Anaerobic decomposition of different parts of Scirpus cubensis: kinetics and gas production.

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ABSTRACT: Anaerobic decomposition of different parts of Scirpus cubensis: kinetics and gas production. We report the anaerobic decomposition of rhizomes, roots and aerial portions (leaves and fertile branches) of Scirpus cubensis, as well as the detritus from the entire plant; in this context, the hypothesis was that the aerial portion of S. cubensis is the most degradable source. Samples of water and plants were collected in the Óleo lagoon (Ecological Station of Jataí; 21° 36' S and 47° 49' W). The labile, soluble and refractory fractions of the fragments were quantified and the contents of carbon, nitrogen and ash were determined. For each kind of detritus, three incubations were prepared (7.0 g DW L^{-1}) in the laboratory, using as controls two prepared with lagoon water. During 140 days, all flasks were maintained under controlled conditions, in the dark, at 20 °C. The results of periodic measurements of CO_2 and CH_4 were analyzed with an ANOVA (Kruskal-Wallis), with the consumptions of detritus being fitted to a 1st order kinetics model. After 140 days, 47% of S. cubensis detritus was mineralized. Conversely the expected, the roots were the most mineralized structure (40%), while the rhizomes were the most refractory (26%); overall, the degradability of detritus was attributed to high ash contents. In addition, the detritus with the highest ash contents (roots) presented the most elevated CH_4 productions (21.7%).

Key-words: aquatic macrophytes, anaerobic decomposition, Scirpus cubensis, methanogenesis, kinetic model.

RESUMO: Decomposição anaeróbia de diferentes partes de Scirpus cubensis: cinéticas e produção de gases. Este estudo visou descrever e discutir a decomposição anaeróbia de rizomas, raízes e porção aérea (folhas e ramos férteis) de Scirpus cubensis, bem como dos detritos desta espécie como um todo; nesse contexto, a hipótese deste trabalho foi que a porção aérea de S. cubensis é a mais rapidamente mineralizada. Amostras de água e de plantas foram coletadas na lagoa do Óleo (Estação Ecológica de Jataí; 21° 36' S e 47° 49' W). As frações lábeis, solúveis e refratárias dos fragmentos foram determinadas e foram estimados os teores de carbono, nitrogênio e cinzas. Para cada tipo de detrito prepararam-se três incubações (7,0 g PS L^{-1}). Outras duas foram preparadas com alíquotas de água da lagoa como controle. Todas as incubações foram mantidas em condições controladas (no escuro e 20 °C) por 140 dias. Periodicamente, determinaram-se as produções CO_2 e CH_4. Os resultados foram submetidos ao teste ANOVA (Kruskal-Wallis) e os consumos de detritos foram ajustados a um modelo cinético de 1ª ordem. Após 140 dias, 47% dos detritos de S. cubensis foram mineralizados. Contrariando o esperado, as raízes foram as estruturas mais consumidas (40%), ao passo que os rizomas foram os mais refratários (26%); no geral, os diferentes consumos relacionaram-se aos conteúdos de minerais dos detritos. Em adição, os detritos com maiores conteúdos de cinzas (raízes) foram os que proporcionaram as maiores produções de CH_4 (21.7%).

Palavras-chave: macrófitas aquáticas, decomposição anaeróbia, Scirpus cubensis, metanogênese, modelo cinético.

Introduction

In shallow lakes aquatic vascular plants can represent the most abundant source of detritus (Wetzel, 1990). Tropical aquatic ecosystems usually present favorable conditions for the growth of aquatic macrophytes, especially with regard temperature and photoperiod (Junk & Piedade, 1993; Camargo & Esteves, 1996).

Under these appropriate conditions, the life cycle of aquatic plants is not well defined, in contrast to that verified in aquatic ecosystems of temperate regions. In this context, a study with Scirpus lacustris pointed out differences in the time it takes for the replacement of rhizomes which can persist for three years and roots which are substituted annually (Hejny, 1960).

