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Root distribution in an Amazonian seasonal forest as derived from $\delta^{13}C$ profiles

Leonel da S.L. Sternberg[1,*], LaVictoria Green[1], Marcelo Z. Moreira[1,2], Daniel Nepstad[3], Luiz A. Martinelli[2] and Reynaldo Victória[2]
[1]*Department of Biology, University of Miami, Coral Gables, FL 33124, USA,* [2]*Centro de Energia Nuclear na Agricultura, University of São Paulo, CP96, 13416-000 Piracicaba, SP, Brazil and* [3]*Woods Hole Research Center, Woods Hole, MA 02543, USA*

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Abstract

Carbon isotope ratios of the main stem in trees, saplings, and seedlings were correlated with their main stem diameter in an Amazonian seasonal forest. This correlation became the basis of using carbon isotope ratios of roots from various levels of the soil profile in order to determine root distribution from emergent, canopy and subcanopy trees, saplings and herbaceous understorey plants. It was observed that the distribution of roots in the soil profile is horizontally and vertically heterogeneous. Pockets of roots from saplings or herbaceous understorey plants were found as deep as 4 m and pockets of roots from emergent trees were found as shallow as 1 m depth.

Introduction

The architecture of the above ground biomass in tropical forests has been studied more than the underground structure (Ackerly, 1995; Fischer, 1986; Hubbell and Foster, 1986; Terborgh, 1992). Most studies have concentrated on the ratios of above ground to below ground biomass and the relationship of this ratio to abiotic factors such as water availability and soil nutrient status (Sanford and Cuevas, 1995). There are fewer studies on rooting depth and the structure of root distribution. Several investigators have observed that the greatest number of roots is found several centimeters above the mineral soil (Sanford and Cuevas, 1995). On the other hand, others have found that water uptake at lower depth of the soil profile may be ecophysiologically important (Jackson et al., 1995; Nepstad et al., 1994). Jackson et al. (1995) using isotopic composition of water from plant stem and the soil profile were able to show that evergreen plants were able to utilize a deeper water source than deciduous plants in a seasonal tropical forest in Barro Colorado, Panama. Nepstad et al., (1994) observed that roots in an Amazonian tropical seasonal forest may reach a depth as low as 18 m below the surface. In addition, they reported that the roots of young vines and palm trees may extend to a greater depth in a shorter period of time than trees (Restom, in press). One of the possible contributing factors for deep rooting in eastern Amazonia may be the soft spots and hollow chambers along the soil profile (Carvalheiro and Nepstad, 1996). These soft spots and hollow chambers allow for easier root penetration than the surrounding soil profile. Based on a soil moisture balance approach, Nepstad et al. (1994) concluded that certain plants in a seasonal forest in the eastern Amazon have an evergreen phenology because they can acquire water from deeper layers of the soil.

In this study we determined whether roots of young understorey, mature canopy, and emergent trees in a tropical seasonal forest are spatially segregated. A plausible example for root segregation in the soil profile would be shown by the limitation of roots from small understorey plants to the top few centimeters of

* FAX No: (305) 284-3039.
E-mail: Lsternberg@umiami.ir.miami.edu

the soil profile. An understanding of root distribution in seasonal tropical forest could be of importance in predicting the relative survival of trees of different size classes during an extended dry season. We developed a set of hypotheses for different types of root distribution and tested them by measuring stable carbon isotope ratios of root material collected at various depth of the soil profile. Stable carbon isotope ratios can be used to determine the source of roots in the soil profile because the δ^{13}C values of plant biomass gradually increase from the forest floor to the canopy (Ehleringer et al., 1986; Martinelli et al., 1997; Medina and Minchin, 1980; Medina et al., 1991; Sternberg et al., 1989). The δ^{13}C values of small understorey plants can be as low as −36‰ and canopy trees have δ^{13}C values as high as −27‰. This increase is caused by differences in stomatal opening due to differences in light availability and vapor pressure deficit, and to differences in $^{13}C/^{12}C$ ratios of ambient CO_2 due to the presence of respired CO_2 (Ehleringer et al., 1986; Medina and Minchin, 1980; Sternberg et al., 1989). The δ^{13}C values of roots will correspond to the δ^{13}C value of their source plant (Medina et al., 1991), therefore roots from emergents will have a high δ^{13}C value compared to those from understorey plants.

