Chapter 21

Periphyton As a Potential Biofertilizer in Intensive Agriculture of the Ancient Maya

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INTRODUCTION

The Yucatecan Maya developed an advanced civilization based on a set of poorly-known systems of intensive agriculture, in a dry tropical region climate and in shallow lithosols and rendzinas, with a low content of organic matter, fast leaching of nutrients, and high phosphorus fixation, that constrain agriculture. In contrast, some Maya communities still practice intensive agriculture using some organic materials as fertilizers. It is assumed that ancient Maya used them too.

The soils of the Yucatán Peninsula are shallow lithosols and rendzinas that are susceptible to biological degradation. In many areas, the soils are very poor in organic matter and have a very high level of phosphorus retention due to their calcimagnesic origin. One interesting problem is how the ancient Maya, over centuries, could provide enough food to sustain a population density in rural areas and urban centers significantly higher than in existence today. Another has been the replenishment of nutrients after several years of milpa cultivation. It has been accepted that the main source of nutrient replenishment came from the natural process of succession known as the

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milpa fallow period. There is no evidence of organic fertilizers in pre-Hispanic times; however, it is assumed that they used them in their intensive agricultural fields (Gómez-Pompa 1978; Wilken 1971). Some present-day Maya communities still practice intensive traditional agriculture and follow agroforestry systems in which they use a number of organic materials as fertilizer (Flores 1983; Gómez-Pompa 1996).

An ancient early Maya settlement has recently been discovered and studied (Fedick 1998) at the El Edén Ecological Reserve (northeast of the Yucatán Peninsula in the state of Quintana Roo, México). Archaeological research has shown a densely populated area that was sustained by agricultural production for several centuries (Morrison 2001). One intriguing discovery has been the presence of a series of rock alignments (some kind of dykes) in the wetlands. It seems that people living in the area were managing the flooding of the wetlands for unknown purposes. One hypothesis that the wetlands were used for agricultural purposes. Unfortunately, up to now, no plant has been found that was worth the effort of these major artificial works in the wetlands, or any proof that plants were grown (or animals raised) in the wetlands.

Agricultural production came from the use of two main groups of soils: (1) those that are seasonally flooded (lowlands and wetlands), and (2) those that are never inundated (uplands). Soils from the upland areas are covered by different kinds of secondary and mature forests on very poor and shallow soils. These soils are the ones used in traditional shifting (*milpa*) agriculture.

The lowland soils are covered by several wetland vegetation types, from seasonally inundated forests and savannas to permanently inundated swamps. One outstanding component of all wetland vegetation is the presence of impressive algal growth (known as periphyton) that covers all submerged substrata. It is composed of an algal mat and an assemblage of microscopic and very small macroscopic organisms that live by attached themselves to a great diversity of submerged substrata, are enmeshed by the attached species, or are feeding among them.

Periphyton is an extremely important component of the wetlands and serve as an important producer in the food web of these ecosystems and also as a nutrient concentrator and producer (Novelo-Maldonado and Tavera 1997). Initial studies by Anaya-Lang (cited by Fedick 1998) on the nutrient content of periphyton showed an outstanding concentration of nutrients, thereby underscoring their potential use as a biofertilizer. The hypothesis that ancient Maya settlers of the El Edén region may have used periphyton as fertilizer is tested, and this paper presents some results on the importance of periphyton as fertilizer. Two methods were used to evaluate its use as a fertilizer: (1) periphyton's chemical properties were analyzed by standard techniques, as well as an elemental analysis by particle-induced X-ray emission (PIXE), and (2) a greenhouse experiment to compare the effect of periphyton on plant growth and arbuscular mycorrhizal fungi colonization with the effect of a chemical fertilizer was conducted.

MATERIALS AND METHODS

Periphyton samples were collected in three different dry seasons: 1997, 1999, and 2000. Triplicate samples of both materials were collectec. For chemical analysis, samples were dried at 50^{o}C, then milled and sieved with 2 mm mesh for edaphological analysis. For PIXE analysis, materials were milled in an agate mortar, sieved with a 100 mm mesh, and tableted (3 x 5-mm) using a hydraulic press.

