Biomarker evidence for recent turf cultivation in Northeast Brazil (Lagoa do Boqueirão, RN)

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ABSTRACT

The first meter of sediments in Lagoa do Boqueirão (RN, Brazil) is characterized by low sedimentation rates during the 1000 BC-1500 AD time period and high sedimentation rate in the top 20 cm, corresponding to the last ten years. Several pentacyclic triterpene methyl ethers (PTME) such as taraxer-14-en-3α-ol ME (crusgallin) and arbor-9(11)-en-3ß-ol ME (cylindrin) are detected in all sediment samples. The major change in sedimentation rates recorded at 20 cm is accompanied by a change in PTMEs concentrations and distribution. Sediments deposited during the 1000 BC-1500 AD time period contain PTMEs at low concentration levels (1.3 µg/g sed) that could constitute a geochemical background of the grasses that naturally developed in the catchment. High concentrations of PTME are recorded during the 1996-2000 time period. They mainly result from high

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concentrations of a compound tentatively identified as arbor-8-en-3βol ME, a potential diagenetic derivative of cylindrin. This increase in PTME corresponds to the beginning of intensive cultivation of *Cynodon dactylon* and *Zoysia japonica* (arundoin and cylindrin producers), for the production of turf to cover Brazilian football stadiums and golf practices. These results constitute a novel application of PTMEs to reconstruct land use changes from lake sediment archives.

Key-words: pentacyclic triterpene methyl ethers, land use, turf, lake sediments.

1. Introduction

In the last decades land-use changes severely altered soil in tropical countries due to increased demography leading to higher demand for food (Houghton et al., 1991; Houghton, 1994; Ojima et al., 1994; Geissen et al., 2009a) but also for leisure (e.g. turf production). The timing and extent to which these changes in land use affected natural ecosystems, soil quality and biodiversity remain to be evaluated. Lacustrine sedimentary archives can potentially record these modifications if suitable tracers allow discriminating between their natural or anthropogenic causes. Molecular biomarkers have revealed useful to track changes ecosystems (e.g. Fisher et al., 2003; Meyers, 2003; Jacob et al, 2008 a; b). Upon molecular biomarkers, pentacyclic triterpenes constitute a highly diversified family of molecules that are mostly produced by higher plants (Das and Mahato, 1983; Mahato

and Sen, 1997). Specifically, pentacyclic triterpenes bearing a methyl ether group at C3 position (pentacyclic triterpene methyl ethers, PTMEs) preserved in lake sediments were shown to be specific of Gramineae (Jacob et al., 2005). One of the first application developed with these compounds was provided by Jacob et al. (2008 a; b) who brought evidence that miliacin (olean-18-en-3β-ol methyl ether) preserved in Lake le Bourget sediments (France) originated from broomcorn millet (*Panicum miliaceum*), an allochthonous plant that was introduced by men in the catchment area of the lake during the Bronze Age. This work proposes another application of PTMEs as biomarkers of grasses to unravel changes in land use and vegetation cover around Lake Boqueirão, northeast Brazil.

2. Study site

Lagoa do Boqueirão is a small lake (catchment area of 250 km^2) located in northeastern Brazil (Rio Grande do Norte State), about 8 km from the Atlantic coast and close to the equator (5° 14' 57.1"N, 35° 32' 42.5"O, Figure 1). The local climate is tropical semi-humid with pronounced seasonality. Precipitation annually reaches 900 mm and mostly occurs during the rainy season, from November to May. The mean annual temperature is 26 °C. The dominant natural vegetation of the region is a grass steppe (restinga). The lake is surrounded by agricultural lands where intensive cultivation of grass is attested by numerous irrigation circles as represented in Figure 1.

3. Materials and methods

Core BOQC0701 (100 cm) was sampled in the centre of the lake with a vibracorer (Martin and Flexor, 1995) and was sliced every 0.7 cm. The samples were dried at 40 °C in an oven, ground and stored.

3.1 Extraction and separation of PTMEs

Lipids were extracted from ca. 0.40 g of sediment by ASE 200 with dichloromethane:methanol 1:1 v/v. The total extract was fractionated by flash chromatography on activated silica. After the elution of aliphatic and aromatic hydrocarbons, PTMEs were eluted with hexane:toluene (1:1 v/v). Fractions were then dried under N_2 and 5α cholestane was added as internal standard before injection.

