[RADIOCARBON, VOL. 38, NO. 2, 1996, P. 191–201]

THE USE OF CARBON ISOTOPES (^{13}C,^{14}C) IN SOIL TO EVALUATE VEGETATION CHANGES DURING THE HOLOCENE IN CENTRAL BRAZIL

L. C. R. PESSENDA,[1] RAMON ARAVENA,[2] A. J. MELFI,[3] E. C. C. TELLES,[1] RENÉ BOULET,[4] E. P. E. VALENCIA[1] and MARIO TOMAZELLO[5]

ABSTRACT. This paper presents carbon isotope data measured in three soil profiles from the Salitre area, Central Brazil. The study forms part of a research project on tropical and subtropical soils in Brazil, in which the main objective is to use carbon isotopes to provide information about vegetation changes that have occurred in relation to climate changes during the Holocene. ^{14}C data from charcoal samples and soil organic matter (SOM) indicate that the organic matter in the soils studied is of Holocene age at least. Furthermore, the presence of a significant amount of charcoal in the soils suggests that forest fire was a significant ocurrence during the Holocene and probably had an important role in determining the dynamics of forest vegetation in the study area. Correspondingly, ^{13}C data indicate that C_3 plants provided the dominant vegetation of the study area, even during the dry periods when savanna vegetation is supposed to have replaced the forest communities. This study contributes to our better understanding of the relation between climatic changes and vegetation in the subtropical region of Brazil.

INTRODUCTION

Reconstruction of past vegetation changes and their relation to climate in tropical and subtropical forest is essential for understanding the response of these ecosystems to future climatic change. Different approaches involving geomorphological (Ab'Saber 1977, 1982; Servant *et al.* 1981; Bigarella and de Andrade Lima 1982), biological, botanical (Haffer 1969; Prance 1973; Gentry 1982) and palynological studies (Absy *et al.* 1991; Ledru 1993) have been used to infer past climatic changes in the Amazonia and Central region of Brazil.

The naturally ocurring isotopes ^{13}C and ^{18}O are used widely as tracers of paleoenvironmental processes, mainly from lacustrine sediments, such as carbonate and organic sediments (Cerling 1984; Hollander, McKenzie and Haven 1992; Aravena *et al.* 1992) and recently this approach has been extended to soils (Schwartz *et al.* 1986; Becker-Heidmann and Scharpenseel 1989, 1992).

The stable carbon isotope composition ($^{13}C/^{12}C$, or $\delta^{13}C$) of soil organic matter (SOM) records information regarding the ocurrence of C_3 and/or C_4 plant species in past plant communities, and their relative contribution to net primary productivity by the plant community (Troughton, Stout and Rafter 1974; Stout, Rafter and Throughton 1975). Such information has been used to document vegetation change (Hendy, Rafter and MacIntosh 1972; Dzurec *et al.* 1985), to infer climate change (Hendy, Rafter and MacIntosh 1972; Krishnamurthy, De Niro and Pant 1982) and to estimate rates of SOM turnover (Cerri *et al.* 1985).

These applications are based on isotope effects occurring during photosynthesis that imprint plants with a carbon isotopic composition that is characteristic of the photosynthetic pathway.

$\delta^{13}C_{PDB}$ values of C_3 plant species range from *ca.* –32 to –20‰, with a mean of –27‰, whereas $\delta^{13}C_{PDB}$ values of C_4 species range from –17 to –9‰, with a mean of –13‰. Thus, C_3 and C_4 plant species have distinct $\delta^{13}C$ values and differ from each other by *ca.* 14‰ (Boutton 1991).

[1]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, 13400-970 Piracicaba, São Paulo, Brazil
[2]Waterloo Center for Groundwater Research, University of Waterloo, Waterloo, Ontario N2L 3G1 Canada
[3]Instituto Astronômico e Geofísico/NUPEGEL, Universidade de São Paulo, 01065-70, São Paulo, SP, Brazil
[4]Instituto de Geociências, Universidade de São Paulo, 05508-900 São Paulo, SP, Brazil
[5]Escola Superior de Agricultura Luiz de Queiróz, Universidade de São Paulo, 13418-260, Piracicaba, SP, Brazil

In this paper we report ^{14}C and $\delta^{13}C$ data measured using SOM and charcoal from three soil profiles collected under a natural forested slope in the Salitre area, central Brazil. The study site is one of several at which applied carbon isotope research is being used to evaluate vegetation and climatic changes during the late Pleistocene and Holocene in tropical and subtropical soils in Brazil.

METHODS

The Salitre site is located *ca.* 300 km W of Belo Horizonte, capital of Minas Gerais State, in the Salitre de Minas (19°S, 46°46'W), region of Central Brazil (Fig. 1). The present climate is characterized by a 4-month dry period and mean winter temperatures above 15°C (Ledru 1993). The native vegetation is Cerrado (wooded savanna) and includes *Byrsonima coccolobifolia, Solanum lycocarpum, Kielmeyera coriacea, Dalbergia mischolobium, Stryphnodedron adstrindens* and *Erythroxylum* spp. (G. Ceccantini, personal communication 1994). Extensive forest areas have been cleared for agricultural use in this region. The site lies within a paleolagoon basin that has been the focus of a paleoclimatic investigation in Central Brazil (Ledru 1993). The soil type according to the Brazilian soil classification is a Latossolo Vermelho Amarelo, and in the American and FAO classification an Oxisol and Ferralsol.