Chemical analysis

Soil and periphyton pH (in H_2O, 1:2.5) were determined according to Soil Conservation Service (1984); organic matter (O.M.) by the dichromate method (Walkley 1947); total nitrogen (T.N.) by the Kjeldahl method (Black et al. 1965); total phosphorus (T.P.) by acid digestion (Black et al. 1965); available phosphorus (A.P.) by the Olsen method (Black et al. 1965); potassium (K) by flame photometry, and soluble calcium (Ca) and magnesium (Mg) by atomic absorption spectroscopy (Richards 1985; APHA-AWWA-WPCF 1992).

PIXE elemental analysis

Tablets of periphyton were bombarded with a beam of particle-induced X-ray emission (PIXE) under air conditions (Johansson and Campbell 1988). The bombardment was at 3 MeV using a proton beam pelletron accelerator at Instituto de Física, Universidad Nacional Autónoma de México (IF-UNAM). Two radiation detectors were used: (1) a hyperpure-Ge detector (GL0055P) with a resolution of 150 eV Full-Width-Half-Maximum (FWHM) to 5.9 KeV of the X-ray line of manganese (Mn), and (2) a Si(Li) detector (XR-100CR) made with 250 eV FWHM to 5.9 eV. The detectors were placed 5 cm from the sample. To calibrate the PIXE measurements, a lake sediment was used as a reference material (Corte, Parr, and Clements 1994). The multielemental analysis was obtained by using the Guelph PIXE software package II (Maxwell, Teesdale, and Campbell 1995). The PIXE technique was applied using the Van de Graaff accelerator 5.5 MeV of the IF-UNAM under vacuum conditions. The Si(Li) detector was used, but not under vacuum conditions, from 180 eV resolution to the X-ray line of Mn.

Greenhouse experiments

Soil, classified as Calcaric Phaeozem, was characterized as follows: medium-alkaline pH (7.70); clay loam texture; high-cation exchange capacity (33.0); low content of organic matter (1.16 percent); low total nitrogen of 0.13 percent; and available phosphorus of 2.30 ppm (parts per million). The experiments were carried out using sets of black plastic trays with pots of 50 g capacity. To avoid compaction, we mixed 30 percent silica sand with the soil.

A preliminary test was carried out to evaluate the efficiency of the indigenous arbuscular mycorrhizal fungi (IAMF); in this case, sterilized soil (IAMF eliminated, but native soil bacteria added) was compared with natural soil (nonautoclaved). The light period was eight hours; humidity was about 70 percent of soil-holding capacity; temperature ranged from about 25^{o}C during the day to 10 to 15^{o}C during the night. The experimental plants were tomato (*Lycopersicum esculentum* Mill., variety: “salades”) and maize (*Zea mays* L., variety: “criollo blanco”).

Experimental design

In our final experiment, only natural soil (non-autoclaved) was used. Seven treatments were applied: (1) control, natural soil alone; (2) low dose of periphyton (1.3 g/kg of soil); (3) medium dose of periphyton (1.65 g/kg of soil); (4) high dose of periphyton (2.3 g/kg of soil); (5) low dose of ammonium sulfate (0.26 g/kg of soil; (6) medium dose of ammonium sulfate (0.325 g/kg of soil); and (7) high dose of ammonium sulfate (0.455 g/kg of soil). Variables evaluated included the dry weight of foliage (dried at 45^{o}C until constant weight); root volume (by measuring the displaced volume in a graduated cylinder); and indigenous AMF root-colonization level (staining the roots by the method of Phillips and Hayman 1970, and measuring by the method of Giovannetti and Mosse 1980). Statistical analysis included ANOVA and Tukey tests (Statistica for Windows® Release 4.3 D 1993).