One of the first events of detritus breakdown, leaching is characterized by the release of organic and inorganic compounds from detritus (Wetzel, 1995). Parallel to leaching, oxidation of reactive organic compounds (labile fraction) occurs. The remaining particulate organic matter (POM) is mainly constituted by cellulose, hemicellulose and lignin, which are the refractory fractions of the detritus. They display slow mass losses, with decomposition rates depending on the microbial enzymatic catalysis (Vrba et al., 2004). The rates and yields of the decomposition of aquatic macrophytes are influenced by the inherent characteristics of the detritus (e.g. C:N ratio, desiccation degree and size) and environmental factors such as temperature, abrasion, pH, available nutrients, dissolved oxygen and the redox potential (Mellilo et al., 1982; Bianchini Jr. & Antonio, 2003).

According to Newell (1993), studies should consider the conditions under which decomposition occurs naturally. Indeed, decomposition experiments frequently use the whole plant tissues as an initial resource, although it has been shown that different plant structures become detritus at different times (Nogueira, 1989; Moschini-Carlos, 1991). Owing the chemical composition of tissues, the leaves usually presents the highs rates of decomposition, in this context, one suppose that in the decay of Scirpus cubensis the leaves will be mineralized faster. Because of the importance of information of the kinetics of decomposition, we aim at investigating the anaerobic decomposition of different structures of Scirpus cubensis compared to the decay of the entire plant detritus.

Material and methods

The samples of water and plants were collected from the Óleo lagoon (21° 36' S and 47° 49' W), located in the municipality of Luiz Antônio (State of São Paulo, Brazil). This lagoon belongs to a group of oxbow lakes on the Mogi-Guaçú river floodplain that have official protection since they are inside of the Jataí Ecological Station (JES). According to Köppen (1931), the climate of the region is characterized as AW, with two well-defined seasons (Ballester & Santos, 2001): one rainy (November to April) and one dry (May to October). Those oxbow lakes are characterized by low depth and intense aquatic macrophytes cover (Nogueira & Esteves, 1990). Anaerobiosis usually occurs during the rainy season in these lagoons (Ballester & Santos, 2001).

To perform the experiments, Scirpus cubensis Poepp & Kunth was used. This Cyperaceae is widely distributed in the oxbow lakes of the Mogi-Guaçu floodplain. Within JES, in the Infernão lagoon, the highest productivity of S. cubensis reached 2.5 kg DW m^{-2} (rainy season) and the submerged structures of S. cubensis corresponded to 68% of the biomass. The death of fertile branches occurred in September, after flowering (Nogueira, 1989; Moschini-Carlos, 1991).

After collected (at the same day), the plants were washed, dried at 50 °C to a constant weight and ground (f = 1.41 mm); Bianchini Jr. & Antonio (2003). Before grinding some specimens were divided into roots, rhizomes and aerial portions (leaves + fertile branches). The carbon and nitrogen contents of the fragments were quantified by elemental analysis (CHN Analyzer Carlo Erba model EA 1110). The ash free dry weight (AFDW) was determined for the initial resources and remaining residues (n: 3) by ignition (550 °C; 2 h) and gravimetric methods (Wetzel & Likens, 1991). The water used in incubations was collected with Van Dorn bottles at different depths of the lagoon: surface, 1.5 and 3.0 m depth. The samples were then mixed to achieve an integrated one. In the laboratory, the water was filtered in glass wool and the organic (DOC) and inorganic carbon (DIC) concentrations were determined by combustion (TOC Analyzer Shimadzu, model 5000A). For each type of resource (integral detritus, roots, rhizomes and aerial portion) three incubations were prepared (7.0 g DW of fragments to 1.0 L of water). Two flasks had only water from the lagoon and were used as controls. All incubations were maintained in the dark, at 20.0 ± 0.15 °C. For 140 days, periodic measurements were performed of CO_2 and CH_4 productions, by sampling the gases enclosed in the incubation headspaces. The samples were taken with a syringe (1.0 ml), from silicon septa coupled on the flask cover. The gas samples were analyzed using gas chromatography (CG Construmaq, model 370; with analytical column PORAPAQ N), with the values obtained being compared with the standard curves for CO_2 and CH_4. After the measurements, the gases were eliminated from incubations by nitrogen bubbling. At the end, the solutions of incubations were centrifuged (978.25 g; 40 min) and filtered in cellulose-ester membranes (f = 0.22 mm) previously washed with distilled water (Stockner et al., 1990); the DOC and DIC concentrations were then determined (TOC analyzer). The particulate material recovered was dried (50 °C) to a constant weigh. These values were used to calculate the correction factors (CF) to incorporate the dissolved fractions to daily production rates (Eq. 1).