Fig. 1. Map showing the location of the study area in Central Brazil

Soil samples were collected from three excavations *ca.* 250 m apart on a forested (mesophytic semideciduous) slope (Fig. 2). We sampled up to 10 kg of soil at 10 cm intervals to a maximum depth of 2 m. The samples were dried to constant weight at 60°C and root and plant remains were discarded by hand-picking. Any remaining plant debris was removed by flotation in HCl 0.01M and the residual soil was then dried to constant weight, sieved to <200 μm to minimize the effects of sample heterogeneity and homogenized for ^{13}C and ^{14}C analyses. The humin fraction was extracted from 2.5-kg aliquots using standard methods (Dabin 1971; Goh 1978; Anderson and Paul 1984), *viz.*, 1) acid digestion in 0.5 M hydrochloric acid at 70°C to 80°C for 4 h followed by washing with distilled water to a pH of 3–4; 2) reaction of the acid-insoluble residue with at least 30 liters (10 liters per extraction) of 0.1M $Na_4P_2O_7$ for *ca.* 36 h (12 h per extraction) followed by washing to a pH of 3–4; 3) hydrolysis of the solid residue with 4 liters of 3M HCl at 100°C for 12 h followed by washing to a pH of 3–4; 4) drying of the solid residue at 40°C for 48 h followed by sieving to recover the <200-μm-size fraction.

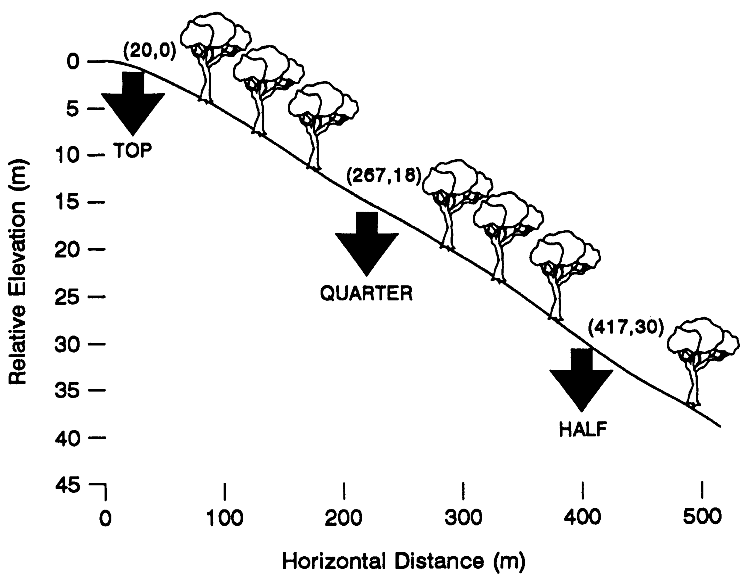

Fig. 2. Sampling points located under the forested slope

Charcoal samples were collected by hand-picking from 10-kg soil samples, oven dried at 90°C, weighed and treated using the conventional acid-alkali-acid treatment. Samples from between 2.20 m and 3.45 m depth were used only for $\delta^{13}C_{PDB}$ measurements. These were collected from small amounts of soil by hand-picking and using a 5-mm sieve.

^{14}C analyses on charcoal and humin samples were carried out at the Radiocarbon Laboratory, Centro de Energia Nuclear na Agricultura (CENA), by liquid scintillation counting of synthesized benzene (Pessenda and Camargo 1991). Benzene samples were counted for at least 48 h in a Packard 1550 low-level liquid scintillation counter. ^{14}C ages are expressed in years BP and reflect normalization to $\delta^{13}C_{PDB}$ of –25‰ (Stuiver and Polach 1977) and an analytical precision of ± 1.0 pMC. ^{13}C analyses were determined by isotope ratio mass spectrometry using CO_2 generated by sample combustion at 900°C in an oxygen atmosphere. These analyses were undertaken at the Stable Isotopes laboratory at CENA and results were expressed in the delta per mil notation, with an analytical precision better than 0.2‰.

The carbon content in soil samples was determined using 1 to 5 gr of the <200 μm soil fraction by combustion in a carbon autoanalyzer or by the wet digestion method. These analyses were undertaken by the Soil Chemistry Laboratory at CENA and the results are expressed as weight percent of the dry sample.

RESULTS AND DISCUSSION

Soil Properties

Physical and chemical properties in the Salitre soil are presented in Figures 3 and 4. These soils are characterized by a high percentage of clay (78% to 83%), with sand and silt being the minor fractions (Fig. 3). Soil organic carbon decreased from *ca.* 4.5% at the surface (except at the half slope location of *ca.* 6%) to 1.5% at 2.0 m (Fig. 4). The relatively good preservation of organic matter in these tropical soils could be a consequence of their high clay content, giving rise to the formation of refractory organo-clay complexes.