Methods

Site description

Samples were taken from a primary seasonal tropical forest in the eastern Amazon (Fazenda Vitória, State of Para; 2°59′ S, 47°31′ W). The area is characterized by a pronounced dry season from the end of May to November. Total rainfall in the area is about 1750 mm, with at least 1500 mm falling during the wet season (Nepstad et al., 1994). The forest canopy reaches approximately 30 m in height and above ground biomass averages 300 Mg/ha (Nepstad, unpublished data). The soils in this location were characterized by Sombroek (1966) as Kaolinitic Yellow Latosols.

Sample collection

An area for collection of samples was chosen where vines and lianas were not abundant. Individual soil cores were taken 1 m from main stems of emergents to avoid obstruction by large roots. Stems of randomly chosen individuals were marked and the diameters of the main stem were measured (M.S.D). We could not take d.b.h. (diameter at breast height) of all individuals sampled, since some saplings and seedlings were below breast level. A small section of each stem (which included the sapwood) was removed from each individual and placed in a paper sac for further processing in the laboratory. Bark from samples was removed in the laboratory and the sapwood material ground in a Wiley mill to mesh 25. Not all of the species sampled could be identified. Following is a list describing the number of individuals sampled and identification to the species, genus or family level: identified to the species level, (2) *Diospyros duckei*, (1) *Holopyxidium jarana*, (1) *Margaritaria nobilis*, (2) *Picrolemma pseudocoffea*, (2) *Pouteria caimitos*; identified to the genus level, (1) *Buchenavia* sp., (1) *Heliconia* sp., (1) *Miconia* sp., (1) *Memora* sp., (2) *Protium* sp.; identified to the family level, (3) Euphorbiacae, (1) Palmaceae, (2) Sapotaceae; and five unidentified individuals.

Root samples were taken with a 10 cm dia. mud auger (AMS). Soil samples from a depth interval of about 10 cm were collected every 0.5 m down to 4 m depth. The volume of these soil samples after unpacking from the auger was approximately 2.5 L. One liter aliquot was taken from soil samples and placed inside plastic bags until the separation of roots. Root separation was done by soaking soil in water to break up mud clods. After about 10 min of soaking, water mixed with root material was decanted and passed through a 20 mesh sieve. The remaining material in the bucket was mixed and the process was repeated until no more roots were found. Root samples were placed in paper bags and sun dried. Root material was hand-picked with a pair of tweezers in the laboratory. Roots with a diameter of 1 mm or less were separated from those with larger diameters. Isotope analysis was only done on fine roots having a diameter of 1 mm or less. Fine roots were either ground in a Wiley mill when there was an abundance of roots or ground with a mortar and pestle when quantities were smaller.

Isotope analysis

Ground plant stem and root samples were sealed in an evacuated 9 mm O.D. × 15 cm Vycor glass ampoule with 1 gm of cupric oxide and 1 gm copper and pyrolyzed at 800 °C for three h. Carbon dioxide was purified from combustion gases by cryogenic distillation. Purified CO_2 was analyzed for $^{13}C/^{12}C$ ratios in a Prism Mass Spectrometer with a precision of ± 0.1‰. δ^{13}C values are expressed relative to the PDB standard.

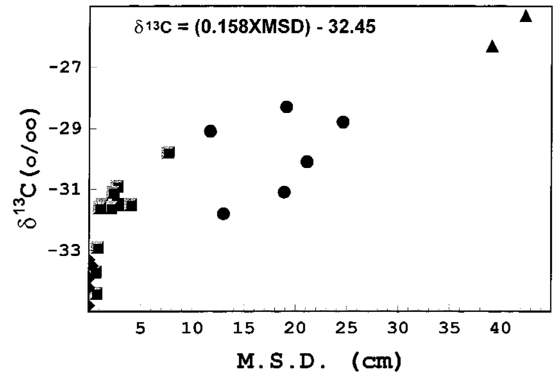

Figure 1. Figure shows the δ^{13}C values of trunk or stem tissue of trees or saplings *versus* their main stem diameter (M.S.D.). Figure also shows δ^{13}C values of stems from herbaceous understorey plants assumed to have a M.S.D. of 0. Symbols are ▲ for emergent trees, • for canopy trees, ■ for understorey trees, and ♦ for understorey herbaceous plants. Also shown in the figure is the linear regression equation for δ^{13}C values of tree or sapling trunks *versus* their trunk diameter. Regression was highly significant with $r^2 = 0.88$ and $P < 0.01$.

