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Root recovery of five tropical tree and shrub species by sieves of different mesh sizes

S. M. Gathumbi[1,2]
[1]*Kenya Forestry Research Institute, P. O. Box 20412, Nairobi, Kenya.;* [2]*MacArthur Agro-Ecology Research Center, 300 Buck Island Ranch Road, Lake Placid, FL 33852, USA; (e-mail: sgathumbi@archbold-station.org)*

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Abstract

Accurate quantitative assessment of roots is key to understanding the belowground plant productivity as well as providing an insight of the plant-soil interactions. In this study, root recoveries by sieves of different mesh sizes (2.0, 1.0, 0.5 and 0.25 mm) were measured for five tropical tree and shrub species grown in monoculture stands: crotalaria (*Crotalaria grahamiana* Wight and Arn.), pigeonpea [*Cajanus cajan* (L.) Millsp.], sesbania [*Sesbania sesban* (L.) Merr.], tephrosia (*Tephrosia vogelii* Hook F.), siratro [*Macroptilium atropurpureum* (DC.) Urb.] and tithonia [*Tithonia diversifolia* (Hemsl.) Gray]. Root samples were take from 0-15 cm soil depth. Recovery of coarser roots (> 1.0 mm) ranged from 70 to 93% and 90 to 98% of the cumulative root length and biomass respectively. The proportion of root length of the finer roots (< 1.0 mm) was greater for pigeonpea (30%), tithonia (22%) and siratro (18%) compared with other species, but contributed negligibly to the cumulative total root biomass for all species. The use of 0.5 mm sieve improved the recovery of root length for most species but had little effect on root biomass. The 0.25 mm sieve was most effective in capturing finer roots (< 0.5 mm) of pigeonpea which represented 16% of cumulative root length and 4% of root biomass recorded for this species. Recovery of roots of different diameter classes depended on species, suggesting that for an improved estimation of root parameters especially when sieves of large mesh sizes (> 0.25 mm) are used, a correction factor could be useful for root length measurements but not root biomass measurements for a particular species in each site and for a specific study.

Introduction

Measurement of belowground plant productivity may be used to explain root growth and distribution as well as the many important root functions in the rhizosphere such as nutrient acquisition and cycling (van Noordwijk and Brouwer 1991), energy allocation, improvement of soil structure and maintenance of soil organic matter. These root functions are important especially in agroforestry systems where selected plant species are expected to rapidly replenish soil fertility by recycling subsoil nutrients through aboveground biomass application. Hence, both qualitative and quantitative estimates of root parameters provide a tool for estimating the overall belowground contribution of plants in the plant-soil system. However, field measurements of tree roots are not only resource demanding but also tedious and time consuming. In fact, many comparisons of the root data among tree species reported on agroforestry systems have been obtained from unreplicated plots (Eastham and Rose 1990) or from few individual trees within a stand (Ruhigwa et al. 1992).

Root length and biomass are commonly measured by auger sampling whereby a known volume of soil containing roots is augered, washed and roots separated from the soil by use of sieves through a process of sedimentation and decantation. The roots are then sorted according to species and/or diameter classes and the root length measured by either the line intercept method or automated scanning, and biomass by dry matter determination.

Despite the pivotal role the very fine roots play in plant nutrient acquisition from the soil (Eissenstat 1992), most studies tend to ignore this fraction of roots because including them would increase the amount of time and resources required for the root preparation and data collection. This often leads to an underestimation of root parameters (Amato and Pardo 1994; Ruhigwa et al. 1992). For instance recent studies conducted in western Kenya reported root parameter estimations based on the roots recovered by 0.5 mm sieve (Gathumbi et al. 2003; Jama et al. 1998; Mekonnen et al. 1997) but ignored part of the fraction of finer roots that passed through the 0.5 mm mesh size sieve. However, Livesley et al. (1998) studied the effects of sieves on root recoveries of grevillea (*Grevillea robusta* A. Cunn. Ex R. Br.) and *Zea mays* L. roots and reported that upto 20% of the total root length of either species could be recovered by the 0.25 mm sieve size. Other earlier studies also reported that finer roots may pass through sieves of larger mesh size, thereby underestimating the accruing root length and biomass estimates (Amato and Pardo 1994; Ruhigwa et al. 1992).