$$CF = \frac{(TOC_i - TOC_f) - (DIC_f - DIC_i)}{C_{gc}} \qquad (1),$$

where TOC_i = initial total organic carbon; TOC_f = final total organic carbon; DIC_i = initial dissolved inorganic carbon; DIC_f = final dissolved inorganic carbon; Cgc = carbon determined by gas chromatography.

The 1st order kinetics model has been used to describe the mass loss of various classes of compounds in detritus (Bianchini Jr., 1997), which allows one to take into account the heterogeneity of the source. In this model, the detritus is constituted by 2 classes of particulate organic carbon, namely labile/soluble (LSPOC) and refractory (RPOC) fractions. Three mineralization routes are assumed, as depicted in Equation 2 through 5):

i) Mineralization of the labile fraction:

$$\frac{dIC_1}{dt} = k_T \times \left(\frac{k_1}{k_T} \times LSPOC \right) \qquad (2),$$

ii) Formation of DOC from leaching, following the DOC consumption:

$$\frac{dDOC}{dt} = k_T \times \left(\frac{k_2}{k_T} \times LSPOC \right) - (k_3 \times DOC) \qquad (3),$$

iii) Mineralization of DOC:

$$\frac{dIC_2}{dt} = k_3 \times DOC \qquad (4),$$

iv) Mineralization of the refractory particulate organic carbon (RPOC):

$$\frac{dIC_3}{dt} = k_4 \times RPOC \qquad (5),$$

where IC_1 = inorganic compounds derived from mineralization of labile organic matter (on carbon basis); IC_2 = inorganic compounds from DOC mineralization (on carbon basis); IC_3 = inorganic compounds produced from refractory tissues mineralization (on carbon basis); k_T = rate constant of LSPOC mass loss (= k_1 + k_2), (d^{-1}); k_1 = LSPOC mineralization rate constant (d^{-1}); k_2 = LSPOC leaching rate constant (d^{-1}); k_3 = DOC mineralization rate constant (d^{-1}); k_4 = RPOC mineralization rate constant (d^{-1}).

For each type of resource, three axenic incubations were prepared (70 mg DW of fragments and 10 ml of sodium azide solution - 0.4 mM) to estimate the LSPOC and RPOC fractions. After 24 hours the solutions were filtered (0.22 mm) and the concentrations of DOC and DIC were determined (TOC Analyzer); after dried (50 °C; 48 hours), the particulate residues were quantified with the gravimetric method. RPOC and LSPOC were estimated from differences between the initial and final (after leaching) masses of the detritus. LPOC (labile fraction of POC) was estimated from differences between LSPOC and DOC.

Based on the results from leaching experiments with aquatic plants (Cunha-Santino & Bianchini Jr., in press) we adopted k_T = 1.5 d^{-1}. The decay coefficients k_3 and k_4 were obtained from the initial (after 24 h of leaching) and final DOC concentrations and RPOC contents (after 140 d), respectively. The total mineralization (IC_{total}) was obtained by the sum of IC (1, 2 and 3) production. The temporal changes of accumulated values of CO_2, CH_4, IC_2 and IC_3 (kinetics) were submitted to Kruskal-Wallis (non-parametric ANOVA) analysis in order to verify for significant differences among treatments ($p < 0.05$).

Results

Taking into account the quality of the plant tissues, the C:N ratio varied from 20.6

(rhizomes) to 85.1 (roots). The ash content varied from 8.6 to 29.7%, respectively for these same structures. The leached DOC varied from 1.8 (roots) to 6.0% (aerial portion), while the refractory fractions (RPOC) always prevailed (86.2 to 91.0%). The highest mineralization yield was verified for the integral detritus of S. cubensis (47.0%), where 51.1% remained as POC and 1.9% as DOC. The rhizomes were the most recalcitrant, 72.4% remained as POM and 1.8% as DOC (Tab. I). The yields of gas formation (CO_2 + CH_4) for those resources were 46.1 and 24.7 %, respectively (Tab. I).