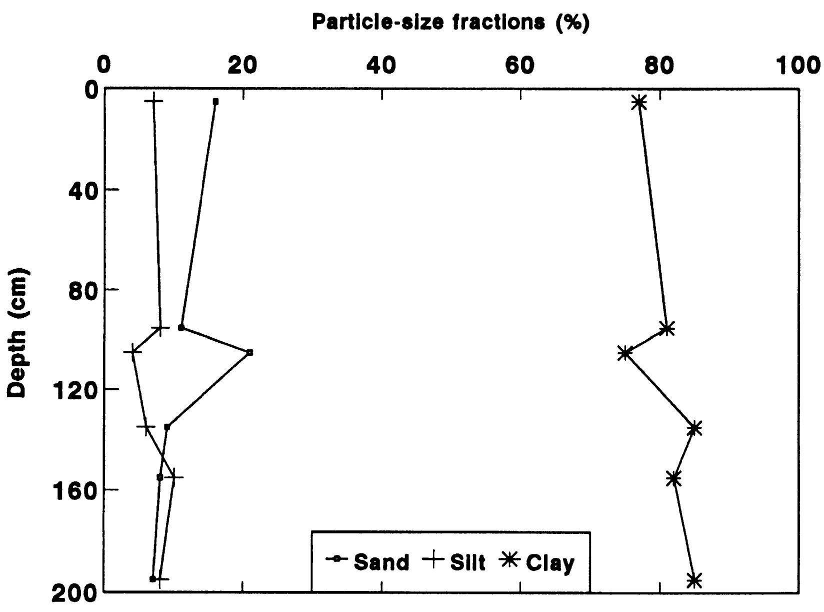

Fig. 3. Particle size fractions of soil from Salitre (half of slope)

Charcoal is present throughout the entire soil profile at each of the three sampling locations (Fig. 5). Peaks are observed at certain soil depths, but without a clear correlation among the three locations. The presence of charcoal in these soils is a clear indication that this area has been affected by frequent forest fires, probably throughout most of the recorded history. The extremely high content of charcoal in some soil horizons indicates that fires were much more prevalent during some periods, perhaps indicating much drier conditions. Charcoal found in forest soils has also been reported in the Upper Rio Negro region of Colombia and Venezuela, indicating the occurrence of frequent and widespread fires in the Amazon Basin, possibly associated with extremely dry periods and/or human disturbance (Saldarriaga and West 1986). Such events range from 6000 BP to the present and several coincide with dry phases recorded in the tropics during the Holocene (Absy 1982; Absy *et al.* 1991; Van der Hammen 1982).