The objective of this study was to investigate the effect of sieve sizes on the recovery and estimation of root parameters of different tropical species planted in monoculture stands. The tested species consist of trees and shrubs with varying rooting patterns and are commonly used in short term improved fallows for soil fertility improvement in the study area.

Materials and methods

The study was conducted on a farmer's field at Nyabeda, western Kenya (0°06'N, 34°34'E, 1330 m above sea level). Rainfall in the study area is distributed in two crop growing seasons per year with an annual mean of 1800 mm. Soils are generally classified as slightly acidic, very fine, kaolinitic, isohyperthermic Kandiudalfic Eutrudox.

This root recovery experiment was carried out for 6-month old tree and shrubby species planted in a larger legume screening experiment established in October 1997 (short rainy season) (Gathumbi et al. 2002). Monoculture stands of *Crotalaria grahamiana*, *Cajanus cajan*, *Sesbania sesban*, *Macroptilium atropurpureum*, *Tephrosia vogelii* and *Tithonia diversifolia* planted in an improved fallow system were sampled for root parameter measurements. All species were planted at an inter-row and intra-row spacing of 0.75×0.75 m. Four replicate samples (soil volume=1178 cm^3) were taken in each fallow plot at 0-15 cm soil depth using a Jarret auger with an internal diameter of 10 cm. Samples were taken from the intersection point of the two diagonal distances from four plants randomly selected within the interior portion of the plot. This was to ensure that the sampled area was representative of the unit area/soil covered by each plant following the suggestions of Rao and Coe (1991).

All samples were soaked in a bucket of water for at least 12 h. The soaked soil-water slurry was then manually stirred and soil-root suspension passed through a stack of four sieves with decreasing mesh sizes, 2.0, 1.0, 0.5, and 0.25 mm with a soft spray of water. To ensure maximum recovery of the very fine roots by the 0.25 mm sieve, the process of sedimentation and decantation was repeated several times before the soil slurry was discarded. Roots and organic debris retained on each sieve were stored at 5 °C in plastic bags containing 17% (v/v) acetic acid solution. Live roots (recognized by their brown colour on the outside and white internally, turgidity and friability) were separated from the dead roots and organic debris within two weeks of sampling. The separated live roots were then stained with methyl violet solution (0.1% in 10% [v/v] ethanol), spread on a glass tray, and scanned with Aldus photostyler image analysis software at a resolution of 95 dots per cm for determination of root parameters. Immediately after scanning, the root samples were oven dried (70 °C, 48 h) and then weighed.

All root data are reported as the actual root parameters measured from the roots recovered by each sieve size. Percent root biomass recovery by each sieve size is the proportion of the total (cumulative) biomass recovered by the four sieve sizes. All data were subjected to one way analysis of variance using Genstat statistical software (Payne et al. 1987). Standard errors of the difference are reported to enable comparison of means (n = 4) of root parameters for the different species recovered by the respective sieves.

Results

Recovery of root length

Total root length and root length density decreased with decreasing sieve mesh size for all the species (Table 1). The proportion of total root length recovered using each sieve size differed among species (Figure 1). The largest sieve size (2.0 mm) recovered the greatest root length (> 60%) for most species except for pigeonpea where it recovered about 45% of the total root length. The coarser root diameter classes retained by the 1.0 mm diameter sieve constituted > 78% of the cumulative root length of all species except for pigeonpea where roots in this diameter class accounted for about 70% of the total root length. The cumulative total root length recovery for the 2.0, 1.0 and 0.5 mm sieve sizes ranged between 84-98% total root length with pigeonpea and sesbania with the smallest and greatest respectively. The greatest root length recovered by the 0.25 mm sieve was recorded for pigeonpea (16%), which also gave the highest root length density (4.4 cm cm^{-3}) compared with the other species.