Table I: Characteristics of Scirpus cubensis tissues and its decomposition yields and parameters.

Resource	**Integral detritus**	**Roots**	**Rhizomes**	**Aerial**
Elemental Analysis				
Ash (%)	15.1	29.7	8.6	8.9
Carbon (%)	47.3	59.6	37.2	46.3
Nitrogen (%)	1.2	0.7	1.8	1.1
C:N	39.4	85.1	20.6	42.1
Leaching assay				
LPOC (%)	7.7	7.2	7.4	7.8
DOC (%)	5.2	1.8	5.9	6.0
RPOC (%)	87.1	91.0	86.7	86.2
Decomposition yields				
MC (%)	47.0	39.6	25.8	28.2
DIC_f (%)	0.9	0.9	1.1	0.5
C-gases (%)	46.1	38.7	24.7	27.7
C-CO_2 (%)	19.0	17.0	17.6	24.7
C-CH_4 (%)	27.1	21.7	7.1	3.0
IC_1 (%)	7.7	7.2	7.4	7.8
IC_2 (%)	3.3	0.0	4.8	5.5
IC_3 (%)	36.6	32.9	11.3	17.3
IC_{total} (%)	47.6	40.1	23.5	30.6
Constant rates				
k_1 (d^{-1})	0.58	1.20	0.83	0.85
k_2 (d^{-1})	0.92	0.30	0.67	0.65
k_3 (d^{-1})	0.01	0	0.01	0.02
k_4 (d^{-1})	0.4´10^{-2}	0.3´10^{-2}	0.1´10^{-2}	0.2´10^{-2}

CO_2 production was detected from the first day, with average rates varying from 0.5 to 0.8 mg g^{-1} d^{-1} (rhizomes and leaves, respectively). It was higher in leaves (24.7%) decomposition and lower in roots (17.0%). The ANOVA indicated that CO_2 yields were considered similar only in rhizomes and roots ($p = 0.8780$). Overall, the CO_2 production can be separated into two stages (Fig. 1). On the first, the highest rates of production were observed (from the 1st to the 10th day), and then a progressive reduction occurred up to the 20th day. The average rates of production in the latter stage varied from 0.4 (rhizomes) to 0.9 mg g^{-1} d^{-1} (leaves). For the decomposition of the leaves, the highest rate of 2.4 mg g^{-1} d^{-1} was observed in the first stage. For the others sources the highest rates were reached in the second stage. The CO_2 production rates varied for rhizomes and integral detritus from 0.3 to 1.9 mg.g^{-1} d^{-1}, respectively (89th and 71st d). The average rates of CO_2 production varied from 0.4 to 0.7 mg g^{-1}.d^{-1} to rhizomes and leaves decay, respectively.

The generation of methane occurred in all incubations, but started earlier for the integral detritus, on the 24th day, than for leaves, on the 44th day. The yields were 27 and 3.0% for the integral detritus and leaves, respectively. The ANOVA analysis indicated significant difference in CH_4 yield ($p = 0.0034$). Nevertheless, methane generation presented similar kinetics, with 3 stages. The first comprised the period when CH_4 was not produced. The second, when the highest rates of CH_4 generation were detected, the values varied from 0.7 (83rd days) to 4.2 mg $g^{-1}.d^{-1}$ (45th days) for leaves and integral detritus, respectively.

The third stage was characterized by a decrease in production rates until a few days before the experiment finished (Fig. 1).

