

Populations and potential activities of methanogens and methanotrophs in rice fields: relations with soil properties

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Abstract

The abundance of cultivable methanotrophs and methanogens, and the potential methanogenic (PMGA) and methanotrophic (PMTA) activities were estimated in 22 rice field soils. These measurements were then related to major soil physico-chemical properties. Methanogens ranged from less than 10^1 g⁻¹ d.w. to about 10^6 g⁻¹ d.w. on H₂/CO₂ and formate, 10^5 g⁻¹ d.w. on methanol, and 10^4 g⁻¹ d.w. on acetate. Methanotrophs ranged from 10^3 to 10^7 g⁻¹ d.w. and were 4– 10^4 times more abundant than methanogens in most soils. Average PMGA over six weeks ranged from 0 to 0.14 mol m⁻² d⁻¹ in control soils and from 0.21 to 0.79 mol m⁻² d⁻¹ in soils enriched with straw. PMTA ranged from 0 to 1.8 mol m⁻² d⁻¹. Correlation studies and principal component analysis indicated that (1) methanogenic or methanotrophic abundance in a dry soil did not reflect its potential to produce or oxidize CH₄ and that activities were mostly substrate dependent, (2) methanogenesis and methanotrophy were positively correlated in terms of populations but not in terms of activities, (3) PMTA was higher than PMGA in most soils, (4) the PMTA/PMGA ratio was mostly governed by PMTA, and (5) soils prone to methanotrophy were above neutrality, rich in available P and with lower clay content.

Keywords: Methanogens, methanogenesis, methanotrophs, methanotrophy, rice, rice field, soil.

Populations et activités potentielles méthanogènes et méthanotrophes dans les sols de rizière: relations avec les propriétés des sols.

Résumé

L'abondance des populations et les activités potentielles méthanogènes (PMGA) et méthanotrophes (PMTA) ont été estimées dans 22 sols de rizière provenant de cinq pays. Les relations avec les propriétés physico-chimiques des sols ont été étudiées. Les densités de méthanogènes vont de $< 10^1$ g⁻¹ p.s. à 10^6 g⁻¹ p.s. sur H₂/CO₂ et formate, 10^5 g⁻¹ p.s. sur méthanol, et 10^4 g⁻¹ p.s. sur acétate. Les densités de méthanotrophes vont de 10^3 à 10^7 g⁻¹ p.s. et sont de 4 à 10^4 fois supérieures aux densités de méthanogènes dans la plupart des sols. Les valeurs de PMGA moyenne sur six semaines vont de 0 à 0,14 mol m⁻² j⁻¹ dans les sols témoins et de 0,21 à 0,79 mol m⁻² j⁻¹ dans les sols enrichis en paille. Les valeurs de PMTA vont de 0 à 1,8 mol m⁻² j⁻¹. Les études de corrélations et l'analyse en composantes principales montrent que (1) l'abondance des méthanogènes ou des méthanotrophes estimée dans un sol sec ne reflète pas son potentiel à produire ou oxyder le méthane et que ces activités dépendent principalement de la disponibilité du substrat, (2) la méthanogénèse et la méthanotrophie sont corrélées positivement en termes de populations mais pas en termes d'activités, (3) la PMTA est supérieure à la PMGA dans la majorité des sols, (4) la valeur du rapport PMTA/PMGA dépend principalement de la PMTA, et (5) les sols favorables à la méthanotrophie ont un pH au dessus de la neutralité, une teneur élevée en P assimilable et une faible teneur en argile.

Mots-clés : Méthanogènes, méthanogénèse, méthanotrophes, méthanotrophie, riz, rizières, sol.



INTRODUCTION

Methane (CH₄) has a high potential for absorbing infrared radiation and is therefore one of the major gases involved in the greenhouse effect. Because of increasing anthropogenic activities, CH₄ concentration in the atmosphere has increased annually by about 1.0–0.8% during the last decades (Steele *et al.*, 1992). The possible effects of this increase on global warming have been widely presented in scientific reviews (Zepp, 1994) and popularization articles.

Waterlogged rice fields are considered as the first or the second anthropogenic source of atmospheric CH₄ (Sass, 1994). The demand for rice will increase by 65% over the next 30 years (IRRI, 1989) and global CH₄ emissions from wetland rice agriculture are likely to increase if cultural practices that will improve rice yield while reducing CH₄ emission from rice field soils are not established.

Methane emission from rice fields results from (1) production by methanogenic bacteria in reduced soil, (2) consumption by methanotrophic bacteria in the oxic zones of the ecosystem (submersion water, water/soil interface, rice rhizosphere—including the inner part of the roots—, and culms) where up to 90% of CH₄ produced is reoxidized, and (3) transfer processes (diffusion, ebullition) through the soil and the rice plant (Schütz *et al.*, 1989). A number of recent studies have identified factors implied in CH₄ emission from rice fields, including soil type, soil pH, soil temperature, and OM incorporation (Lindau *et al.*, 1993). It has been shown that CH₄ emission from rice fields can be reduced by introducing temporary drainage periods during the crop cycle (Sass *et al.*, 1992; Watanabe A. *et al.*, 1995), and by using mineral fertilizers rather than organic and using selected rice varieties (Neue *et al.*, 1996).