Root biomass

As with the root length, recovery of root biomass decreased with the decreasing sieve sizes and differed among species (Table 1). Cumulative total root biomass recovery by the 2.0, 1.0, and 0.5 mm sieves was > 96% of the total biomass for all the species. Cumulative biomass for coarser roots (> 1.0 mm) ranged between 720 and 1450 mg per core (Table 1). The very fine roots that passed through the 0.5 mm sieve but trapped by the 0.25 mm sieve contributed a negligible proportion of the total root biomass for most species (Figure 2). However, the proportion of fine root biomass recovered by the 0.5 and 0.25 mm sieves was greater for pigeonpea (13%), compared with crotalaria (3%), sesbania (2%) and tephrosia (3%) but similar to siratro (8%) and tithonia (10%).

Discussion

Larger sieve sizes (2.0 and 1.0 mm) recovered the bulk of the root length and biomass for all the species tested suggesting that there were more coarser roots in the 0-15 cm soil depth sampled than the fine roots. Similar root recovery findings were reported for

Table 1. Total root length, root length density, and biomass of different species as determined with sieves of 2.0, 1.0, 0.5 and 0.25 mm mesh sizes in western Kenya.

Species[a]	Root length (m)				Root length density (cm cm^{-3})				Root biomass (mg $core^{-1}$)			
	> 2.0 mm	1.0-2.0 mm	0.5-1.0 mm	0.25-0.5 mm	> 2.0 mm	1.0-2.0 mm	0.5-1.0 mm	0.25-0.5 mm	> 2.0 mm	1.0-2.0 mm	0.5-1.0 mm	0.25-0.5 mm
Crotalaria	40.2	5.2	3.3	1.4	29.9	4.4	2.8	1.2	1206	33	26	9
Pigeonpea	22.1	10.2	6.4	5.2	18.7	8.6	5.5	4.4	590	134	79	33
Sesbania	26.8	5.6	2.6	0.6	26.9	7.0	2.2	0.6	1388	63	24	11
Siratro	10.5	2.4	1.4	1.6	9.0	2.1	1.2	1.4	215	23	13	8
Tephrosia	32.6	8.2	1.6	1.0	27.7	6.9	1.4	0.9	703	84	17	8
Tithonia	19.8	5.9	4.7	2.9	16.8	5.0	5.5	2.4	1224	111	112	33
SED[b]	11.4	2.6	2.2	1.5	8.3	2.4	2.2	1.3	279	40	30	17

[a]Species scientific names *Crotalaria grahamiana; Cajanus cajan; Sesbania sesban; Macroptilium atropurpureum; Tephrosia vogelii; Tithonia diversifolia;* [b]Standard error of the difference between treatment means.

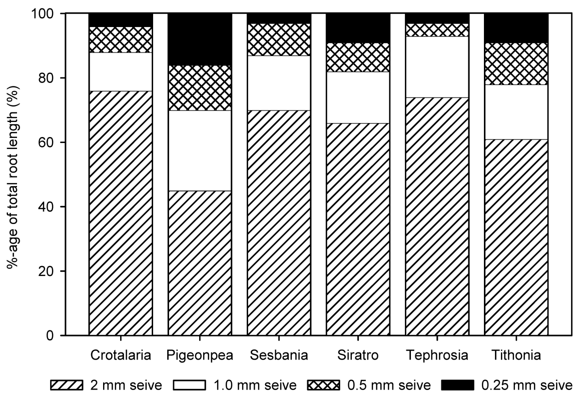

Figure 1. Proportion of the total root length recovered by the 2.0, 1.0, 0.5 and 0.25 mm sieve mesh sizes for five tropical trees and shrubs in western Kenya. Species scientific names are *Crotalaria grahamiana*; *Cajanus cajan*; *Sesbania sesban*; *Macroptilium atropurpureum*; *Tephrosia vogelii*; *Tithonia diversifolia.*