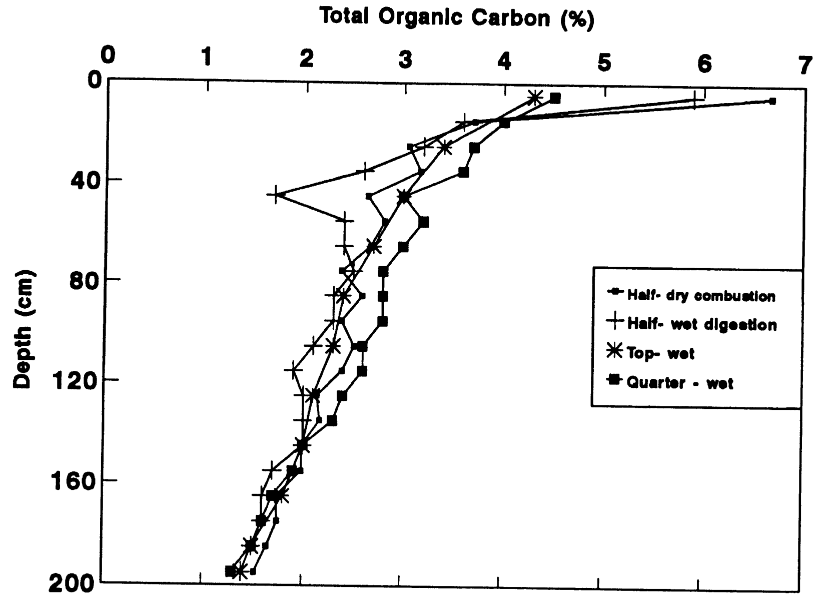

Fig. 4. Total organic carbon of soil samples

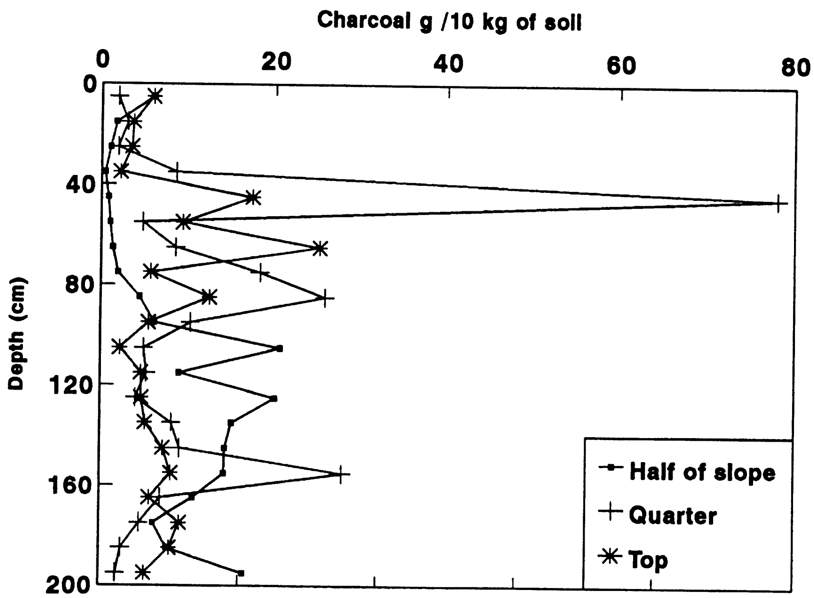

Fig. 5. Charcoal distribution in relation to soil depth

The presence of charcoal throughout the entire sampled depth of the Salitre area soil profiles indicates that translocation of organic matter has been a significant process during the development of these soils. Erosion is sometimes attributed to the formation of soil horizons (Oliveira, Menk and Rota 1985). However, the ^{14}C dates on charcoal described in the following section eliminate this possibility for the Salitre soils. The most likely mechanism is translocation of organic matter due to biological activity (Boulet *et al.* 1995). This mechanism has been postulated to explain the presence of charcoal in soils from Amazonia (Soubies 1980) and in the dark horizon of soils from the Botucatu region, Brazil (Miklos 1992).

Carbon Isotope Data

^{14}C Results

Charcoal ^{14}C ages range from 160 yr BP near the soil surface to 8790 yr BP at 2.0 m depth (Fig. 6). From the age/depth gradient, charcoal at the deepest locations should date to at least 12 ka BP. No clear differences are observed in the ^{14}C depth profiles at the three sampling locations, except that below 150 cm, the charcoal sample from the "quarter" location is older than charcoal at similar depth in the other locations. These data also show that no significant age differences are observed over certain depth intervals. This effect is most obvious at the top location between 150 and 180 cm, suggesting mixing of charcoal at this depth interval. ^{14}C dates of charcoal and humin do not show significant age differences to a depth of 155 cm (Fig. 7). It can be assumed therefore that the humin is a useful fraction for dating SOM. At 200 cm the humin is *ca.* 2000 yr younger than the charcoal, indicating a significant translocation of younger carbon into the deepest part of soil profile. ^{14}C dates of charcoal indicate that these materials represent vegetation that grew in the study area for at least the last 12 ka BP.

^{13}C Results

$\delta^{13}C_{PDB}$values obtained for SOM from the "half", "quarter" and "top" slope locations range, respectively, from −24.7 ‰ to −21 ‰, −26.7 ‰ to −21.7 ‰ and −26‰ to −21‰ (Fig. 8). This general