3.2 Gas Chromatography-mass spectrometry (GC-MS)

The fraction containing PTMEs was analyzed by GC–MS with a TRACE-Polaris-GCQ. The gas chromatograph was fitted with a Rtx-5MS column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The GC operating conditions were: temperature held at 40 °C for 1 min, then increased from 40 to 120 °C at 30°C min^{-1}, 120 to 300 °C at 3 °C min^{-1}, with a final isothermal hold at 300 °C over 30 min. The sample was injected splitless, with the injector temperature set at 280 °C. Helium was the carrier gas. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV ionization energy and scanned from m/z 50 to 600. PTMEs were identified by using standard compounds (Jacob et al., 2005). Due to possible coelutions with other compounds, the PTME concentrations were estimated by

measuring the areas of their peaks on ion specific chromatograms. PTMEs concentrations were estimated after calculating a correction factor between the peak area on the ion specific chromatogram and the peak area on the Total Ion Current (TIC) on authentic standards.

4. Results and discussion

The age model was first established from nine AMS ^{14}C radiocarbon dates performed on total organic matter at the Laboratoire de Mesure du Carbone 14, Gif sur Yvette, France. These data were calibrated by using the Calib 5.0.2 program (Stuiver, Reimer and Reimer, 2009). Two top-core samples were dated by ^{210}Pb at the Laboratory of Radioecology and Global Changes (LARAMG) in University of State Rio de Janeiro, Brazil (Table 1).

Lead dating of the upper part of the core (0 to 19 cm) indicates a mean sedimentation rate of 1.6 cm/yr (Figure 1). Sediments from the bottom core (20 to 100 cm) display an average sedimentation rate of 0.033 cm/yr, estimated from ^{14}C dates. These two distinct sedimentation rates indicate a strong change in sedimentation that happens around 20 cm depth. This drastic change not only resulted in a large hiatus (estimated between ca. 1500 and 1994) but also in an increase in sediment input.

Ether fractions of sediment extracts contain several PTMEs upon which taraxer-14-en-3bol ME (crusgallin) and arbor-9(11)-en-3bol ME (cylindrin) could be identified with authentic standards (Figure 2). The mass spectrum of compound 1, detected at 44.3 min, is characterized by intense m/z 273 and 241, M^+ at 440, $[M^+-32]$ at m/z 408, $[M^+-15]$ at m/z 425 and $[M^+-32-15]$ at m/z

393, i.e. undistinguishable from the mass spectra of cylindrin, arundoin and related compounds (Jacob et al., 2005). Based on relative retention times between arundoin, fern-8-en-3βol ME and cylindrin (Ohmoto et al., 1970) with those of our standards and samples (Figure 2), we tentatively attribute an arbor-8-en-3β-ol ME structure to compound 1, although it has never been reported yet.

The evolutions of crusgallin and cylindrin concentrations with time are depicted in Figure 3. High concentrations of compound 1 (2.5-11.7 µg/g sed) are recorded in sediments dated back to the 1996-2000 period whereas the sediments deposited during the 1000 BC-1500 AD are characterized by lower concentrations in compound 1, cylindrin and crusgallin (average 0.1, 0.2 and 0.3 µg/g sed, respectively). PTMEs concentrations found in the lower part of the core could therefore be regarded as the natural background of the grass steppe vegetation.

Compound 1 concentration is on average 20 times higher in the upper part of the core than in the natural background. This increase observed from 1996 to 1999 coincides with the beginning of grass cultivation around Lagoa do Boqueirão in the 90s, in order to provide turf for soccer, golf and polo fields. Upon the three species cultivated in the area, *Cynodon dactylon* is reputed to produce arundoin and *Zoysia japonica* to produce both arundoin and cylindrin (Ohmoto et al., 1970). Since concentrations in cylindrin are below detection level in sediments dated back to the 1996-1999 period, we therefore interpret compound 1 as a probable diagenetic derivative of the

cylindrin produced by *Zoysia japonica*. The acidic-catalysed migration leading to Δ^8 structures from their $\Delta^{9(11)}$ isomers has been demonstrated for arundoin and for fern-9(11)-en-3βol (Ageta et al., 1987; Nishimoto et al., 1968). Accordingly, compound 1 could result from the acidic conversion of cylindrin under acidic conditions that presently prevail in the sediment, although this double bond migration has not been demonstrated yet. The higher sensibility of arundoin to acidic conditions (as compared to cylindrin; Nishimoto et al., 1968) could explain the absence of this compound and of its diagenetic derivatives in the sediments from this period.