The presence of methanogenic and methanotrophic bacteria in rice soils has been demonstrated indirectly by many authors who measured CH₄ production and oxidation, but microorganisms involved are still very poorly characterized, both qualitatively and quantitatively.

Methanogens are strict anaerobic Archaea. They constitute the last step in the electron transfer chain generated by the anaerobic degradation of organic matter (OM) (Garcia, 1990). They use a restricted range of substrates produced during this degradation: H₂/CO₂, acetic acid, formate, methyl compounds like methanol and trimethylamine, and some secondary alcohols. Acetate and H₂/CO₂ are the two major substrates for methanogenesis in waterlogged ricefields (Takai, 1970; Schütz *et al.*, 1989); they originate from (1) rice residues, algae and aquatic plants incorporated into the soil, (2) soil humus, and (3) autolysis products and exudates of the rice roots (Neue & Roger, 1994). Twenty genera of methanogens have currently been described, but only a few studies report the isolation of methanogens from rice fields.

Four genera, *Methanobacterium*, *Methanosarcina*, *Methanobrevibacter* and *Methanoculleus* have been isolated (Raimbault, 1981; Rajagopal *et al.*, 1988; Asakawa *et al.*, 1993; Fetzer *et al.*, 1993; Jouliau *et al.*, 1996 and in press).

Methanotrophs are a subset of the physiological group of methylotrophs, which utilize a variety of one-C compounds (Hanson & Hanson, 1996). Two types of CH₄ oxidation in soils are known (Bender & Conrad, 1992). The first type (high-affinity activity) is observed at atmospheric CH₄ concentration (≤ 12 ppm) and is apparently ubiquitous in soils. The second type (low-affinity activity) occurs at CH₄ concentrations higher than 40 ppm and is performed by the bacteria called methanotrophs (Whalen *et al.*, 1990). Methane concentration in the soil water of submerged rice soils (110 ppm v/v according to Conrad & Rothfuss, 1991) and in the soil atmosphere when the soil is being drained, is often likely to be significantly higher than the threshold of 11–45 ppm (v/v) CH₄ established by Bender & Conrad (1992) for the methanotrophic activity *sensu stricto*. Therefore, CH₄ oxidation in rice fields is mostly of the "low affinity" type. Thus far, only two strains of methanotrophs have been isolated from rice fields: *Methylosinus sporium* (Bowman *et al.*, 1993) and *Methylocystis* sp. (Takeda, 1988). Quantitative estimates are also scarce. This is probably due to the difficulties encountered in enumerating and isolating methanotrophs (Escoffier *et al.*, 1997).

This paper presents the results of a study where we estimated the abundance of cultivable methanotrophs and major trophic groups of methanogens and the potential methanogenic and methanotrophic activities in 22 rice fields soils. These measurements were then related to major soil physico-chemical properties.

MATERIALS AND METHODS

Soils

Soils were collected from 22 rice fields in five countries (Australia, France, Philippines, U.S.A., Trinidad), constituting a sample with a broad range of physico-chemical properties (table 1). Soil analyses were performed by the Analytical Services Laboratories of IRRI, according to standardized procedures. Soils were collected at the end of the crop cycle as a composite sample, air dried at ambient temperature as large clods, and stored at room temperature. Before use, dry soils were crushed and passed through a 5 mm sieve.

Measurement of potential activities

The potential methanogenic activity (PMGA) was measured on duplicated samples of 15 g of dry soil, amended or not with 1% of rice straw, with 20 ml of anaerobic distilled water in 120 ml flasks under an N₂ atmosphere. Flasks were incubated

Table 1. - Ranges of physico-chemical properties of 21 of the 22 rice field soils*

Soils properties		Mean	Median	Maximum	Minimum	Mean for Asian soils**
pH		6.1	6.1	7.9	3.8	6
Organic C	%	1.6	1.5	3.7	0.8	1.4
Total N	%	0.2	0.2	0.4	0.1	0.1
Available P	ppm	10.4	5.6	45	0.2	3.8
Available Zn	ppm	1.7	0.9	13	0	n.a.
Active Fe	%	1.7	1.3	7.2	0.3	n.a.
Activr Mn	%	0.06	0.04	0.2	0.001	n.a.
Clay	%	39	35	76	19	38
Silt	%	44	63	22	28	28
Sand	%	15	11	39	2	34

* The peat soil, because of its unusual properties (C, 15.7%; N, 1.5%; available P, 102 ppm; clay, 17%), is not included.

