diameters can be made on the sample spread out on a grid, by measuring on every *x*th interception, using a binocular microscope with an ocular micrometer. At least 20 readings per sample are required (assuming three samples per treatment per layer).

D.2.8 Mycorrhizas

It is desirable to have some measure of the type and intensity of mycorrhizal infection in each treatment. At peak root biomass take approximately 60 cm of fresh clean root at random from each depth and stain to visualize fungal hyphae. The method of Phillips and Hayman (1970) can be followed with small adaptations: short (ca. 2 cm) lengths of roots (stored in alcohol-acetic acid mixture after washing) are heated for about 1 hr at 90°C in 10% KOH (or left for 2-3 days at ambient temperature), washed in water, if necessary (dark tissue) bleached in alkaline peroxide (3% NH_4OH in 3% H_2O_2, prepare daily), washed in water, acidified for 3 min in 1% HCl, stained for 5 min at ambient tempearture in 0.05% trypan blue (Merck Art

No. 11732) in lactic acid and destained in clear lactic acid. In the original description lactophenol (1:1:1:1 mixture of phenol, lactic acid, glycerol and water) was used instead of lactic acid. As phenol is toxic it should not be used, unless staining results are unsatisfactory. A modified procedure was described by Kormanik and McCraw (1982). For roots with a high lignin content the KOH treatment may have to be intensified. After staining the samples are inspected under a (dissecting) microscope. Vesicular arbuscular mycorrhiza (VAM) infection is characterized by the formation of unseptated hyphae outside the root and inter- and/or intracellular hyphae in the cortical cell layers of the root (Sieverding, 1991). The percentage of root sections which has mycorrhizal structures is assessed. Ectomycorrhizas are characterized by a fungal sheath and/or Hartig net; the percentage of root tips with such structures (which can often be recognized macroscopically and without staining) is assessed.

See also Appendix C "Mycorrhizas".

D.2.9 Sampling frequency

The amount of living and dead root in the ecosystem fluctuates as a balance of new root growth and root death and decay. Given the large number of replicate samples required to obtain a reasonable (let alone accurate) estimate of root density at a given moment, a regular sampling programme to monitor changes during the growing season easily becomes unmanageable. For TSBF site characterization a minimum of two samples per year is required.

For perennial vegetation some prior knowledge of the phenology of the root system will allow sampling to coincide with the likely peaks and troughs in root biomass ; root growth often alternates with periods in which reproductive growth is a major sink for carbohydrates. Where no prior knowledge exists samples should coincide in herbaceous vegetation with maximum and minimum above-ground biomass, and in forest with the months of minimum and maximum rainfall. For annual crops maximum root development often coincides with flowering (transition from vegetative to generative stage).

Sampling at harvest time may lead to a considerable amount of dead roots. Sampling before planting a new crop is relevant for estimating the rate of decay of dead roots from previous crops.

D.3 ESTIMATION OF TOTAL ROOT PRODUCTION

To estimate total root production the biomass estimates obtained (corrected for sampling schemes and dry matter losses) should be corrected for root turnover between sampling dates. Due to the large variability of results from destructive sampling it is not possible, in practice, to obtain turnover estimates from frequent sampling schemes.

The frequent sampling method derives from the following scheme with two pools (live and dead roots) and three rates (production, mortality and disappearance):

Production (R_p) --> Live root mass (L_t) --> Mortality (R_m) --> Dead root mass (D_t) --> Disappearance (R_d)