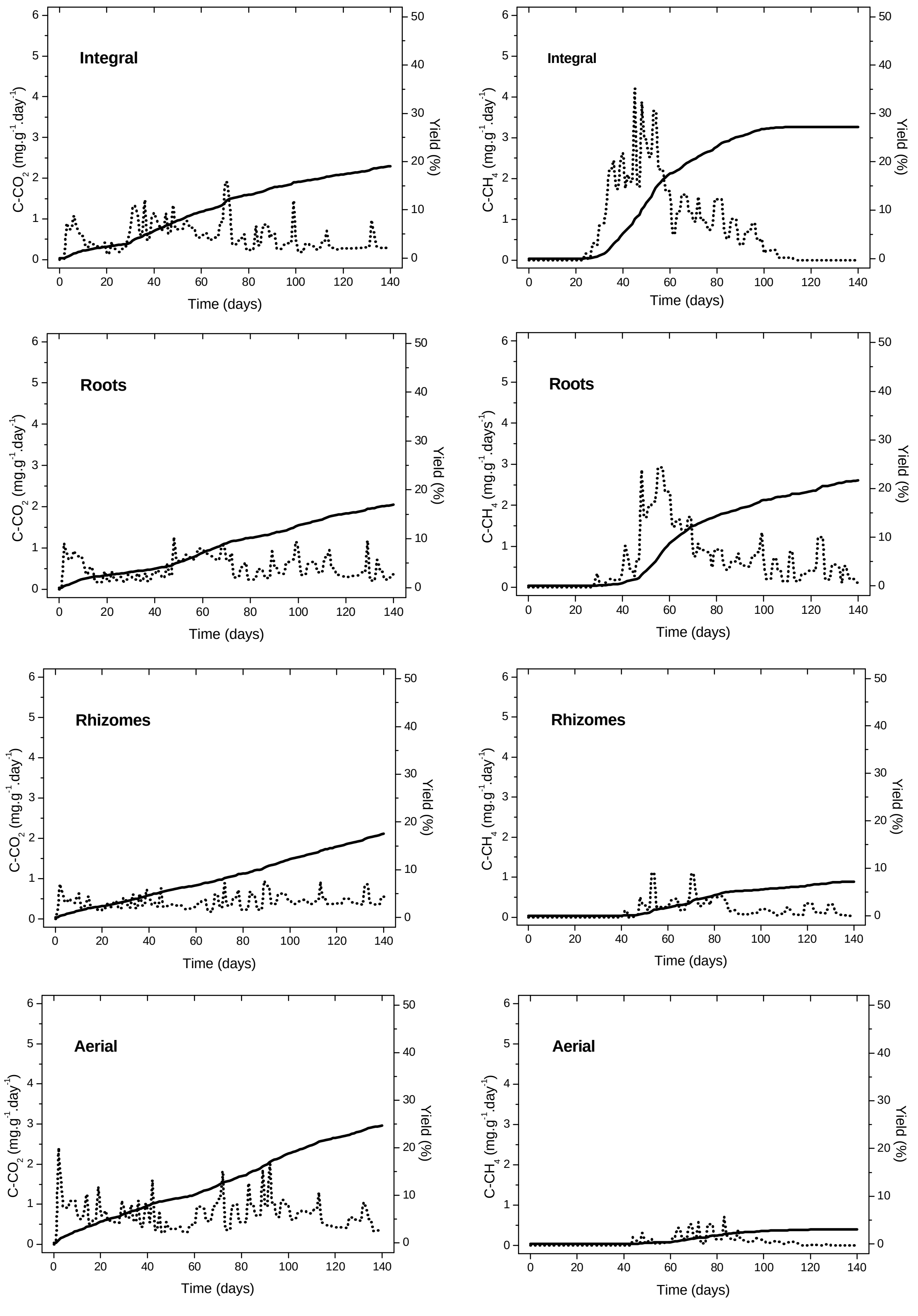

Figure 1 : CO_2 and CH_4 produced in anaerobic decomposition of Scirpus cubensis (integral, roots, rhizomes and aerial portion); kinetics (solid lines) and daily production rates (dotted lines).