** Data from Kawaguchi & Kyuma, 1977. n.a., not available.

at 37°C (optimum for most methanogens) for six weeks. Methane produced was measured weekly after shaking to release gas entrapped in the soil. Flask atmosphere was replaced by an N₂ atmosphere after each measurement. Results were expressed in mol m⁻² day⁻¹ (extrapolated on the basis of 1200 t of rice soil ha⁻¹) as (1) average activity over six weeks and (2) maximum activity recorded during the six weeks of incubation.

The potential methanotrophic activity (PMTA) was estimated by incubating soil samples under a mixture of air/CH₄ (80/20, v/v). This concentration, which is most frequently used (Bender & Conrad, 1992; Megraw & Knowles, 1987), is adequate to enhance the "low affinity" methanotrophy occurring in rice fields, avoid frequent replacement of the gas phase in the incubation flasks, and avoid the hazard of an explosive mixture of CH₄ in air (between 5 and 15%). Incubations were performed using 50 g of soil of water holding capacity in 600 ml cylindrical flasks (bottom area 50 cm²) bearing a lateral tubing closed with a butyl rubber stopper. The flask atmosphere was replaced daily and analysed after each replacement. Flasks were incubated at 30°C, which is optimal for most methanotrophs (Whalen *et al.*, 1990).

MPN counts of methanogenic and methanotrophic populations

The composition and anaerobic preparation of the media used for methanogenic counts were described by Jouliau *et al.* (1996). Selective media for enumeration were prepared by adding to a common enriched medium one of the following methanogenic substrates: formate (40 mM), methanol (40 mM), or acetate (20 mM) (sterile and anaerobic stock solution) and H₂/CO₂ (80/20 v/v, 2 bars) which was directly injected into the gas phase.

The medium used for methanotrophic counts was derived from the original salt medium NMS of Whittenbury *et al.* (1970) by using 0.04 g l⁻¹ EDTA-Ferric-Sodium salt as iron source instead of the

Sequestrene iron complex, as suggested by Henry & Grbic-Galic (1991). The pH of the medium was adjusted to 6.8 before autoclaving at 120°C for 20 min. Two ml of sterile phosphate buffer solution (mixture of Na₂HPO₄ and KH₂PO₄, 15 g in 300 ml distilled water) were added per litre of cooled medium, after autoclaving.

We used the most probable number (MPN) method with triplicate tubes to estimate the methanotrophic and methanogenic populations. The serial 1/10 suspension-dilutions were prepared from a 10⁻¹ dilution obtained by stirring 10 g of dry soil in 90 ml of sterile water containing 9 g NaCl l⁻¹ (under anaerobic conditions for methanogens) for one hour. MPN tubes were inoculated with 10% of suspension-dilution.

The major trophic groups of methanogens were counted in duplicate using dilutions from two soil subsamples and four selective media. Anaerobic techniques (Hungate, 1969; Macy *et al.*, 1972) were used throughout the experiments. Methanogenic growth was assayed by measuring CH₄ produced after 8 weeks of incubation at 37°C. Inoculated tubes containing medium with no substrate added served as control. A tube was considered positive when CH₄ produced was at least 5% higher than in the control.

Methanotrophs were counted in triplicate using dilutions from three soil subsamples. Tubes were flushed with an air/CH₄ (80/20, v/v) mixture and incubated at 30°C for 8 weeks. A tube was considered positive when more than 20% of the CH₄ initially present was oxidized. The method was discussed by Escoffier *et al.* (1997).

Gas analysis

Methane production was measured with a flame ionization detector gas chromatograph (Girdel, série 30, column SP 1000 with chromosorb WAW and 1% H₃PO₄) with N₂ as carrier gas. Temperatures were as follows: injector, 200°C; column, 150°C; detector, 200°C.

Methane and O₂ consumption, and CO₂ production were measured using a gas chromatograph (Chrompack CP 9000) equipped with two thermal conductivity detectors and a double column set up allowing to simultaneously analyse O₂, N₂, CH₄, and CO₂. The first column (1.5 m × 2 mm) was packed with silicagel 60-80 mesh, the second column (1.5 m × 2 mm) was packed with a molecular sieve 5Å 60-80 mesh. An empty tubing (1.5 m × 2 mm) inserted between the columns improved the separation of CO₂. Carrier gas was helium (15 ml min⁻¹). Temperatures were as follows: injector, 50 °C; columns, 50 °C; detectors, 150 °C.

Statistical analysis

Data were analysed with (1) functions implemented in Microsoft Excel for routine analysis and cross-correlations, and (2) ADE4 (Thioulouse *et al.*, 1997) for principal component analysis, cluster calculation and dendrograms to define grouping of the projections of the points. Dendrograms were constructed using Ward's method of pooling. In the results section, groups are presented according to the hierarchy of the dendrogram. Replicated counts of methanotrophs (Escoffier *et al.*, 1997) and methanogens (data not shown) showed a log-normal distribution of the data. Therefore, analysis were performed on the logarithms of the counts. Activities exhibiting a coefficient of variation >80% were transformed ($y = \log_{10}(x + 1)$) before analysis. Correlations were considered "highly significant" for $p < 0.01$; "significant" for $0.01 < p < 0.05$ and "weak" for $0.05 < p < 0.10$.