RESULTS AND DISCUSSION

Chemical soil properties

The analysis revealed that all soils, which correspond to lowlands and low and medium high forest near to them, were very rich in organic matter (O.M.), so they have to be considered as organic soils. Values ranged from 17.9 to 26.9 percent (Table 21.1); the lowest value corresponded to Rancho Santa María, and the highest values to two samples of acahual low forest (burned areas ± 25 and ± 10 years old, respectively) in El Edén. Similar values were found by Anaya-Lang, Palacios-Mayorga, and González-Velázquez (1997). These authors also found the highest O.M. contents in soils from burned areas.

In our sites, the exchangeable Ca and Mg were lower than the levels detected by Anaya-Lang, Palacios-Mayorga, and González-Velázquez (1997), but K values were higher; lower pH values were also found. The higher values of total phosphorus (TP) corresponded to burned areas because of the effect of ash (Boyer and Dell 1980), but the highest value (6.29 percent) corresponded to Rancho Santa María, an archaeological area. The highest values of K corresponded to low forest from burned area. The total nitrogen (TN) levels varied from 0.26 to 1.42 percent, which are very high and relate to the richness of OM. These values were also similar to those mentioned by Anaya, Palacios-Mayorga, and González-Velázquez (1997). TP was high and available phosphorus (AP) was very low (Table 21.1) as occurs in calcaric soils (Duchaufour 1984).

Chemical periphyton properties

The chemical analysis (Table 21.2) shows a very rich organomineral complex with a neutral pH in which the exchangeable Ca and Mg were very

TABLE 21.1. Chemical properties of El Edén soils.

Locality	**Site**	**pH**	**O.M.**	**T.N.**	**T.P.**	**A.P.**	**K**	**Ca**	**Mg**
			%				**mg kg^{-1}**		
El Edén Medium high forest	Well Preserved	6.8	22.8	1.42	3.34	0.45	16	61.89	3.23
Acahual Low forest	Burned area (±25 years old)	7	25.5	1.28	3.59	0.35	5	48.90	1.40
Acahual Low forest	Burned area (±10 years old)	7.2	26.9	0.95	4.11	0.20	27.2	58.39	2.32
Rancho Santa María Low forest	Archaeological Area	7	17.9	0.26	6.29	0.35	9.8	37.39	2.39

OM = organic matter; TN = total nitrogen; TP = total phosphorus; AP = available phosphorus high and the K was high; the percent of O.M. was high, similar to an organic horizon of a tropical forest soil. T.N. and T.P. were very high.

According to these results, periphyton are richer in total nitrogen than any other manure or compost used at present time, and similar (and sometimes richer) in total phosphorus than chicken manure (Monroy-Hernádez and Viniegra-González 1990). Periphyton analysis by PIXE indicates a similar pattern of total macronutrients concentration: Ca > P > S > K (Table 21.3). For micronutrients, only Mn and Fe presented a similar pattern concentration (Fe > Mn). Cu and Zn were present (Zn > Cu) in most sites where both were present (Table 21.3 and Figure 21.1); it was also found that most trace elements show a different pattern across the spectrum (Figure 21.1).

Effect of periphyton on foliage dry weight

With this variable, the best response of both crop plants (tomato and maize) was obtained with dose 3 (D3) (Figure 21.2a and 21.2d); foliage dry weight was significantly higher when compared with control (C) and dose 3 (D3) of ammonium sulfate.

Effect of periphyton on root volume

The most significant increase was obtained in tomato with dose 3 (D3) of periphyton, while a smaller effect was observed with the same dose of ammonium sulfate (Figure 21.2b). Nevertheless, a response was observed in maize root volume only with dose 3 (D3) of periphyton, compared with the control (C) (Figure 21.2e).

TABLE 21.2. Chemical properties of periphyton from El Edén.

Periphyton Type	**pH**	**O.M.**	**T.N.**	**T.P.**	**A.P.**	**K**	**Ca**	**Mg**
			%				**mg kg^{-1}**	
Ordinary	7	21.8	1.22	1.43	0.35	60.0	59.77	3.10
Rich in Chara	6.6	33.8	1.10	4.89	0.10	35.4	67.01	2.68

OM = organic matter; TN = total nitrogen; TP = total phosphorus; AP = available phosphorus

TABLE 21.3. Elemental analysis of periphyton samples by PIXE [mg kg^{-1}].