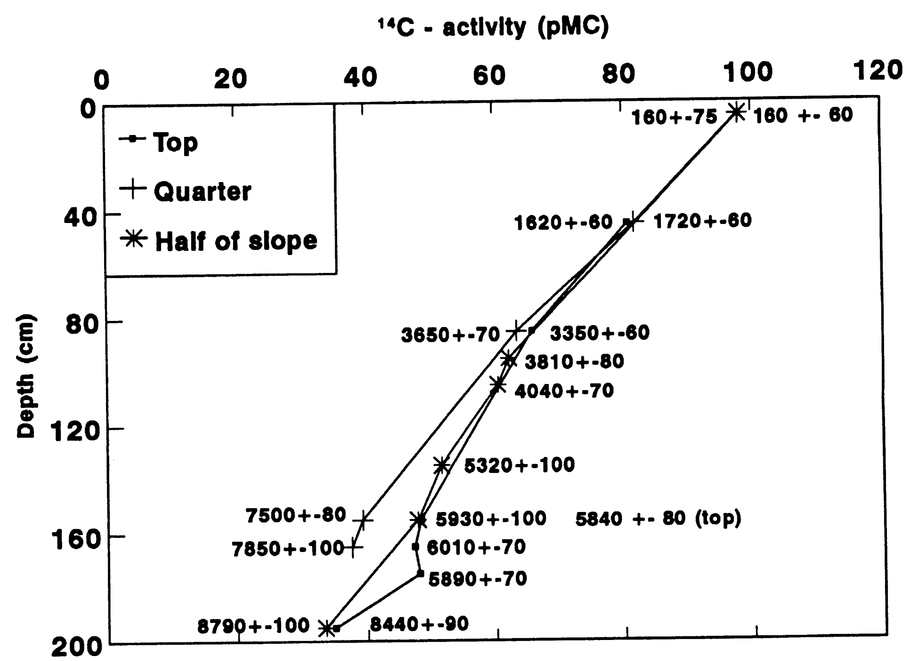

Fig. 6. ^{14}C dates (yr BP) of charcoal samples in relation to soil depth

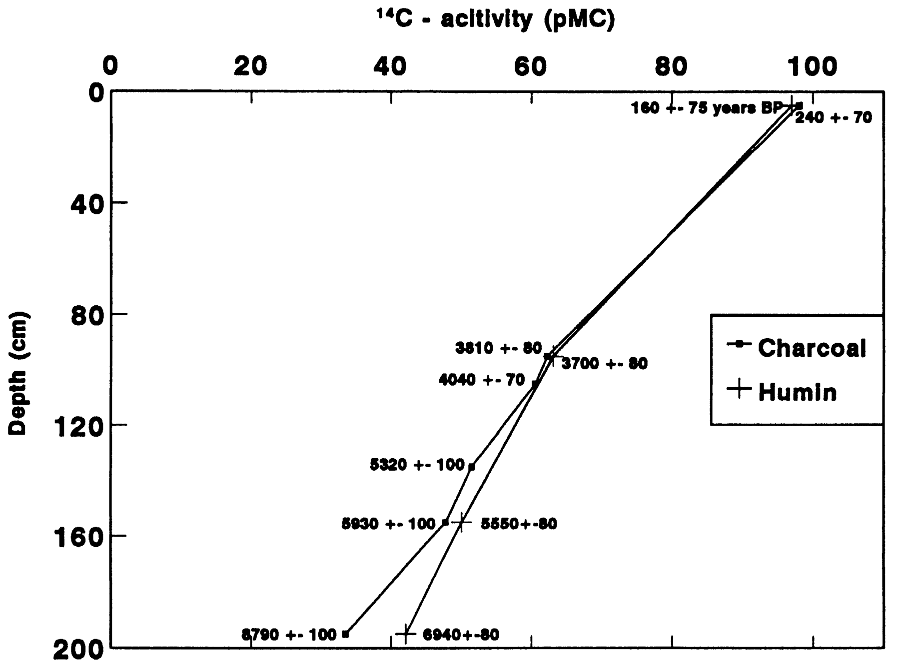

Fig. 7. ^{14}C dating of charcoal and humin samples (half of slope)

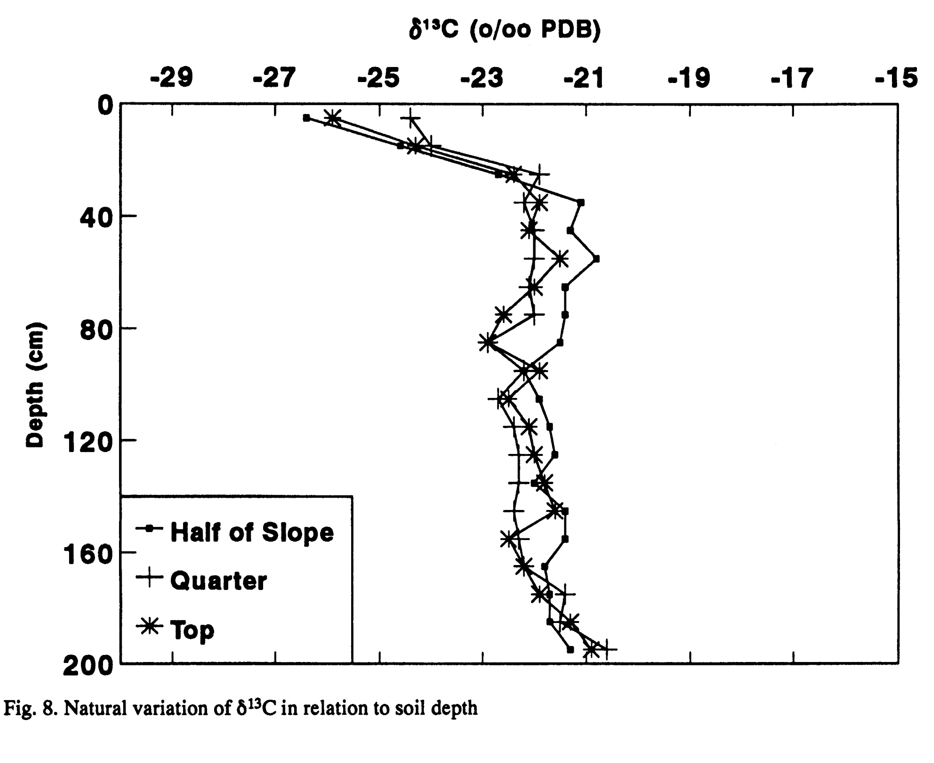

Fig. 8. Natural variation of $\delta^{13}C$ in relation to soil depth

trend toward heavier ^{13}C enrichment with depth could be due to isotope effects occurring during decomposition of SOM (Nadelhoffer and Fry 1988; Becker-Heidmann and Scharpenseel 1992). The pattern is certainly typical for SOM generated by C_3-type vegetation (Cerri *et al.* 1985; Boutton 1991; Pessenda *et al.*)

The $\delta^{13}C$ values of the charcoal show a wide variability within the three soil depth profiles, ranging between –28 to –25 ‰ (Fig. 9). Their ^{13}C values clearly indicated that C_3 plants have dominated in the area, probably for at least the last 12,000 yr. This pattern concurs with the interpretation of the independent ^{13}C data measured for SOM.