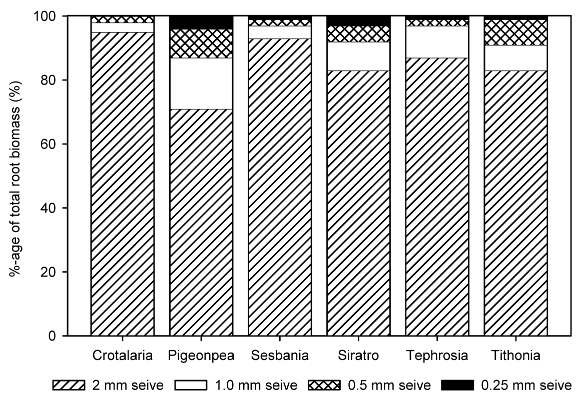

Figure 2. Proportion of the total root biomass recovered by the 2.0, 1.0, 0.5 and 0.25 mm sieve mesh sizes for five tropical trees and shrubs in western Kenya. Species scientific names are *Crotalaria grahamiana*; *Cajanus cajan*; *Sesbania sesban*; *Macroptilium atropurpureum*; *Tephrosia vogelii*; *Tithonia diversifolia.*

tree roots by Amato and Pardo (1994) and Livesley et al. (1998) using sieves of coarser mesh sizes ($>$ 0.5 mm). The 0.5 mm sieve greatly improved the recovery of root length and biomass for all the species. However, the very fine roots recovered by the 0.25 mm sieve contributed a significant proportion of the measurable root length but had little effect on the total root biomass for most species (Figure 1 and Figure 2), suggesting that the 0.5 mm sieve was sufficient for the biomass measurements. The use of the 0.25 mm sieve size recovered similar proportions of root length as the 0.5 mm sieve in most cases, and hence doubling the values obtained for the proportion of roots passing through the 1.0 mm sieve. While the use of sieve sizes of 2.0 and 1.0 mm was recommended by Amato and Pardo (1994), the results of this study suggested that this would lead to an underestimation of root length estimates by about 30% (pigeonpea), 22% (tithonia), 18% (siratro), and 12% (sesbania and crotalaria), if roots passing through the 1.0 mm sieve were not accounted for. Similar concerns were raised by Livesley et al. (1998) in a study investigating the effect of sieve sizes on root recovery for grevillea and maize plants.

The majority of nutrient uptake from the soil is believed to occur through the fine root and root hairs primarily due to larger uptake surface area and hence inclusion of this root fraction in the estimation of root length density is of paramount importance. Root morphology and size is dependent on species, nutritional status of the soil in which the plant is growing, and age of the plant (Fitter 1991). However, fragmentation of the roots may also occur during root sampling and processing which may lead to loss of distinct root integrity and increased amounts of roots passing through a given sieve (Amato and Pardo 1994). This concern could in part be minimized by adequate soaking of roots in water to facilitate easier root disaggregation from the soil and application of a mild water spray throughout the washing process. However, it is worth noting that the use of sieves of finer mesh sizes in root studies substantially increase both time and labour costs (Livesley et al. 1998).

From the results of this study it appears that the 0.5 mm sieve is adequate for recovery for the majority of roots. However, the fraction of the very fine roots passing through the 0.5 mm sieve but trapped by the 0.25 mm sieve can improve the accuracy of the estimation of root length measurements and should be recommended for use in root length measurements. Since the root fraction recovered by the 0.25 mm sieve differed among species, correcting root parameters of the very fine root fraction should be done for each species during each sampling time. Again, it should be noted that development of a universal correction factor for the fine root estimation may not be appropriate since its application could be limited because plant root morphology and size is dependent on species, growth stage and other physical and environmental factors.

Recovery of root length and biomass by sieves of different mesh sizes differed among the species. The minimum sieve mesh size to use may depend on the root parameters to be measured and the species being studied. The 0.5 mm sieve appeared to be adequate for root biomass estimation for most species while the 0.25 mm sieve was advantageous in root length estimations. For improved accuracy of root length measurements in a specific study, I suggest that few additional replicated root samples should be taken (or a replicated subset of samples) and roots passing through the 0.5 mm but retained by the 0.25 mm sieve be measured. The accruing estimates of the very fine roots can then be used to correct root length estimates obtained for the rest of the sample recovered by the 0.5 mm sieve for a specific plant species at a particular location during each sampling time.

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