The following equations hold:

$$L_{t+1} = L_t + \int R_p$$

$$D_{t+1} = D_t + \int R_m - \int R_d$$

If live and dead roots cannot be separated:

$$(L+D)_{t+1} = (L+D)_t + \int R_p - \int R_d$$

Thus:

$$\int R_p \geq (L+D)_{t+1} - (L+D)_t$$

Sampling errors in determining L_t and D_t play a dominant role, however. In part of the literature estimates of root production are based on statistically significant increments of root mass. Statistical significance, however, not only depends on the size of the difference but also on the sampling intensity. Although this method has been used in past decades, and the literature contains many estimates based on this method, a methodological study by Singh *et al.* (1984) showed that considerable over and under estimates can occur. For a full discussion of the biological and statistical aspects of the topic, including frequency of sampling in a range of systems, consult McClaugherty *et al.* (1982), Fairley and Alexander (1985), Goltz *et al.* (1984), Lauenroth *et al.* (1986), Hansson and Andren (1986), Singh *et al.* (1984) and Vogt *et al.* (1986). More reliable methods for estimating root turnover are based on separate study of root growth and root decay processes.

D.3.1 Root decay

Root decay (both on a root length and a root weight basis) can be studied in a modified litterbag method, incubating known amounts of excised roots (e.g. collected by sieving soil at harvest time) in a ceramic pot filled with sieved, root-free soil and placing the pots in the field (possibly at two depths). A screen cover on top of the pot may prevent soil fauna from removing root tissue. At regular intervals pots are retrieved and washed on a fine mesh sieve. Intact roots and root debris are collected separately.

D.3.2 In-growth cores

Seasonal patterns of root growth can be studied by the in-growth core method (Steen, 1984; Fabiao *et al.*, 1985). Soil from the depth at which the in-growth core will be placed is collected from the site, air-dried and roots removed by sieving. In-growth bags may be made from plastic sacking of a minimum of 4 mm mesh, or from more rigid polypropylene cylinders (4 to 10 cm diameter, sealed at the bottom and top with mesh). For each bag an amount of soil is weighed according to the required bulk density of the soil. Auger holes are prepared, and the sacks or cylinders are filled (*in situ*) with soil, compacting cm by cm, loosening the surface before new soil is added. The right procedure to obtain the required bulk density can be found by trial and error. At regular intervals (say 1 month) in-growth

bags (minimum 3) are retrieved and washed over a fine mesh sieve. Data allow periods with rapid root growth to be identified.

Because of the altered physical, chemical and biological conditions in the sieved and repacked soil, growth of the roots into the bag may not accurately represent growth in the bulk soil, particularly on compacted or clay-rich soils. Periods of active root growth in different layers, however, should be accurately reflected.

The technique can also be used to record root response to localised fertiliser application or other heterogeneities in the soil (Cuevas and Medina, 1988; Hairiah *et al.*, 1991). Fertiliser application should be made by mixing the soil with a fertiliser solution prior to filling the bags.

D.3.3 Minirhizotrons

Simultaneous processes of root growth and root death or decay can only be quantified if the fate of individual roots can be followed. The simplest method uses a glass sheet against a soil profile wall, tracing roots on transparent polyethylene sheets. By using different colours of marking pens both new root growth and disappearance of roots can be quantified. An intermediate (but tedious) technique uses glass tubes inserted into the soil and regularly inspected (on grid lines in the glass) for roots using a mirror and a torch. More sophisticated versions use a fibreoptic system with a camera (Figure D.3) and flash light or a video system (Taylor, 1987).

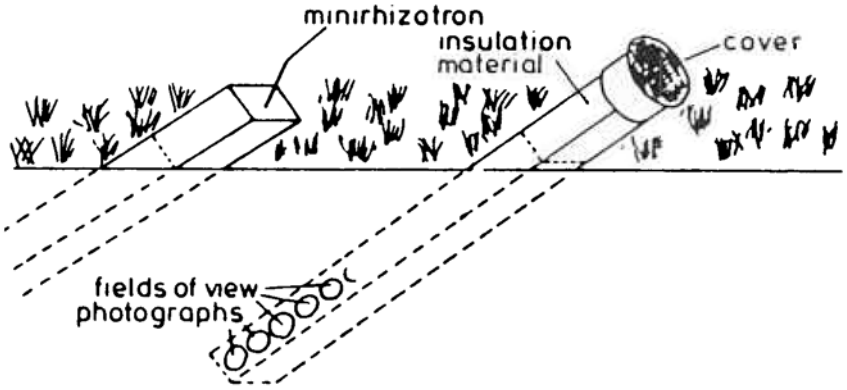

Figure D.3 Minirhizotron system to observe dynamics of root growth and decay under field conditions; at each time of observation a series of images with increasing depth is taken; these images can be analysed as shown in Figure D.4.