	Periphyton 1997							**Periphyton 1999**		**Periphyton 2000**	
Element	1	2	3	4	5	6	7	1	2	1	2
P	83.5	63.5	35.9	84.6	84.3	84.9	72.9	87.4	82.3	54.5	59
S	23.6	22.6	20.3	22	22.5	23.1	17.4	24.4	27.9	5.8	9.5
Cl	3.1	6.6	5.3	5.7	4.5	12.2	4.5	6.8	1.9	2	5.6
K	1.2	127	4	9.1	7	7.5	41.3	4.2	3.3	10	15.1
Ca	498.8	402.6	212.5	433.5	426.1	453.8	425.2	497.2	503.2	598.3	580.1
Ti	281	66	3.3	⊗	⊗	37	⊗	32	36	⊗	⊗
V	66	61	93	47	31	⊗	⊗	⊗	52	⊗	428
Cr	56	61	177	58	⊗	⊗	⊗	⊗	42	533	278
Mn	258	488	2.1	570	472	275	602	516	616	1.4	635
Fe	4.9	1.4	41.3	1.3	1	809	1.5	1.5	2.4	2.8	2.1
Co	⊗	⊗	256	⊗	⊗	⊗	⊗	⊗	⊗	2	4
Ni	203	⊗	45	557	372	220	149	93	83	⊗	⊗
Cu	⊗	16	49	19	⊗	⊗	⊗	⊗	⊗	3	2
Zn	64	36	84	71	21	44	⊗	⊗	⊗	2	1
Ga	⊗	56	64	43	55	⊗	⊗	⊗	⊗	⊗	⊗
As	⊗	⊗	20	⊗	42	⊗	⊗	⊗	⊗	⊗	⊗
Br	100	⊗	176	⊗	56	⊗	⊗	⊗	⊗	6	9
Rb	243	⊗	211	83	⊗	⊗	⊗	⊗	⊗	2	3
Sr	⊗	⊗	605	⊗	⊗	⊗	200	⊗	⊗	49	50
Y	⊗	⊗	⊗	⊗	⊗	1.2	⊗	697	⊗	⊗	⊗

TABLE 21.3. (continued)

	Periphyton 1997							Periphyton 1999		Periphyton 2000	
Element	1	2	3	4	5	6	7	1	2	1	2
Zr	⊗	⊗	465	⊗	290	100	⊗	⊗	⊗	⊗	⊗
Ba	327	⊗	⊗	⊗	43	⊗	⊗	⊗	200	7	18
Pb	⊗	⊗	⊗	166	⊗	⊗	181	⊗	⊗	⊗	⊗

⊗ = out of minimum detectable level (MDL).