RESULTS AND DISCUSSION

Physico-chemical properties of the soils

Analysis showed that the peat soil (soil 22) markedly differed from other soils because of its very high content in OM (organic C, 15.7%; total N, 1.5%) but also in available P (102 ppm). Peat soils can be used for rice cultivation, but represent only a few percent of the global area planted to rice. When the peat soil was not included in the statistical analysis, the low difference between means and medians of the data indicated a regular distribution of most physico-chemical properties of the soils (table 1). Average values were quite close to those reported by Kawaguchi & Kyuma (1977) for 410 rice field soils from South East Asia (table 1).

Correlations observed between the physico-chemical properties of the soils (table 2, area 1,1; 10,10) agreed with reports on rice soil properties from the literature. A highly significant positive correlation between organic C and total N is usually observed in rice soils which rarely exhibit unusual C/N values. It is known that a negative correlation between available

Zn and pH results from the increase of Zn adsorption with soil pH (De Datta, 1981). A negative correlation between soil pH and active Fe is common in wetland soils (Roger *et al.*, 1995) where ferrous iron decreases by 100 times when pH increases from 6.25 to 7.25 (De Datta, 1981). The negative correlation between clay and sand/silt contents resulted from the high clay content of ricefield soils. When the peat soil was included in the analysis, an unusual correlation between soil OM (C and N) and available P was observed (data not shown). This was obviously due to the unusually high available P content of this soil (102 ppm; average of asian rice soils, 10.4 ppm).

Principal component analysis of the 10 physico-chemical properties of the soils was performed on the three first axis representing about 71% of the total variability. When the peat soil was included in the analysis (fig. not shown), 18 of the 22 soils were aggregated within a centred narrow ellipsoidal cloud oriented according to the "pH - clay" axis. An excentered group comprised of three acidic soils was characterized by a high content of available Zn and active Fe. The peat soil was strongly isolated by its very high C, N and available P content. When the peat soil was not included in the analysis, available P was not correlated with soil organic C, and a more detailed discrimination of soils allowed one to distinguish four groups (fig. 1): a group of seven clay soils comprised of two subgroups distinguished by their active Fe and available Zn content, a group of 12 soils richer in silt and sand (contribution to the hierarchy node [c.t.n.]: 78%), and two soils with higher contents in OM and available P.

The 22 soils used for the study constituted a representative sample of rice field soils comprised of 19 "average" soils mostly discriminated by their texture and their content in available Zn, two alkaline soils rich in available P and OM, and one peat soil, with high OM content but also unusually rich in available P. Because of its physico-chemical characteristics, correlation studies and principal component analysis were performed with and without including the peat soil. We present in tables and figures the results of the analysis performed without the peat soil. When noticeable differences were observed by including the peat soil in the analysis, we indicate it.

Methanogenic and methanotrophic populations

Counts

Populations of methanogens, counted on the four selective substrates, ranged from a minimal value <10¹ bacteria g⁻¹ soil d.w. independent of the substrate, to a maximum value of about 10⁶ on H₂/CO₂ and formate, 10⁵ on methanol, and 10⁴ on acetate (table 3). Hydrogenotrophs were dominant in 13 of the 22 studied soils. Hydrogenotrophs

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Table 2. - Correlations between physico-chemical and microbiological properties of the soils.

		pH	C	N	P	Zn	Fe	Mn	Clay	Silt	Sand	Hydro.	Form.	Methyl.	Aceto.	15	16	17	18	19	20	
1	pH																					
2	Organic C %													+								+
3	Total N %													+								+
4	Available P ppm													+								+
5	Available Zn ppm																					
6	Active Fe %																					
7	Active Mn %																					
8	Clay %																					
9	Silt %																					
10	Sand %																					
11	Hydrogenotrophs nb. g ⁻¹ d.w.		+	+				+														
12	Formatotrophs nb. g ⁻¹ d.w.																					
13	Methylotrophs nb. g ⁻¹ d.w.																					
14	Acetotrophs nb. g ⁻¹ d.w.																					
15	Max. PMGA (control) mol m ⁻² d ⁻¹																					+
16	Max. PMGA (+straw) mol m ⁻² d ⁻¹																					+
17	Av. PMGA (control) mol m ⁻² d ⁻¹																					+
18	Av. PMGA (+straw) mol m ⁻² d ⁻¹																					+
19	Methanotrophs mol m ⁻² d ⁻¹												+	+	+						+	
20	PMTA mol m ⁻² d ⁻¹																					

p < 0.01
 0.01 < p < 0.05
 0.05 < p < 0.10

Peat soil included

Peat soil not included

and formatotrophs were mostly rods belonging to the genus *Methanobacterium*; acetotrophs and methylotrophs were mostly sarcinae belonging to the genus *Methanosarcina* (Joulian *et al.*, in press). Recorded values are similar to those reported in 29 Senegalese rice field soils by Garcia *et al.* (1974). In an Italian rice field, Schütz *et al.* (1989) and Mayer & Conrad (1990) counted 10⁴-10⁵ acetotrophs and 10⁵-10⁶ hydrogenotrophs g⁻¹ soil d.w.