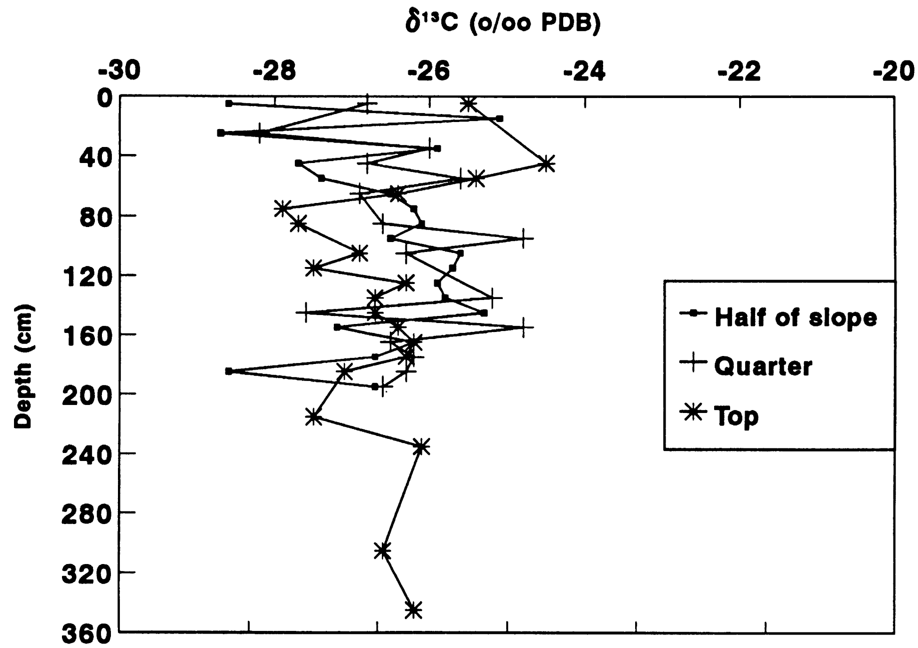

Fig. 9. Natural variation of $\delta^{13}C$ of charcoal samples in relation to soil depth

Implication for Reconstruction of Paleovegetation

Reconstruction of paleovegetation and its relation to past climatic changes is one of the key research areas for our better understanding the response of tropical and subtropical forest to any future climate change in the Amazonia and Central regions of Brazil (Absy 1982; Absy *et al.* 1991; Ledru 1993; Pessenda *et al.*, in press). In the Salitre area, Central Brazil, Ledru (1993) used pollen analyses to record vegetational changes during the last 32,000 yr BP. She postulated two major episodes of forest retreat that were probably associated with very dry climatic conditions. These occurred between 11,000 and 10,000, and 6000 and 4500 yr BP. Dry periods have also been reported in the Central Amazon Basin and other areas of South America during the Holocene (Absy 1982; Van der Hammen 1982). The most significant of these phases appear to fall between 7500 and 6000; 4200 and 3500; 2700 and 2000; 1500 and 1200; and 700 and 400 yr BP (Bigarella 1971; Fairbridge 1976; Absy *et al.* 1991).

In the case of Salitre area, it is possible to expect that during the drier periods the vegetation was mainly composed of C_4 grass species, since these are more resistant to low soil moisture (Tieszen *et* *al.* 1979; Korner, Farquhar and Roksandic 1988). However, our ^{13}C data from charcoal and SOM samples suggest that C_4 grasses were not the predominant vegetation at any time in the SOM record, including the drier periods postulated by Ledru (1993). It seems that C_4 grass has not been the dominant vegetation during the drier periods in the tropical Amazon region (Martinelli *et al.*, in press). However, ^{13}C data from subtropical soils (Pessenda *et al.* 1995) suggest that C_4 plants provided a significant part of the vegetation during the early and middle part of the Holocene in the subtropical region of Brazil. This in turn indicates that in the case of tropical regions the "dry phase" savanna vegetation was dominated by C_3 grasses and/or woody vegetation. On the basis of pollen analyses, Absy (1991) postulated this hypothesis for vegetation changes in the Carajas, Amazon region during a dry phase between 7500 and 6000 BP. An alternative explanation is that the replacement of the forest by savanna vegetation was restricted to small areas. The presence of abundant charcoal in the soils in the Salitre area even during the dry periods postulated by Ledru (1993) suggests that forest and woody vegetation were present in this region at least throughout the entire Holocene. The presence of C_4 plants inferred from the ^{13}C data in subtropical regions (Pessenda *et al.* 1995) could indicate that during past episodes of climatic change this region was much drier than the tropical region.