The DOC mineralization (route IC_2) was statistically different ($p = 0.001$). This pathway was responsible for mineralization of 3.3 and 5.5% of integral detritus and leaves, respectively (Tab. I). The roots presented the lowest DOC generation (1.8%) and this fraction was not decomposed (k_3=zero). For the others resources, k_3 varied from 0.01 d^{-1} (integral detritus and rhizomes) to 0.02 d^{-1} (leaves). Consequently, the half-life times varied from 39.1 to 98.3 days (leaves and integral detritus). The RPOC mineralization (route IC_3) varied from 11.3 to 36.6%, for rhizomes and integral detritus, respectively (Tab. I). k_4 changed from $1.0x10^{-3}$ to $4.0x10^{-3}$ d^{-1} (half-life times: 695 and 182 days, respectively). The lowest k_3:k_4 ratio was found for the integral detritus and the highest for the rhizomes. The ANOVA indicated similarity between IC_3 routes on integral detritus and roots ($p = 0.3585$) as well as on rhizomes and aerial portion ($p = 0.3239$). The yields of mineralization (IC_{total}) were statistically different ($p = 0.0006$), except for rhizomes and leaves ($p = 0.3145$).

Discussion

In opposite the hypothesis the leaves of S. cubensis not presented the highest mineralization rates, it was observed to roots. The initial ratio C:N has frequently been considered an important predictor about detritus breakdown (Melillo et al., 1982; Esteves & Barbieri, 1983); detritus with low C:N ratios presents high decay rates. However, this event do not apply to the mineralization of S. cubensis structures, since roots presented the highest ratio C:N and the highest detritus consumption; in this context, other studies, with different species, verified similar results (Godshalk & Wetzel, 1978; Puriveth, 1980; Wrubleski et al., 1997). Some studies that considered the leaves decay in stream, the lignin content of the detritus was a better predictor decomposition rates (e. g. Gessner & Chauvet, 1994; Royer & Minshall, 2001). Under this condition, the decay was not limited by nutrients. The decompositions of cellulose and hemicellulose may be limited by the presence of lignin, which makes it difficult for microorganisms producers of cellulases and hemicellulases to access the fibers (Prescott et al., 2004). It is possible that the higher decomposition of the roots observed in the present study was related to their lower lignin contents compared to the rhizomes and aerial portions. This hypothesis is corroborated by the results obtained from the kinetic model which presented just one route where the roots had a higher yield than any of the other plant structures. Indeed, the mineralization of refractory fractions of roots was at least twice higher than for other structures. This fact can be associated with the supporting function of rhizomes and leaves tissues. The slow decomposition of support structures was also verified by Esteves & Barbieri (1983) for Nymphoides indica (petioles and blades), by Darwich (1995) for Echinocloa polistachya (leaves and stems) and by Gessner (2000) for Phragmites australis (stems and leaves); these studies determined that decay coefficients were at least twice higher for leaves than the other structures considered. Also to P. australis decomposition in lakes, it was observed after 33 months the mass losses of 82 to 90% of the leaves while the stems presented 39 to 43% of mass loss (Dinka et al., 2004); the authors also noted a more intense decay for the leaves fibers.

The quantitative differences in the mineralization of S. cubensis structures may also be related to the mineral contents. In this context, the roots presented ash contents up to three times the contents in rhizomes. Taken together with the low DOC formation by leaching (7.2%), this result allows us to infer that the roots were rich in mineral elements. In a study with leaves (Ficus microcarpa, Quercus robur and Alchornea triplinervia) Schoenlein-Crusius et al. (1999) observed that its colonization by aquatic hyphomycetes was directly correlated to the ion contents of the detritus. According to these results, it is possible to infer that the availability of minerals was a key factor to enhance mineralization of roots, especially the particulate fraction, and of the integral detritus of S. cubensis. In addition, the ash content probably linked with the methane production; in this context, the increments of CH_4 production has been related with additions of nickel, boron, vanadium, iron, zinc and cobalt (Banik et al., 1996; Basiliko & Yavitt, 2001). However, the presence of micronutrients has not affected fermentations and anaerobic respirations, suggesting that the methanogenesis was the most element-trace limited process. In this context, Kaesler & Schönheit (1989) reported that methanogenic archaea required at least 1.0 mM of Na^+ for ATP formation from the Na^+/K^+ bomb. Overall, the results obtained in this study indicate that the higher decomposition rates and methanogenic activity are related with low lignin content and mineral availability.

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