Populations of methanotrophs ranged from 10³ to 10⁷ bacteria g⁻¹ soil d.w. (table 3). Data available for comparison are 10⁴ methanotrophs g⁻¹ d.w. in a Japanese rice field soil (Watanabe I. *et al.*, 1995), and 4 x 10⁶ methanotrophs g⁻¹ d.w. in an Italian rice field soil (Bender & Conrad, 1992).

Population of methanotrophs were more abundant (4-10⁴ times) than populations of methanogens in most soils. Only four soils (Camargue, Pila, Balagbag, and Capenang) exhibited populations of methanogens and methanotrophs of the same order of magnitude (table 3). These soils did not have specific common physico-chemical properties.

Indeed, as the methods of enumeration only recorded culturable organisms, only part of the bacteria of both groups were probably recorded, which may

have weakened some of the correlations, especially those obtained with potential activities.

The study of the correlations between populations (table 2, area 11,11; 14,14) showed highly significant positive correlations between the four trophic groups of methanogens, indicating that their relative abundance varied in the same way among soils, without implying proportionality, and that all trophic groups were represented in soils rich in methanogens.

Methanotrophs were positively correlated with methanogens. The correlation was significant with acetotrophs and methylotrophs and weak with hydrogenotrophs and formatotrophs (table 2, area 11,19; 14,19). Such correlations obviously resulted from the fact that the first group produces the substrate used by the second. However methanotrophs were usually more abundant than methanogens. The difference was especially marked in soils from California and Australia, with a ratio of 10³-10⁴. In rice fields from both areas, a single annual crop is performed and fields are submitted to a long dry fallow (instead of the double cropping often performed in South East Asia). The dry fallow causes soil cracking and oxygenation of the deeper soil, which is favourable to aerobic methanotrophs and detrimental to anaerobic methanogens.

Table 3. – Methanogenic and methanotrophic populations (cells g⁻¹ d.w.) and potential activities (mol m⁻² d⁻¹) of the 22 rice field soils.