Conclusion

^{14}C concentrations in charcoal and SOM collected from three soil profiles in the Salitre area, Central Brazil, indicate that the organic matter in these soils represents the Holocene at least. The presence of significant amounts of charcoal in the soil profiles suggests that forest fires were a significant process throughout the Holocene and probably had an important role in determining the dynamics of the forest vegetation in the study area. ^{13}C data from both charcoal and SOM indicate that C_3 plants provided the dominant vegetation, even during the dry periods postulated by Ledru (1993). This study provides additional information to our understanding of the relationship between climatic changes and vegetation in the tropical region of Brazil.

Acknowledgments

We gratefully acknowledge financial support from São Paulo Foundation for Research (FAPESP), grant no. 91/3518-0. Most of the laboratory work was done by Maria Valéria L. Cruz, Paulo Ferreira, Cláudio Sérgio Lisi, Gláucia Pessin and Márcio Arruda.

References

Ab'Saber, A. N. 1977 Espaços ocupados pela expansão dos climas secos na América do Sul, por ocasião dos períodos glaciais quaternários. *Paleoclimas* (São Paulo) 3: 1–20.

____1982 The paleoclimate and paleoecology of Brazilian Amazonia. *In* Prance, G. T., ed., *Biological Diversification in the Tropics*. New York, Columbia University Press: 41–59.

Absy, M. L. 1982 Quaternary palynological studies in the Amazon Basin. *In* Prance, G. T., ed., *Biological Diversification in the Tropics*. New York, Columbia University Press: 67–73

Absy, M. L., Cleef, A., Fournier, M., Servant, M., Siffedine, A., Silva, M. F. F., Suguio, K., Turcq, B. and Van der Hammen, T. 1991 Mise en évidence de quatre phases d'ouverture de la forêt dense dans le sud-est de l'Amazonie au cours des 6000 dernières années. Première comparaison avec d'autres régions tropicales. *Compte Rendus de l'Académie des Sciences.* 2nd series, 312: 673–678.

Anderson, D. W. and Paul, E. A., 1984 Organo-mineral complexes and their study by radiocarbon dating. *Soil Science Society of America Journal* 48: 298–301.

Aravena, R., Warner, B. G., MacDonald, G. M. and Hanf, K. I. 1992 Carbon isotope composition of lake sediments in relation to lake productivity and radiocarbon dating. *Quaternary Research* 37: 333–345.

Becker-Heidmann, P. and Scharpenseel, H. W. 1989 Carbon isotope dynamics in some tropical soils. *In* Long, A. and Kra, R. S., eds., Proceedings of the 13th International ^{14}C Conference. *Radiocarbon* 31(3): 672–679.

____1992 The use of natural ^{14}C and ^{13}C in soils for studies on global climate change. *In* Long, A. and Kra, R.

S., eds., Proceedings of the 13th International ^{14}C Conference. *Radiocarbon* 34(3): 535–540.

Bigarella, J. J. 1971 Variações climáticas no Quaternário Superior do Brasil e sua datação radiométrica pelo método do carbono 14. Instituto de Geografia–Universidade de São Paulo. *Paleoclimas* 1:1–22 .

Bigarella, J. J. and de Andrade-Lima,D. 1982 Paleoenvironmental changes in Brazil. *In* Prance, G. T., ed., *Biological Diversification in the Tropics*. New York, Columbia University Press: 27–40.

Boulet, R., Pessenda, L. C. R., Telles, E. C. C. and Melfi, A. J. 1995 Une évaluation de l'accumulation superficielle de matière par la fauna du sol à partir de la datation des charbons et de l'humine du sol. Exemple des latosols des versants du lac Campestre, Salitre, Minas Gerais, Brésil. *Comptes Rendus de l'Académie des Sciences de Paris*. 2nd series, 312: 287–294.

Boutton, T. W. 1991 Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine and freshwater environments. *In* Coleman, D. C. and Fry, B., eds., *Carbon Isotope Techniques*. San Diego, Academic Press: 173–185.

Cerling, T. E. 1984 The stable isotopic composition of modern soil carbonate and its relation to climate. *Earth and Planetary Science Letters* 71: 229–240.

Cerri, C. C., Feller, C., Balesdent, J. Victoria, R. and Plenecassagne, A. 1985 Application du traçage isotopique naturel en ^{13}C, a l'étude de la dynamique de la matière organique dans les sols. *Comptes Rendus de l'Académie des Sciences de Paris* 2nd series, 300: 423–428.

Dabin, B. 1971 Etude d'une méthode d'extraction de la matière humique du sol. *Science du Sol* 1:47–63.

Dzurec, R. S., Boutton, T. W., Caldwell, M. M. and Smith, B. N. 1985 Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. *Oecologia* 66: 17–24.

Fairbridge, R. W. 1976 Shellfish-eating preceramic Indians in coastal Brazil. *Science* 191: 353–359.

Gentry, A. H. 1982 Phytogeography patterns as evidence for a Chocó refuge. *In* Prance, G. T., ed., *Biological Diversification in the Tropics*. New York, Columbia University Press: 112–135.