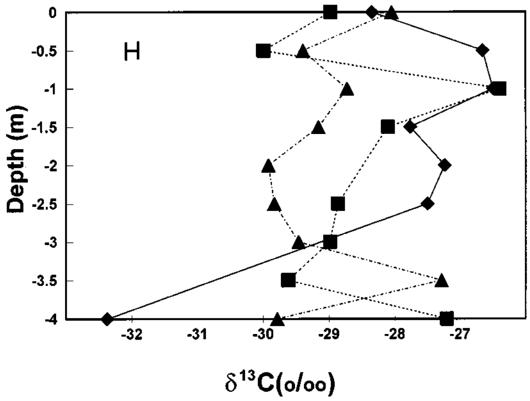

Figure 2. δ^{13}C values of root material in relation to depth of sample from 1 L of soil aliquots for three separate cores represented by different symbols and lines. Also shown in the figure is the precision of δ^{13}C measurement.

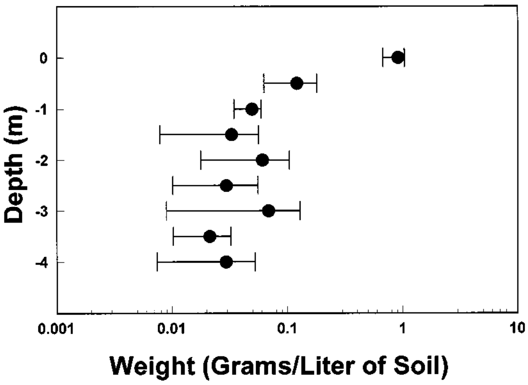

Figure 3. Average and range of weight of dried root mass per liter of soil at various depth in the soil profile.

Results

The δ^{13}C values of stem material was clearly related to the diameter of the main stem (Figure 1). A value of 0 for main stem diameter was assigned for herbaceous understorey perennials, but these values were excluded from the regression analysis. Given the regression equation on Figure 1, it should be possible to calculate the approximate main stem diameter of the source plant using the δ^{13}C value of single root samples from the soil profile.

Average δ^{13}C values of stem material from emergents, canopy and sub-canopy trees, and small trees and understorey herbaceous plants are shown on Table 1. A significant difference existed between each class of plant size, with emergent trees having the highest average value of −25.8‰ and the small trees and understorey herbaceous vegetation having the lowest average value of −32.0‰ and −34.1‰, respectively.

The δ^{13}C values of root material collected at various depth of the soil profile (shown in Figure 2) vary by as much as 6‰. There are no particular patterns noted on this variation except that at the surface the isotopic values tend to converge toward δ^{13}C values between −28‰ and −29‰. The lowest δ^{13}C value of root material (−32.4‰) was recorded at the depth of 4 m, while the greatest value (−26.5‰) occurred at the depth of 1 m. Figure 2 also documents the precision of the analysis. The average range of values for several (6) repetitive analyses of ground roots from the same sample averaged 0.18‰.

Average weight and the range in weight of dried root mass at each level of the soil profile is shown on Figure 3. With the highest root mass at the top 0.5 m of the soil profile and stabilizing at the weight of approximately 0.02 g/L of soil throughout the rest of the soil profile.

Discussion

Results presented in Figure 1 and Table I indicate the feasibility of using stable isotopes to trace the source of roots in the soil profile. Emergents had significantly higher δ^{13}C values than canopy and sub-canopy trees; these in turn had significantly higher δ^{13}C values than small trees and understorey herbaceous perennials.

