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COMPARISON OF PROCEDURES FOR SAMPLING THE BELOWGROUND BIOMASS IN SHORTGRASS PRAIRIE

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ABSTRACT

To facilitate reconciliation of observations on root biomass and composition as recorded by Grassland Biome investigators, comparison was made of three procedures currently in use for root studies at the Pawnee site. Biomass (i.e., organic carbon) recoveries by the three methods were not greatly dissimilar; they were ranked as Pawnee wet < Pawnee dry < ARS dry. All three methods were amenable to macroscopic and rapid sorting procedures permitting compartmentalizations of root material. Compositionally, the several fractions differed markedly in C:N ratios, in free and bound amino acids, and in free and bound carbohydrates. Discrepancies concerning the composition of the root biomass at Pawnee are believed to stem largely from extrapolating to the total biomass the unique characteristics of relatively minor fractions of that total. The extent to which compositional attributes of root compartments may be used as indicators of the active or functional fraction of the total root biomass is discussed.

INTRODUCTION

During the several years that studies under the US/IBP Grassland Biome Program have been in Progress at the Pawnee site in northeastern Colorado, a number of investigators with diverse interests have made root biomass measurements. Their methods of sampling and of sample handling have varied considerably. Although some of the data that have been compiled on root biomass are not greatly dissimilar (Sims and Singh, 1971; Clark, 1975), other data, such as that concerning root nitrogen (Bokhari and Singh, 1974; Clark, Warren and O'Deen, 1974) or functional root biomass (Singh and Coleman, 1975; Clark, 1975) are sufficiently dissimilar as to suggest that a reconciliation of the different measurements is highly desirable and that this might best be accomplished by direct comparison of several different operational procedures. Hopefully, in such a comparison, useful information might be compiled concerning differences in root composition dependent on whether roots were washed from soil or given dry separation. Also, further light might be shed on the extent to which meaningful separations of the total root biomass into compartments could be routinely achieved. It seems ironic that of the total biomass, the 10 or 15 percent that is found aboveground is divided into as many as five compartments (standing green, this year's dead, old dead, loose litter, humic litter) while the far larger biomass belowground is commonly lumped together simply as root biomass.

Our purpose here is to report on the total yields of root biomass, the extent to which compartmental separations within the total can be achieved, and the compositional differences among compartmentalized root samples to be observed when using dissimilar procedures.

METHODOLOGY

The three root sampling methods discussed herein are identified as the Pawnee wet, the Pawnee dry, and the ARS dry.

The Pawnee wet method involves machine washing of individual soil core in screen-walled cylindrical containers. The containers are mounted on a revolving drum (capacity 48 cans) in such fashion that they pass through water during the lower part of their cycle, while in the upper part they are struck with water jets. Root materials and sand or rocks not passing the 40-mesh screens are removed and placed in water, after which the roots are decanted from the gravel and oven-dried. Routinely this harvest is weighed and asked as a unit and recorded as the first fraction of the root biomass yield. The second fraction consists of the organic debris that escapes from the cans during washing and leaves the assembly in the overflow water. It is trapped therefrom on a 60-mesh screen. The total recovery on this screen is divided by the number of cans on the drum and that quotient added to the individual first fractions to give the total root yield from an individual soil core.

For purposes of this study harvests from the individual cans were composited into a single gross sample and this was divided into two parts, one designated as "wet fibrous" and the other as "wet trashy." The former included the more filamentous roots that tended to tangle together and the latter, the more fragmented root material that remained in the gross sample after the more fibrous roots had been picked out by hand. The overflow root material collected on the 60-mesh screen was not subdivided; it was simply labeled as "overflow trashy."

The Pawnee dry method involves pulverization of all cloddy soil followed by processing of the dry soil and its contained root materials through a seed-cleaning type of machine. During operation there is side delivery into a bucket of root materials and coarse sand and gravel; there is also pan entrapment of airborne root materials within the machine itself. In the application of the Pawnee dry method in the present study, it was noted that the major portion of the organic material in the root bucket was fibrous root material, and the remainder, trashy. In the root pan, the major portion of the root material was trashy, but there was also some fibrous root material present. The fibrous material from both the bucket and the pan was handpicked and labeled as "dry fibrous;" the remainder in both containers was labeled as "dry trashy." Routinely at Pawnee, personnel have picked for chemical analyses a dry fibrous sample corresponding to what is herein labeled as "dry fibrous." In the Pawnee dry coring and sampling on August 26, 1974, performed specifically for this comparative study, all soil coming from the cleaning machine was saved for examination in the ARS laboratory. There it was found to have heavy contamination with organic material. Accordingly, it was given the ARS air suction and water flotation treatments (see below) and the root materials obtained were labeled as "ARS additional."