Sediments dated back to the 2000-2008 period have lower PTMEs concentration than that of natural background and of compound 1. This drop might result from the withdrawal of most of the natural vegetation cover to create fields for the cultivation of grass as well as intensive agriculture practices (cultivation of coconut, tomato and banana).The decrease in compound 1 concentration around 1996 might result from a better treatment of waste derived from the turf exploitation to the tailings pond.

Conclusion

The detection and quantitation of grass specific biomarkers in sediments of Lake Boqueirão covering the last 3000 years allowed us to distinguish between natural background and geochemical signature of anthropogenic activities and to unravel recent land use around the lake, in agreement with sedimentary dynamics. A new compound, tentatively identified as arbour-8-

en-3βol ME could be a diagenetic derivative of cylindrin produced by *Zoysia japonica*, a grass cultivated for turf production around the lake.

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References

Ageta, H., Shiojima, K., Arai Y., 1987. Acid- induced rearrangement of triterpenoids hydrocarbons belonging to the hopane and migrated hopane series. Chemical and Pharmaceutical Bulletin 35, 2705-2716.

Das, M.C., Mahato, S.B., 1983. Triterpenoids. Phytochemistry 22, 1071–1095.

Fisher, E., Oldfield, F., Wake, R., Boyle, J., Appleby, P., Wolff, G.A., 2003. Molecular marker records of land use change. Organic Geochemistry 34, 105-119.

Geissen, V., Sánchez-Hernández, R., Kampichler, C., Ramos-Reyes, R., Sepulveda-Lozada, A., Ochoa-Goana, S., Jong, B.H.J., Huerta-Lwanga, E., Hernández-Daumas, S., 2009. Effects of land-use change on some properties of tropical soils - An example from Southeast Mexico. Geoderma 151, 87-97.

Houghton, R.A., 1994. The worldwide extent of land-use change. BioScience 44, 305-313.

Houghton, R.A., Skole, D.L., Lefkowitz, D.S., 1991. Changes in the landscape of Latin America between 1850 and 1985. II. Net release of CO_2 to the atmosphere. Forest Ecology and Management 38, 173-199.

Jacob, J., Disnar, J.R., Boussafir, M., Sifeddine, A., Albuquerque, A.L.S., Turcq, B., 2005. Pentacyclic triterpene methyl ethers in recent lacustrine sediments (Lake Caçó, Brazil). Organic Geochemistry 36, 449-461.

Jacob, J., Disnar, J.R., Arnaud, F., Chapron, E., Debret, M., Lallier-Vergès, E., Desmet, M., Revel-Rolland, M., 2008. Millet cultivation history in the French Alps as evidenced by a sedimentary molecule. Journal of Archaeological Science 35, 814-820.

Jacob, J., Disnar, J.R., Bardoux, G. 2008. Carbon isotope evidence for sedimentary miliacin as a tracer of Panicum miliaceum (broomcorn millet). Organic Geochemistry 39, 1077-1080.

Mahato, S.B., Sen, S., 1997. Advances in triterpenoid research 1990-1994. Phytochemistry 44, 1185–1236.

Martin, L., Flexor, J.M., Suguio, K., 1995. Vibrotestemunhador leve. Construção, utilização e potencialidades. Revista IG. São Paulo 16, 59-66.

Meyers, P.A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Organic Geochemistry 34, 261-289.

Nishimoto, K., Ito, M., Natori, S., Ohmoto, T., 1968. The structures of arundoin, cylindrin and fernenols. Triterpenoids of fernane and arborane groups of Imperata cylindrical var. koenigii. Tetrahedron 24, 735-752.

Ojima, D.S., Galvin, K.A., Turner, B.L., 1994. The global impact of land-use change. BioScience 44, 300-304.

Ohmoto, T., Ikuse, M., Natori, S., 1970. Triterpenoids of the Gramineae. Phytochemistry 9, 2137-2148.

Stuiver, M., Reimer, P.J., Reimer, R. 2009. Calib, version 5.02. http://calib.qub.ac.uk/calib/

Table captions

Table 1: ^{14}C dates and ^{210}Pb data used for the establishment of core BOQC0701 age model.