Soils	Methanogenesis						Methanotrophy		PMTA/av.PMGA			
	Counts on				Maximum activity	Average activity		Counts	Activity	Control + straw		
	H ₂ /CO ₂	Formate	Methanol	Acetate	Control + straw	Control + straw						
1 Camargue	9.6 × 10 ³	1.8 × 10 ³	2.5 × 10 ³	5.0 × 10 ³	0.13	0.90	0.06	0.42	1.6 × 10 ⁴	1.80	31.5	4.3
2 Maahas	7.0 × 10 ⁴	1.0 × 10 ³	2.9 × 10 ²	1.3 × 10 ²	0.06	0.67	0.04	0.36	2.0 × 10 ⁶	0.19	5.3	0.5
3 Pila	1.3 × 10 ⁵	7.0 × 10 ³	1.8 × 10 ²	5.0 × 10 ²	0.07	0.73	0.05	0.38	1.1 × 10 ⁵	0.91	19.7	2.4
4 Luisiana	1.9 × 10 ³	9.0 × 10 ³	5.0 × 10 ¹	1.2 × 10 ²	0.38	0.84	0.14	0.46	5.5 × 10 ⁴	0.10	0.7	0.2
5 Urdaneta	7.3 × 10 ²	6.0 × 10 ¹	6.5 × 10 ¹	1.3 × 10 ²	0.12	0.62	0.06	0.29	1.4 × 10 ⁵	0.66	11.5	2.3
6 Binalonan	4.2 × 10 ³	2.1 × 10 ²	1.2 × 10 ²	2.4 × 10 ²	0.10	0.59	0.04	0.32	3.2 × 10 ⁵	0.33	7.9	1.0
7 Bugallon	3.0 × 10 ²	6.7 × 10 ¹	2.0 × 10 ¹	2.7 × 10 ¹	0.12	0.61	0.07	0.33	7.2 × 10 ⁵	0.44	6.0	1.3
8 San Dionisio	1.3 × 10 ²	6.0 × 10 ¹	2.0 × 10 ²	< 10 ¹	0.24	0.62	0.13	0.26	9.0 × 10 ³	1.12	8.8	4.4
9 Amurao	5.7 × 10 ²	1.1 × 10 ³	2.7 × 10 ²	2.3 × 10 ²	0.11	0.54	0.07	0.37	2.1 × 10 ⁵	0.35	4.9	1.0
10 Lal-lo	2.3 × 10 ²	5.3 × 10 ¹	< 10 ¹	4.7 × 10 ¹	0.03	0.31	0.02	0.21	2.5 × 10 ⁴	0.10	5.8	0.5
11 Maligaya	1.8 × 10 ⁴	4.3 × 10 ²	2.3 × 10 ²	1.8 × 10 ²	0.02	0.38	0.00	0.26	1.1 × 10 ⁵	0.22	48.6	0.8
12 Trinidad	7.9 × 10 ³	n.d.	2.9 × 10 ²	8.0 × 10 ¹	0.02	0.63	0.00	0.36	6.2 × 10 ⁵	0.90	185.6	2.5
13 Australia	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	0.11	0.89	0.06	0.42	5.5 × 10 ⁴	0.90	6.4	0.8
14 California 3	1.0 × 10 ²	1.3 × 10 ²	2.8 × 10 ²	1.8 × 10 ²	0.00	0.71	0.00	0.37	6.3 × 10 ⁶	0.48	∞	1.3
15 California 2	1.2 × 10 ³	1.0 × 10 ³	7.0 × 10 ²	< 10 ¹	0.00	0.67	0.00	0.34	6.0 × 10 ⁶	0.34	∞	1.0
16 Tiaong	2.3 × 10 ⁶	1.6 × 10 ⁶	1.9 × 10 ⁵	2.2 × 10 ⁴	0.21	0.71	0.15	0.44	3.5 × 10 ⁷	0.84	5.7	1.9
17 Balagbag	5.5 × 10 ⁵	5.0 × 10 ²	6.0 × 10 ²	1.2 × 10 ³	0.06	0.58	0.03	0.33	7.2 × 10 ⁵	0.72	22.8	2.2
18 Capenang	4.9 × 10 ³	3.1 × 10 ³	6.5 × 10 ²	< 10 ¹	0.00	0.51	0.00	0.26	1.5 × 10 ³	0.10	∞	0.4
19 Bay Clay	4.5 × 10 ⁴	3.6 × 10 ²	5.0 × 10 ²	1.5 × 10 ²	0.24	0.66	0.13	0.38	5.5 × 10 ⁵	0.28	2.1	0.7
20 Pili	1.5 × 10 ⁴	3.0 × 10 ¹	< 10 ¹	< 10 ¹	0.10	0.60	0.04	0.33	5.5 × 10 ⁴	0.27	7.2	0.8
21 Maahas alkaline	2.1 × 10 ⁵	n.d.	6.0 × 10 ⁵	1.5 × 10 ⁴	0.00	0.81	0.00	0.31	9.5 × 10 ⁵	0.17	∞	0.6
22 Peat	7.0 × 10 ⁴	8.0 × 10 ¹	2.1 × 10 ⁴	1.6 × 10 ²	0.87	1.33	0.49	0.79	3.7 × 10 ⁶	1.22	2.5	1.6
Minimum	< 10 ¹	< 10 ¹	< 10 ¹	< 10 ¹	0	0.31	0	0.21	1.5 × 10 ³	0.10	0.7	0.2
Maximum	2.3 × 10 ⁶	1.6 × 10 ⁶	6.0 × 10 ⁵	2.2 × 10 ⁴	0.87	1.33	0.49	0.79	3.5 × 10 ⁷	1.80	185.6	4.4
Mean	1.6 × 10 ⁵	8.2 × 10 ⁴	3.7 × 10 ⁴	2.0 × 10 ³	0.13	0.68	0.07	0.36	2.6 × 10 ⁶	0.54	21.3	1.5
Median	6.4 × 10 ³	3.4 × 10 ²	2.9 × 10 ²	1.4 × 10 ²	0.10	0.64	0.04	0.35	2.7 × 10 ⁵	0.35	6.8	1.0

av.: average; n.d.: not determined.

Potential methanogenic and methanotrophic activities

Average PMGA (estimated over six weeks after soil submersion) ranged from 0 to 0.14 mol m⁻² d⁻¹ (table 3). Maximal values were observed any time from the first to the sixth week of incubation, depending upon soil, and ranged from 0 to 0.87 mol m⁻² d⁻¹. Rice straw incorporation very markedly increased CH₄ production: average PMGA ranged from 0.21 to 0.79 mol m⁻² d⁻¹ and maximum PMGA ranged from 0.31 to 1.33 mol m⁻² d⁻¹. The peat soil exhibited the highest PMGA. The variations between PMGA among soils (maximal and average) were much higher for controls (coefficient of variation higher than 100%) than for soils where straw was added (coefficient of variation around 30%). This may indicate that methanogenesis in rice soils probably depends more upon substrate availability than the methanogenic microflora.

PMTA ranged from 0 to 1.8 mol m⁻² d⁻¹ (table 3). They were higher than PMGA in most control soils (no straw added) where the ratio between both activities ranged from 2 to 190 (median: 8). In soils with straw added, PMTA was equal to or higher than PMGA in 11 of the 22 soils tested.