The ARS dry method consists of hand sorting from pulverized soil of the major portion of fibrous root material, air-suction pickup of the more highly fragmented fibrous roots and miscellaneous particles of soil-borne litter, and finally, a water flotation of the fine particles of organic matter remaining in the soil after the air-suction treatment. Root materials recovered by the hand, air-suction and flotation treatments have been arbitrarily designated as live, senescent and detrital

roots, respectively. In general, the live roots compartment includes the non-brittle, longer, and quite uniformly light-colored root material. In the separation by air suction of the so-called senescent roots, approximately 0.5 kg soil is spread on a metal tray 38 by 53 cm, and the under side of the tray is repeatedly stroked with a hand-held, electric vibrator tool of the type commonly used to pack chromatographic columns. Thereafter, the end of a household-type vacuum cleaner hose, covered with fine nylon gauze, is held parallel to and about 1 cm above the soil surface, and passed repeatedly back and forth over that surface. Particulate organic material drawn onto the gauze is removed and the vibration and suction treatments are repeated until the yield of particles on the gauze becomes negligible. The operation is then performed on additional aliquots until all of a gross soil sample has been processed. In the separation of detrital roots, soil that has been given handsorting and air suction treatments is slowly poured into a container of water, and the particulate debris floating to the water surface is skimmed off with a 100-mesh screen. Stirring and skimming is continued until float yield becomes negligible. The ARS method was developed by Clark and associates for use in a longterm isotopic nitrogen experiment at the Pawnee site. Root biomass data from that experiment, initiated in 1971 and still in progress, has been drawn on for purposes of this report.

Specifically for this report, two series of soil cores were taken on 26 August 1974 from the experimental area used for the ^{15}nitrogen study, but not from within the mini-plots themselves. Soil coring of the 0-10 cm profile was performed by C. E. Dickinson and associates.

These workers subjected one series to the standard Pawnee wet method and the second, to the Pawnee dry method, for separation of root material. All root materials recovered were given to Clark and associates for analytical determinations.

Chemical determinations made on the root materials were as follows: Total organic carbon was determined by combustion in an automated electric furnace. Total nitrogen was determined by standard micro-Kjeldahl procedure. For extraction of the soluble constituents from root materials, triplicate samples of 0.5 g each were subjected to five successive 15-minute extractions with boiling ethanol at concentrations of 80, 80, 50, 50, and 80 percent, v/v. Soluble sugar content was determined by adding 5 ml anthrone solution (0.2 g in 100 ml conc. H_2SO_4) to 1 ml of ethanol extract solution and 2 ml of deionized water. Optical density was measured at 630 nm after 7.5 minutes in a boiling water bath. Values obtained were expressed as glucose equivalents. Free amino acid content was determined by the method of Rosen (1957) except that KCN was used instead of NaCN. Values obtained were expressed as tyrosine equivalents. Hydrolysis of polymers was accomplished by exposure of the ethanol-extracted residues to 7 ml of 6 N HCl at 105 C for 24 hours. Amino acids and oligosaccharides released during hydrolysis were then measured as already noted.

The question of just how much of a root component is lost when roots are subjected to washing was investigated by measurements on root nitrogen. A gross lot of fibrous roots dry-sorted from soil was divided into four aliquots. One aliquot was analysed without any pretreatment; a second was soaked one hour in water before analysis;

a third was oven-dried for 2 hours and then soaked 1 hour before analysis; and a fourth, oven-dried, ground to pass a 40-mesh screen, and then soaked 1 hour prior to analysis. A closely similar experiment was done with dry-sorted live and senescent roots, with one aliquot of each analyzed unwashed; a second, dried and washed; and a third, dried, ground, and washed.

RESULTS

Oven-dry weights of the diverse root materials recovered were recorded but they are not particularly meaningful because of the differing amounts of mineral soil contamination carried by the roots. Comparison of biomass recoveries based on total organic carbon recovery are shown in Table 1. Also shown are total nitrogen recoveries and the C/N ratios of the several root samples.