	^{210}Pb age information				
Laboratory code	Depth (cm)	Material dated	Age (Yr AD)		Age Yrs cal BP
Boqc0701-1	0	Total organic matter	2007		-1
Boqc0701-2	11.2	Total organic matter	2000		-8
	^{14}C age information				
Laboratory code	Depth (cm)	Material dated	^{14}C yr BP	$\delta^{13}C$ (‰)	Intercept ages cal yr BP
SacA 11586	21.7-22.4	Total organic matter	660 ± 30	-26.7	563
SacA 11587	31.5-32.2	Total organic matter	975 ± 30	-26.2	804
SacA 11588	40.4-40.9	Total organic matter	1660 ± 30	-25.1	1425
SacA 11589	50.9-51.4	Total organic matter	1645 ± 30	-21.8	1422; 1455; 1518
SacA 11590	59.9-60.4	Total organic matter	1915 ± 30	-23.0	1745; 1820
SacA 11591	69.9-70.4	Total organic matter	2000 ± 30	-20.7	1988
SacA 11592	79.9-80.4	Total organic matter	2160 ± 30	-24.4	2080
SacA 11593	90.9-91.4	Total organic matter	2425 ± 30	-24.4	2353
SacA 11594	99.4-99.9	Total organic matter	2860 ± 30	-26.6	2885; 2910; 2922

Figure captions

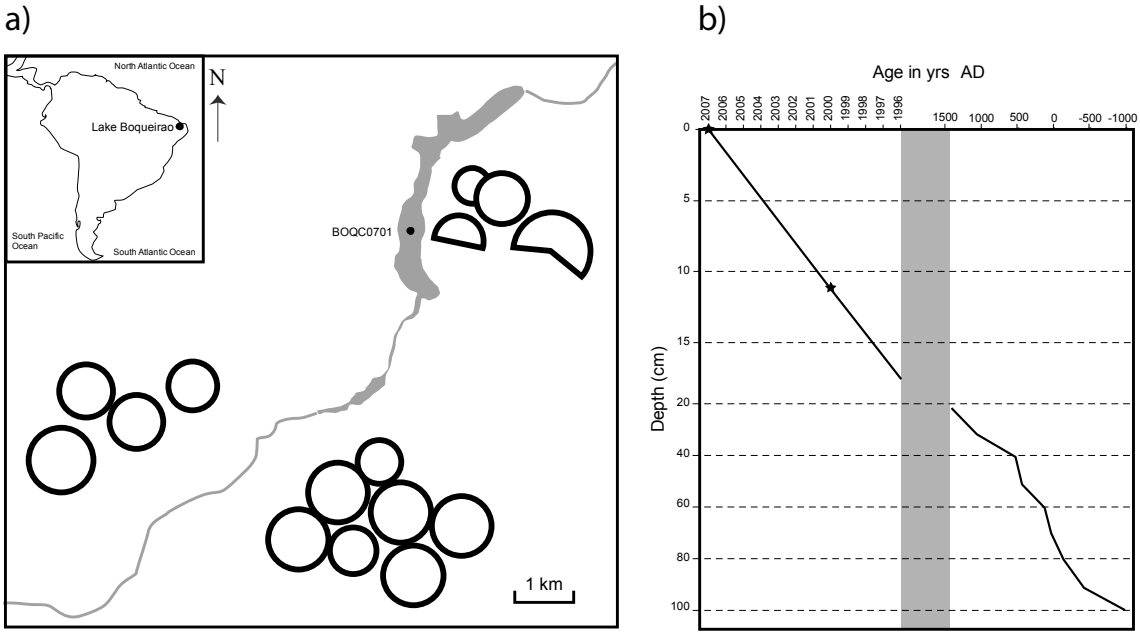


Figure 1: (a) Map of Lake Boqueirão region emphasizing numerous irrigation circles that attest to intensive agriculture in the catchment. The location of core BOQC0701 is indicated by a full circle. (b) Age-depth relationship in core BOQC0701 based on lead and carbon isotopes illustrating the strong change in sedimentation rate at 20 cm.