Goh, K. M. and Molloy, B. P. J. 1978 Radiocarbon dating of paleosols using organic matter components. *Journal of Soil Science* 29(4): 567–573.

Haffer, J. 1969 Speciation in Amazonian forest birds. *Science* 165: 131–137.

Hendy, C. H., Rafter, T. A., MacIntosh, N. W. G. 1972 The formation of carbonate nodules in the soils of the Darling Downs, Queensland, Australia, and the dating of the Talgai cranium. *In* Rafter, T. A. and Grant-Taylor, T., eds., Proceedings of the 8th International ^{14}C Conference, Wellington, Royal Society of New Zealand: D106–D126.

Hollander, D. J., McKenzie, J. A. and Haven, H. L. 1992 A 200 year sedimentary record of eutrophication in Lake Greifen (Switzerland): Implications for the origin of organic-carbon rich sediments. *Geologia* 20: 825–828.

Korner, Ch., Farquhar, G. D. and Roksandic, C. 1988 A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* 74: 623–632.

Krishnamurthy, R. V., DeNiro, M. J. and Pant, R. K. 1982 Isotope evidence for Pleistocene climatic changes in Kashmir, India. *Nature* 298: 640–641.

Ledru, M. P. 1993 Late Quaternary environmental and climatic changes in Central Brazil. *Quaternary Research* 39: 90–98.

Martinelli, L. A., Pessenda, L. C. R., Valencia, E. P. E., Camargo, P. B., Telles, E. C. C., Cerri, C. C., Aravena, R., Victoria, R. L., Richey, J. E. and Trumbore, S. 1996 Carbon-13 variation with depth in soils of Brazil and climate change during the Quaternary. *Oecologia* 106: 376–381.

Miklos, A. A. W. (ms.) 1992 Biodynamique d'une couverture pédologique dans la région de Botucatu (Brésil – SP). Ph. D dissertation, University of Paris: 247 p.

Nadelhoffer, K. J. and Fry, B. 1988 Controls on natural nitrogen-15 and carbon-13 abundance in forest soil organic matter. *Soil Science Society of America Journal* 52: 1633–1640.

Oliveira, J. B., Menk, J. F. R. and Rota, C. L. 1985 Solos do parque estadual de Campos do Jordão. Silvicultura em São Paulo. *Revista do Instituto Florestal* 9: 125–155.

Pessenda, L. C. R. and Camargo, P. B. 1991 Datação radiocarbônica de amostras de interesse arqueológico e geológico por espectrometria de cintilação líquida de baixa radiação de fundo. *Química Nova* 14(2): 98–103.

Pessenda, L. C. R., Valencia, E. P. E., Camargo, P. B., Telles, E. C. C., Martinelli, L. A., Cerri, C. C., Aravena, R. and Rozanski, K., 1995 Natural radiocarbon measurements in Brazilian soils developed on basic rocks. *Radiocarbon*, this issue.

Prance, G. T. 1973 Phytogeographic support for the theory of Pleistocene forest refuges in the Amazon basin, based on evidence from distribution patterns in Caryocaraceae, Chrysobanaceae, Dichapetalaceae and Lecythidaceae. *Acta Amazonica* 3(3): 5–28.

Saldarriaga, J. G. and West, D. C. 1986 Holocene fires in the northern Amazon basin. *Quaternary Research* 26: 358–366.

Schwartz, D., Mariotti, A., Lanfranchi, R. and Guillet, B. 1986 $^{13}C/^{12}C$ ratios of soil organic matter as indicators of vegetation changes in the Congo. *Geoderma* 39: 97–103.

Servant, M., Fontes, J.-C., Rieu, M. and Saliège, X. 1981 Phases climatiques arides holocènes dans le sud-ouest de l'Amazonie (Bolivie). *Comptes Rendus de l'Académie des Sciences de Paris*. 2nd series, 292: 1295–1297.

Soubies, F. 1980 Existence d'une phase sèche en Amazonie brésilienne datée par la présence de charbons dans le sols (6000–3000 ans B. P.). *Cahier ORSTOM série Géologie* 11 (1): 133–148.

Stout, J. D., Rafter, T. A., Throughton, J. H. 1975 The possible significance of isotopic ratios in paleoecology. *In* Suggate, R. P. and Cresswell, M. M., eds., *Quaternary Studies*. Wellington, Royal Society of New Zealand, 279–286.

Stuiver, M. and Polach, H. A. 1977 Discussion: Reporting of ^{14}C data. *Radiocarbon* 19(3): 355–363.

Throughton, J. H., Stout, J. D. and Rafter, T. 1974 Long-term stability of plant communities. *Carnegie Institute of Washington Yearbook* 73:838–845.

Tieszen, L. L., Snyimba, M. M., Imbamba, S. K. and Throughton, J. H. 1979 The distribution of C_3 and $_4$ grasses and carbon isotope discrimination along an attitudinal and moisture gradient in Kenya. *Oecologia* 37: 337–350.

van der Hammen 1982 Paleoecology of tropical South America. *In* Prance, G. T., ed., *Biological Diversification in the Tropics*. New York, Columbia University Press: 60–66.