If a series of images has been obtained analysis can proceed as in Figure D.4: for each depth in the soil images are compared step by step (T1 with T2, T2 with T3 etc.) and the number of new root intersections with a grid is scored plus the number of intersections which has disappeared since the last observation. By adding up all new root intersections the actual root intensity on the observation plane can be expressed as a fraction of the total annual

production. Curve fits (e.g. logistic) of root growth and root decay per layer can be obtained. If at one point in time, by destructive sampling, a reasonable estimate of standing root mass was obtained, these relative figures can be used to estimate annual root production. The assumption need not be made that root length on the observation surface has a known or constant relation to root length density in the soil. Calibration lines do in fact differ between soil horizons, crops, soil types etc. (Taylor, 1987). The main assumption needed for estimates of total root production are that the relative pattern of root growth and decay on the observation surface represents that in the soil. When glass or perspex (rigid) walls are used for the observation structure (mini-rhizotron), gaps between this surface and the soil may be unavoidable and roots grow and die in a "gap" environment. Gijsman *et al.* (1991) described an inflatable minirhizotron system which reduces the problem of gap formation.

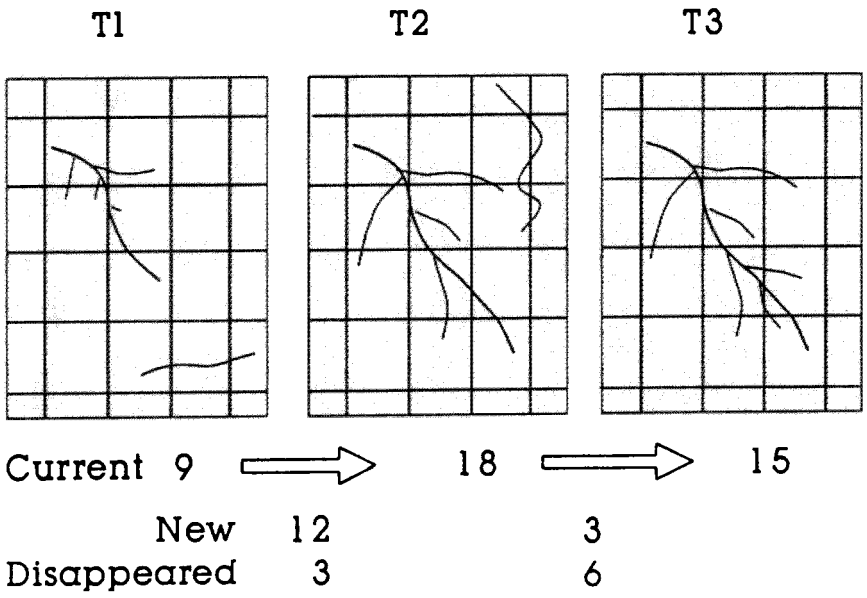

Figure D.4 Analysis of sequential images to derive root turnover.

D.4 ESTIMATE OF TOTAL CARBON INPUT TO THE SOIL

In addition to structural root tissue, carbon inputs to the soil include sloughed root cap cells, mucilage produced at the root tip, decayed root hairs and other cellular material, soluble carbohydrates, amino acids and other exudates, and CO_2 from root respiration. These root-rhizosphere transitions form a continuum, the study of which usually is based on ^{14}C labelling and sophisticated laboratory equipments.

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