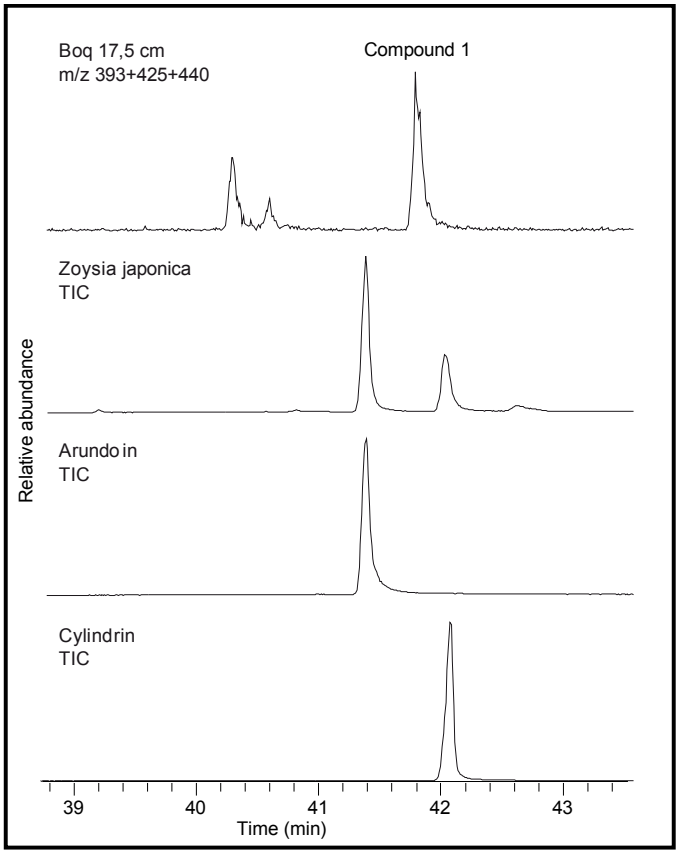


Figure 2: Distribution of PTMEs in the sediments of Lake Boqueirão as illustrated by the partial m/z 393+425+440 chromatogram of sample collected at 17.5 cm (upper part of core, 1996 yrs AD) compared to the TIC of *Zoysia japonica* extract (showing arundoin and cylindrin) and to TIC traces of arundoin and cylindrin standards.

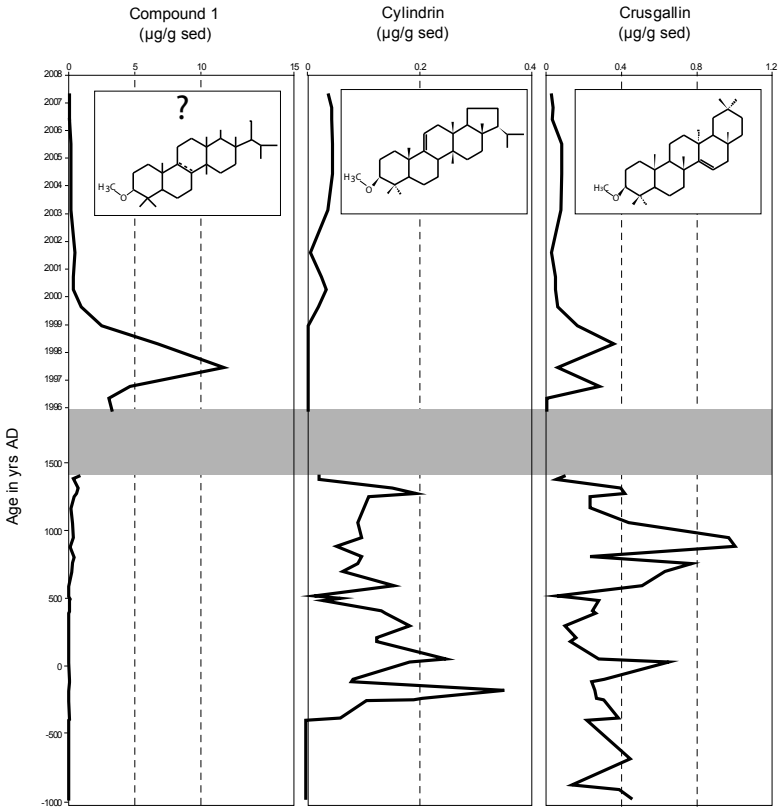


Figure 3: Evolution of compound 1, cylindrin and crusgallin concentrations with time in core BOQC0701.