Average and maximum PMGA were highly positively correlated, in both control and straw enriched soils (table 2, area 15,15; 18,18). Activities in controls were positively correlated with those in soils enriched with straw. These correlations were significant when the peat soil was or was not included in the analysis. No correlation was found between PMGA and PMTA (table 2, area 15,20; 18,20) except when the peat soil was included in the analysis (table 2, area 20,15; 20,18).

The results indicated that PMTA was frequently higher than PMGA. Their interpretation and extrapolation must however take into account that: (1) the measurements of potential activities were performed under conditions that cannot occur simultaneously in a rice field (anaerobiosis vs. field capacity), (2) soil enrichment with 1% rice straw corresponds to a maximum level of straw incorporation unusual in rice fields.

Relations between populations and activities

PMTA was independent of the density of methanotrophs (table 2, area 19,20). PMGA of control soils was usually independent of the density

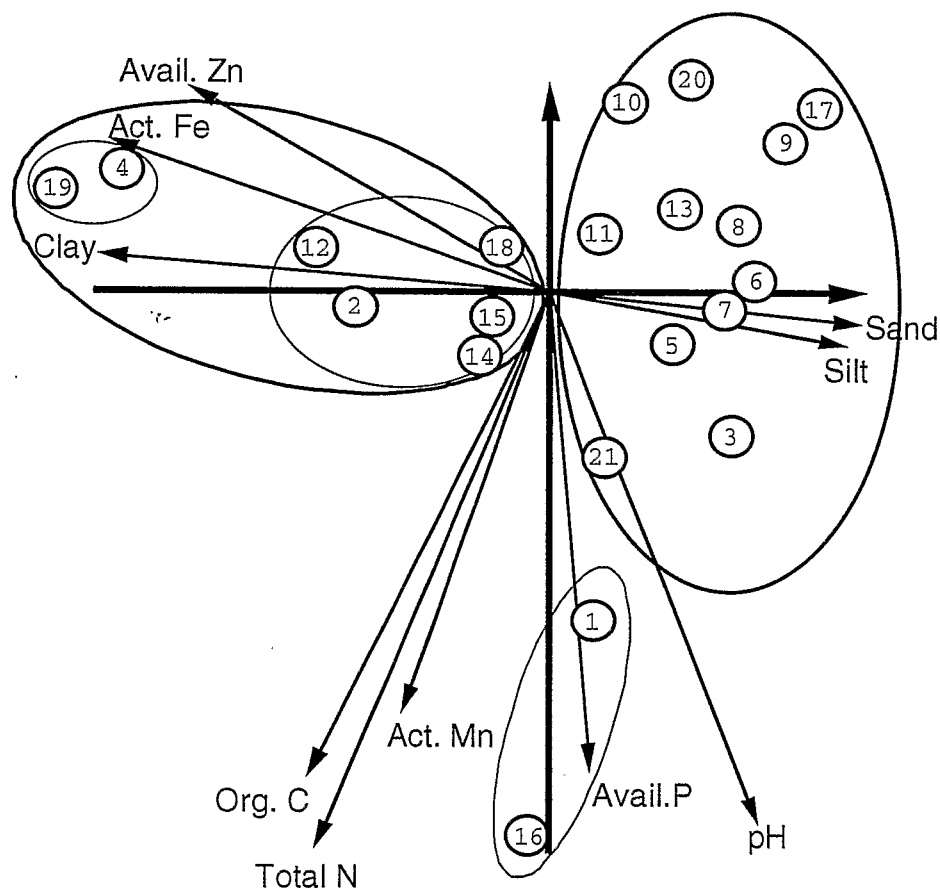


Figure 1. - Principal component analysis of the physico-chemical properties of the 21 rice field soils (peat soil not included). The first axis was negatively loaded on clay (-87%), active Fe (-85%), and available Zn (-70%), and positively on sand (60%) and silt (57%). The second axis was negatively loaded on total N (-80%), pH (-76%), organic C and available P (-69%) and active Mn (-60%).

of methanogens (table 2, area 11,15; 14,18). In soils enriched with straw, a weak positive correlation was observed between maximum PMGA and methylotrophs. When the peat soil was included in the analysis, positive correlations were observed between PMGA (maximum and average) of straw enriched soils and methylotrophic populations.

Principal component analysis of the populations and activities (peat soil not included) was performed on the three first axis, representing about 74% of the total variance. The population and activity vectors formed quite broad angles (fig. 2) which agreed with the absence of correlation observed between populations and activities in most cases (table 2). Four soil groups were distinguished (fig. 2). Groups 1 and 2 were discriminated from groups 3 and 4 by seven microbiological properties with no clear effect of specific factors (single c.t.n.: 12-18%). Group 1 (seven soils) was discriminated from group 2 (four soils) by very low PMGA in controls (c.t.n.: 76%). Group 2 was characterized by high PMGA. Within

this group, soil 16 (Tiaong) was individualized by its highest population of methanogens and methanotrophs (c.t.n.: 91%). Group 4 (three soils) was discriminated from group 3 (seven soils) by its very low PMGA and PMTA (c.t.n.: 80%). When the peat soil was included in the analysis (data not shown) 19 of the 22 soils were grouped within two close clusters. Two soils (16 and 21) were individualized by their high microbial populations. The peat soil was notable by its highest PMGA (c.t.n.: 90%).