Table 1. Average δ^{13}C values ± SEM, range of diameters, and approximate heights for different growth stages of trees and herbaceous understory plants in a seasonal tropical forest. Number in parentheses represent the number of replicates

Plant type	Emergents	Canopy and sub-canopy	Small trees	Understorey herbaceous plants
Diameter	30–50 cm	10–29 cm	0–9 cm	0
Height	>30m	15–25 m	<10 m	<1 m
δ^{13}C ± SEM	−25.3‰, −26.3‰ (2)	−29.9 ± 0.5‰(6)	−32.0 ± 0.4‰(12)	−34.1 ± 0.3‰(4)

The results are consistent with several other studies which reported a gradient in δ^{13}C values of leaf and wood tissue correlated to height in tropical forests (Ehleringer et al., 1986; Martinelli et al., 1997; Medina and Minchin, 1980; Medina et al., 1991; Sternberg et al., 1989). δ^{13}C values of root material can be used to determine the size class of its source only if δ^{13}C values of roots are reflective of the δ^{13}C value of stem material. Analysis of data from a previous study in the Luquillo National Forest, (a tropical rainforest in Puerto Rico), indicate that there is a significant difference between δ^{13}C values of roots and stem, but on the average δ^{13}C values of roots were only 0.47‰ higher than those of their source stem (Medina et al., 1991). However, further studies will need to calibrate the relationship between δ^{13}C values of stem and root tissue at this particular locale.

Figure 2 indicates that there are no systematic patterns in the δ^{13}C values of root material *versus* depth. The lowest δ^{13}C value of −32.4‰, representing roots from small understorey trees or a mixture of understorey and other size class individuals, was found at the lowest depth sampled (4 m). It appears that the roots of small individuals in the forest are capable of penetrating the soil profile to the depth of 4 m. The greatest δ^{13}C value of −26.4‰, representative of roots from emergents, were found only 1 m below the soil surface. The only noticeable pattern was the apparent convergence of the δ^{13}C values of roots from all three cores to values between −29 to −28‰ towards the surface of the soil.

Roots collected at any particular depth in the soil profile may either represent roots from a single individual, a mixture of several individuals from the same size class, or a mixture of several individuals from different size classes. The latter case may complicate our interpretation of rooting depth of different size classes based on δ^{13}C values of root biomass. For example, if the root sample collected at 4 m depth with a δ^{13}C value of −32‰ represents a mixture of several individuals of different size classes, then the contribution of roots from herbaceous understorey individuals having δ^{13}C values below −34.1‰ must be substantially greater compared to the case where the root sample represent roots from a single understorey individual or a mixture of individuals from the same size class. Therefore, the assumption that roots collected at any particular soil depth represent roots from a single individual or individuals from the same size class will give a conservative estimate on the extent of how deep roots from understorey plants penetrate the soil profile. Likewise, these assumption will lead to a conservative estimate on the occurrence of roots from canopy individuals in the upper layers of the soil.

Four different types of root distribution and their expected δ^{13}C profile of root material are shown schematically in Figure 4. The first case in which there is both horizontal and vertical homogeneity of roots throughout the soil profile, abbreviated as (H+V+), occurs where the roots from emergents, canopy, and understorey individuals are well mixed. In the second case there is only vertical homogeneity of roots (H−V+). This would occur if, for example, a single individual dominated the area where the core was taken and the dominance was maintained throughout the soil profile. In the third case, there is only horizontal homogeneity of roots (H+V−). An example of such a distribution would be if the surface layers of the soil were dominated by roots from smaller understorey individuals, and deeper layers were dominated by more mature individuals. In the last type of root distribution there is no homogeneity in either the horizontal or vertical distribution of roots (H−V−). This latter case would imply the presence of pockets of roots from emergents, canopy (or mixture of roots from emergents and understorey individuals), or understorey individuals throughout the soil profile. These different types of root distribution would yield dis-