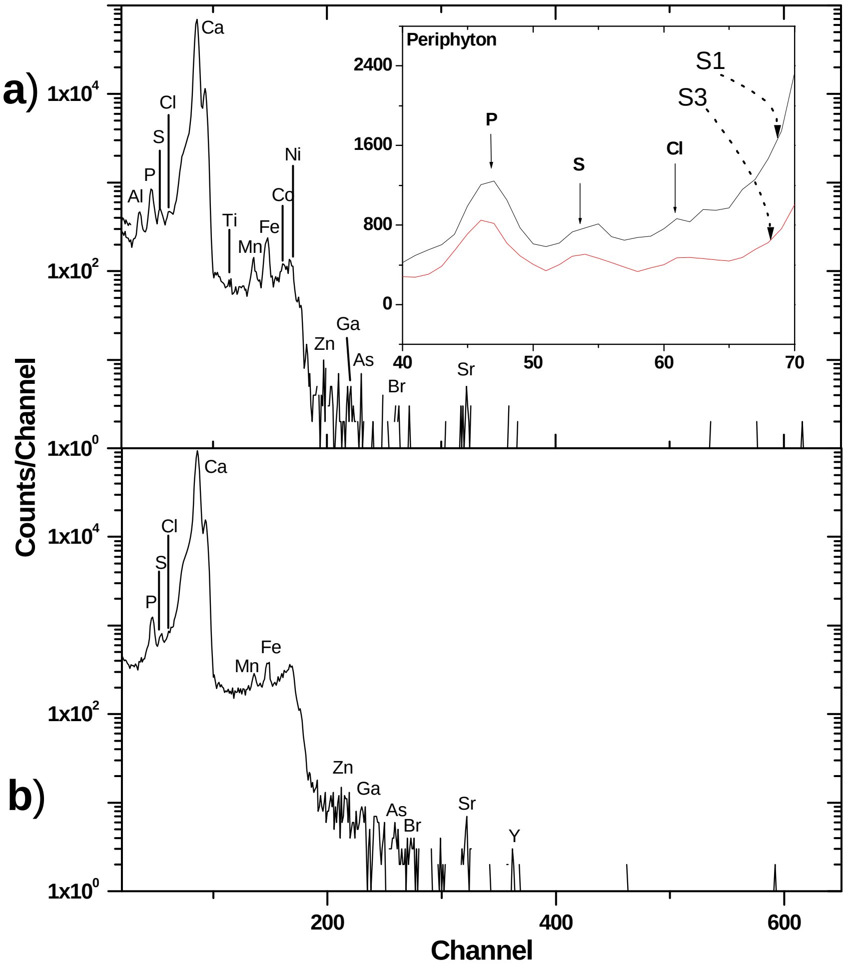

FIGURE 21.1. Comparison of the PIXE spectra of two samples of periphyton: S3 (site 3, 1997) and S1 (site 1, 1999). S3 has a higher phosphorus (P) content (amplification square, Figure 1a); nevertheless, a fingerprint of P, S, Cl, and Ca is observed among sites (Figures 1a and 1b).

Effect of periphyton on indigenous AMF root-colonization level

A significant positive effect was observed in tomato with all doses of both periphyton and ammonium sulfate (Figure 21.2c); in the case of maize, a similar effect was detected with dose 1 (D1) and dose 3 (D3) of periphyton, and only with dose 2 (D2) of ammonium sulfate (Figure 21.2F). The negative effect of ammonium sulfate on indigenous AMF reported by some authors (Hayman 1987; Chambers, Smith, and Smith 1980) is still not clear; in this context, our results could be controversial, due to the fact that we detected a positive effect with ammonium sulfate as well as with periphyton. However, the effects can vary from one soil site to another and may depend on the availability of phosphorus (Sieverding 1991).