Table 2 shows the carbon and nitrogen contents of the root samples, the free and polymerized amino acids expressed per gram of root nitrogen, and the free and polymerized sugars expressed per gram of root carbon.

Nitrogen contents in the root washing experiments are expressed relative to root nitrogen in the freshly collected undried and unwashed aliquots. Relative nitrogen contents in the first batch of roots were as follows:

Freshly collected, undried and unwashed fibrous roots	1.00
Not dried, but washed	.92
Dried and washed	.91
Dried, ground, and washed	.67

The second batch of roots yielded the following data:

Freshly collected live roots	1.00
Dried and washed	.91
Dried, ground and washed	.68
Freshly collected senescent roots	1.00
Dried and washed	.94
Dried, ground and washed	.89

Table 1. Carbon and nitrogen recoveries and C:N ratios of compartmentalized root materials obtained by dissimilar methods of sampling.

	mg root C from 7.2 kg of soil	Compartmental C / Total C	mg root N from 7.2 kg of soil	Compartmental N / Total N	Root C / Root N
Pawnee Wet Method:					
wet fibrous	4635	.22	86.4	.09	53.6
wet trashy	9674	.45	438.8	.47	22.0
overflow trashy	7083	.33	407.1	.44	17.4
Total	21392	1.00	932.3	1.00	22.9
Pawnee Dry Method:					
dry fibrous	3202	.14	52.4	.05	61.1
dry trashy	2323	.10	73.7	.07	31.5
(ARS additional)	17518	.76	886.4	.88	19.8
Total	23043	1.00	1012.5	1.00	22.8
ARS dry Method:[1/]					
live	4522	.18	115.6	.09	39.1
senescent	14413	.57	731.0	.59	19.7
detrital	6517	.25	395.6	.32	16.5
Total	25452	1.00	1242.2	1.00	20.5

[1/] Based on 12 samplings over 4 growing seasons and involving 160 kg of soil; recalculated to 7.2 kg soil basis for comparative presentation.

Table 2. Certain compositional characteristics of compartmentalized root materials obtained by dissimilar methods of sampling.

	Percent C	Percent N	Free amino acids, µg/g N	Bound amino acids µg/g N	Free sugars mg/g C	Structural carbohydrates mg/g C
Pawnee Wet Method						
wet fibrous	42.18	.786	642.3	5001	55.0	131.1
wet trashy	28.33	1.285	185.1	5871	33.1	94.2
overflow trashy	17.44	1.003	127.6	8426	18.4	183.9
Pawnee Dry Method						
dry fibrous	40.78	.667	514.1	5310	82.2	222.2
dry trashy	26.70	.847	249.4	6673	57.8	170.5
(ARS additional)	14.39	.728	199.0	6386	23.2	264.2
ARS Dry Method [1/]						
live roots	32.38	.806	418.9	4202	74.9	291.4
senescent roots	19.78	.933	135.3	5084	30.5	220.1
ditrital roots	16.71	1.017	211.9	5929	22.6	295.3

[1/] Based on 18 June 1974 field samples of the isotopic nitrogen experiment. All determinations shown in the four righthand vertical columns were made concurrently.

DISCUSSION

Based on parallel corings, the Pawnee dry method yielded only 24 percent of the root biomass yielded by the Pawnee wet method, insofar as the Pawnee procedures in themselves were concerned. It was only when the ARS additional was summed with the Pawnee dry yield that the Pawnee dry outscored the Pawnee wet method. Neither of the Pawnee procedures yielded as much root biomass as did the ARS procedure. Because only limited coring on a single day was used as the data base for the Pawnee methods, such yield differences as were encountered must be viewed as indicative rather than firm. Also, although the Pawnee dry method yields only a minor fraction of the root material actually present, this in itself is no indictment of the procedure, inasmuch as it is not used routinely to determine root biomass, but simply to obtain unwashed root samples for chemical analyses. In this respect a very serious indictment does exist in that the composition of this minor fraction differs markedly from that of the total biomass. Prior to fuller discussion of this point, let us compare the compartmentalizations achieved by using the three methods.

The best agreement quantitatively between compartmental separations in the three methods is that existing between the Pawnee wet method and the ARS dry method. The three compartments in the former account for 22, 45, and 33 percent of the biomass total, and in the latter, 18, 57, and 25 percent. The 14 percent of fibrous roots in the Pawnee dry total approaches the 22 and 18 percentage values for fibrous and live roots in the other two methods. This value of 14 percent accounts for most of the root recovery in the Pawnee dry method as routinely used. The soil

pulverization and machine shaking involved in that method apparently convert most of the trashy or brittle and senescent roots into finely fragmented debris that reaches the soil bucket and not the root bucket in the separational process.