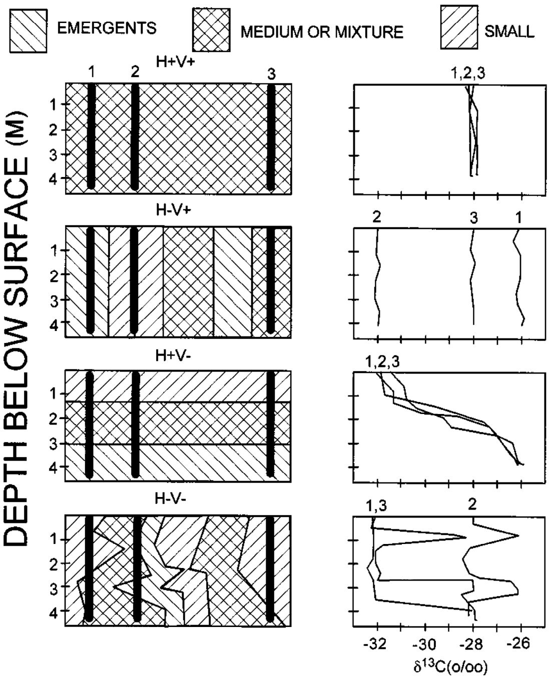

Figure 4. Possible forms of root packing in a forest. We considered three types of roots with distinct isotopic signatures: roots from emergent trees having an approximate δ^{13}C value of −26‰, from canopy trees or a mixture of emergents and small trees having an approximate δ^{13}C value of −28‰, and from small trees having an approximate δ^{13}C value of −32‰. Figure shows all the permutations on the type of horizontal (H) and vertical (V) distribution in which a plus sign signifies homogeneity and a negative sign signifies heterogeneity. Dark bars represent hypothetical cores taken at different locations in the forest. Also shown are hypothetical graphs of δ^{13}C values of roots in relation to the depth of the root for each type or root packing.

tinct profiles for δ^{13}C values of root material collected throughout the soil profile as shown in Figure 4.

Given a specific sample size and measurements of δ^{13}C values of root material at different depths of the soil profile, how does one test to determine which of the four root distribution discussed in Figure 4 is occurring at this site? If the distribution of roots in the soil profile were of the type H−V+ or H+V−, than ANOVA would detect a significant difference in δ^{13}C values between cores or between different sampling depths. Our results indicate no significant differences between δ^{13}C values of roots from different cores or depths, (F = 0.763, and P = 0.483 and F = 0.850, P = 0.541 for differences between cores and for different depths, respectively). A sensitivity analysis (Sokal and Rohlf, 1981) indicates that, for this number of replicates, if there are significant differences in the order of 3‰ between different cores or levels in the soil profile, then there is a greater than 80% chance of finding these differences at a highly significant level ($P < 0.01$). Thus root distribution at this site is either of the first (H+V+) or last (H−V−) type. Discrimination between these two types of root distribution is done by calculating the variance in δ^{13}C values with depth. If the variance falls within the precision of our measurements of root material in one liter of soil than we must consider the first hypothesis as true. If, however, the variance is greater than our precision of measurement, then we must consider the fourth case as representative of root distribution. Repeated measurements of roots collected from a single soil aliquot gave on the average a range of 0.18‰. Yet the range of variation with actual measurements of roots throughout the soil profile was on the order of 6‰; much greater than our precision of measurement. We must, therefore, conclude that there is lack of horizontal and vertical homogeneity of root distribution in the soil profile for those soil samples collected within the area and depth interval of this study. At this site, pockets of roots from different size classes of plants are distributed throughout the soil profile. One of the factors that may contribute to this heterogeneity may be the presence of soil soft spots and hollow chambers heterogeneously distributed throughout the soil profile at this site (Carvalheiro and Nepstad, 1996). The findings reported here are consistent with the observations of Restom (in press) regarding below ground horizontal overlap between tree roots at this same site. Restom (in press) observed 3 times more below ground overlap between tree roots than that of their respective crowns, leading her to the conclusion that there is considerable heterogeneity in the soil profile. There is, however, a clear deviation in the root distribution pattern compared to the four possibilities shown on Figure 4, since δ^{13}C values of surface samples from the three cores gradually converge to values between −29 and −28‰. This convergence may indicate that there is some horizontal homogeneity of root distribution on the top 20 to 10 cm of the soil profile, although at deeper levels root distribution is heterogeneous.

Conclusions

Root distribution in a seasonal tropical forest was observed to be heterogeneous. With pockets of roots from understorey, canopy, and emergent plants distributed throughout the soil profile from surface down to 4 m. The average quantity of roots for each size class at each depth interval, however, was not addressed by this study. It was observed that roots from small saplings or herbaceous understorey plants may reach as low as 4 m below the surface. The ecological implications of this finding are important in interpreting the drought resistance of understorey plants to the pronounced dry season of the eastern Amazon region.

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