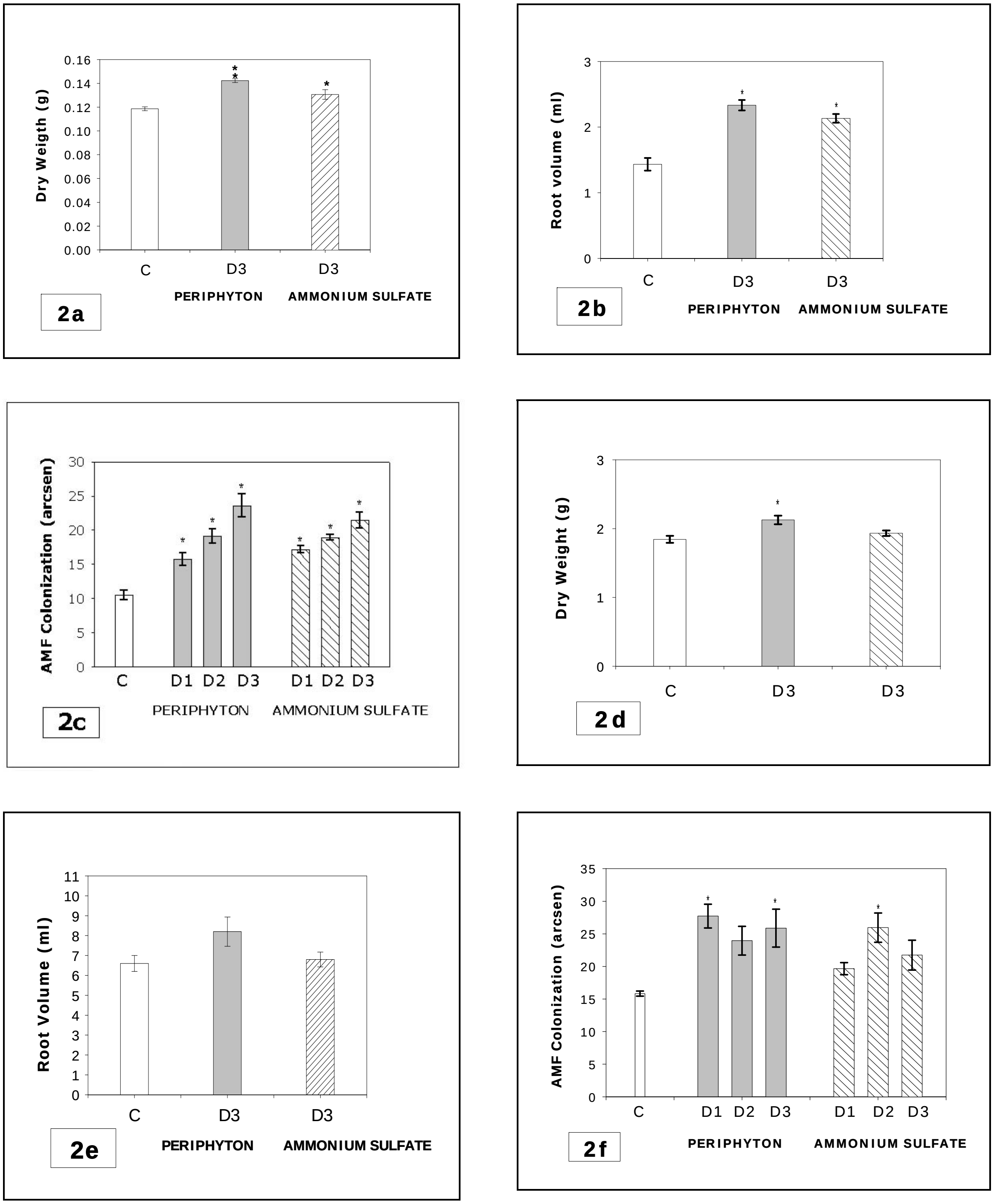

Figure 21.2. Greenhouse experiments. Effect of periphyton and ammonium sulfate on tomato foliage dry weight (2a); on tomato root volume (2b); on indigenous AMF colonization of tomato (2c); on maize foliage dry weight (2d); on maize root volume (2e); on indigenous AMF colonization of maize (2f). C = control, D1 = (low), D2 = (medium), D3 = (high). * = Significant differences with respect to control, $p < 0.05$. ** = Significant differences with respect to homologous dose, $p < 0.01$.

CONCLUSIONS

A number of important conclusions can be drawn from our research. First, the chemical analysis of periphyton indicated that this biological complex could be used as a natural source of two of most important macronutrients—nitrogen and phosphorus. Thus, periphyton are as good as (or better than) modern organic fertilizers. Second, PIXE analysis detected that periphyton have a complex chemical composition. Therefore, periphyton play a very important role in biogeochemical cycling because they act as a natural source of micronutrients in the ecosystem.

Third, periphyton are a biological complex that absorb and concentrate macronutrients and micronutrients; therefore, they could also be useful to detect the natural distribution of some trace elements in wetland ecosystems. According to these properties, PIXE analysis could be a useful technique to obtain an elemental "fingerprint" of different types of periphyton, plants, soils, and sediments.

Fourth, our greenhouse experiments showed that most of the periphyton doses applied to plants produced a positive effect on plant growth, which was equivalent (and sometimes superior) to the effect of ammonium sulfate. Finally, periphyton improved indigenous arbuscular mycorrhizal symbiosis.

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