Turning now to compositional characteristics of the compartmentalized root materials and looking first at C:N ratios (Table 1), one notes extremes of 61.1 and 16.5, the former for fibrous roots of the Pawnee dry method, and the latter for detrital roots of the ARS dry method. Herein lies the first serious indictment of making chemical analyses on a portion of the Pawnee dry sort and then using those values as indicative of the composition of the sevenfold larger amount of root material yielded by the Pawnee wet sort. In contrast to the 61.1 C:N ratio of the Pawnee dry sort are the C:N ratios of 22.9, 22.8, and 20.5 encountered for the total root recoveries (Table 1). The values for free amino acid or free sugar contents (Table 2) also emphasize that wide compositional differences exist among different fractions of the total root biomass and that extrapolation of values from any one compartment to the total is ill-advised.

The compositional data given in Table 2 can be used to evaluate the extent to which the total root biomass consists of functional roots. Estimates now in press range from 36 percent (Clark, 1975) to 62 percent (Singh and Coleman, 1975). Clark's estimate was based on the ^{15}nitrogen contents of live, senescent and detrital roots. Recently, some calculations based on root dehydrogenase measurements (unpublished data of the writers) showed that 36 percent of the total root biomass was functional. The close agreement of this estimate with the isotopic

nitrogen estimate suggested a further series of calculations based on such compositional attributes as free sugars or free amino acids. Table 3 summarizes the calculated activity values for the total root biomass for diverse compositional characteristics of the several compartmentalizations shown in Tables 1 and 2.

From the range of activity values shown in Table 3 it is obvious that dissimilar estimates of the proportion of functional roots in the total biomass can be obtained depending upon the root property used in the calculation. The values in Table 3 together with the 36 percent estimate based on isotopic nitrogen (Clark, 1975) and the 62 percent estimate based on movement of ^{14}C photosynthate into the root system (Singh and Coleman, 1975) suggest that measurements based on root nitrogen tend to give lower estimates of the functional root percentage than do measurements based primarily on root carbohydrates. Taken collectively, all the values in Table 3 indicate that 47.3 ± 7.4 percent of the total root biomass can be viewed as functional.

The data concerning the loss of nitrogen during root washing indicate that washing of unground roots causes loss of less than 10 percent of the total nitrogen. Grinding prior to leaching greatly magnifies movement of nitrogen into the leachate. To obtain estimates of the total root biomass nitrogen, it would appear preferable to use the nitrogen values on unground washed roots and accept the error involved rather than to extrapolate the nitrogen content of a minor fraction (i.e., dry-sorted fibrous roots) of the total root biomass to that total and in so doing incur an error several times greater than if washed roots had been used. One need only to look in Table 2 at the free amino acid values of

Table 3 . Percentage estimates of functional roots in the total root biomass.

Basis used for calculating activity	Pawnee wet sort of 26 Aug. 74	Pawnee dry sort of 26 Aug. 74	ARS dry sort of 5 Aug. 74	ARS dry sort of 18 June 1974
Free amino acid content	41.6	48.4	41.8	48.9
Nonlabile/labile amino acids	34.0	34.7	37.9	41.3
Free sugar content	59.9	42.3	43.1	48.9
Nonlabile/labile sugars	67.2	41.3	47.4	55.7
Root C/root N	51.2	43.8	---	57.3
Dehydrogenase activity	--	--	39.7	--

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from 642 to 128 μg/gN for different root compartments to appreciate why generalization to the total should not be made from the value for any single compartment.

Data such as those given in Tables 1 and 2 emphasize that the root biomass should be viewed as consisting of dissimilar compartments and that differences between these compartments can be as striking as the differences between compartments commonly recognized in the aboveground biomass (e.g., standing green versus aboveground litter). Although compartmentalization belowground may not be made with the same precision as aboveground, nevertheless the approximate conformity of the compartmentalizations in the Pawnee wet sort, the Pawnee dry sort, and the ARS dry sort does suggest that root biomass separations can routinely and quickly be achieved. Finally, root productivity and decomposition values based on seasonal changes in the total root biomass should be much more informative if concurrently with measuring changes in the total biomass compartmental changes were also measured.