Results indicated that the density of methanogens or methanotrophs in dry soils did not reflect their potential to produce or oxidize CH_4 . Results showing no correlation between microbial populations densities and the corresponding activities are not unusual. Using data presented by Garcia *et al.* (1974), we found no correlation between CH_4 production and methanogenic populations in a sample of 11 non-saline rice soils from Senegal. Mayer & Conrad (1990) already reported that methanogenesis initiation appeared to be limited by the substrate and not by the number of methanogens,

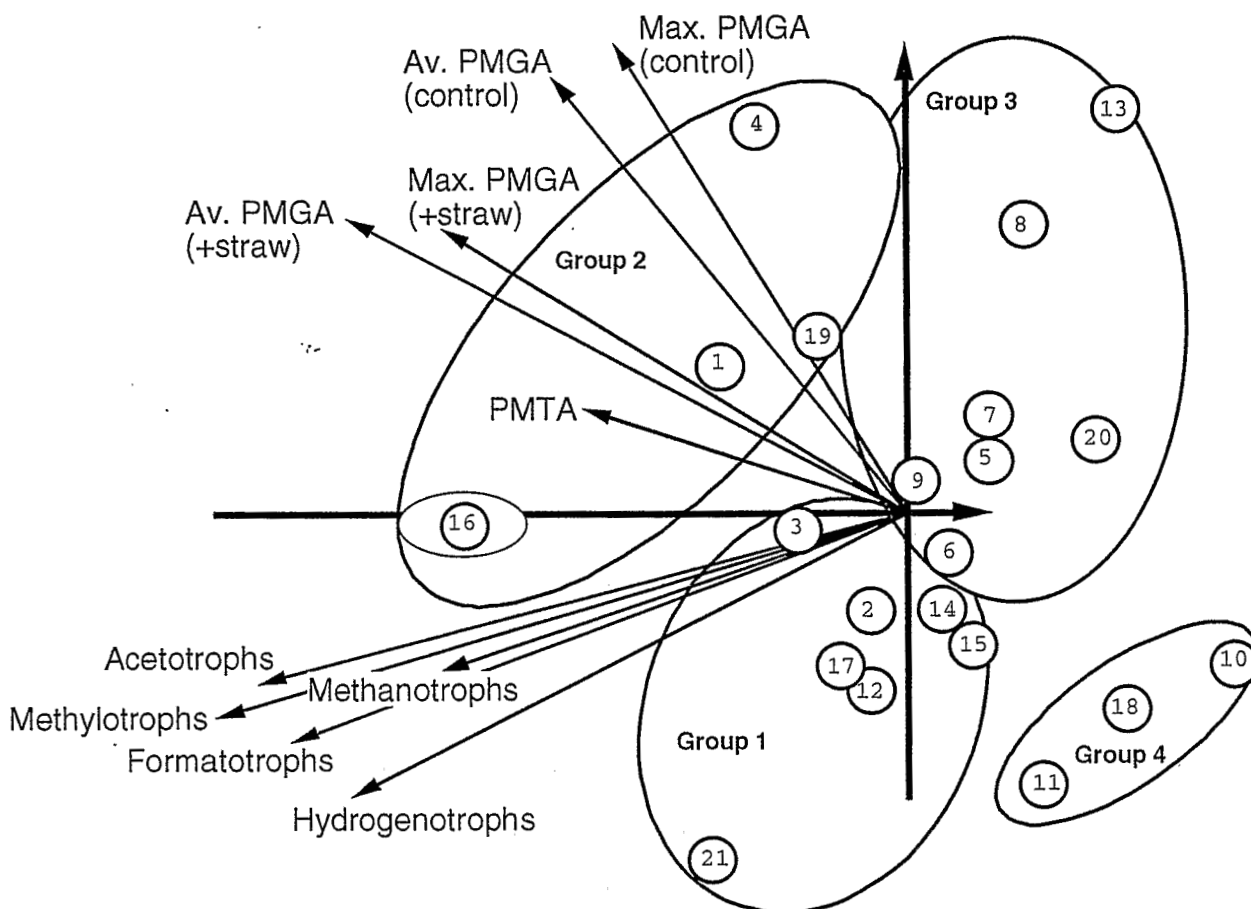


Figure 2. – Principal component analysis of the microbiological properties of the 21 rice field soils (peat soil not included). The first axis was negatively loaded on methylotrophs (–83%), acetotrophs and formatotrophs (>–70%), PMGA (average and maximum) of enriched soils, hydrogenotrophs, and methanotrophs (–55 to –70%). The second axis was positively loaded on PMGA (average and maximum) of control soils (75 and 80%), and average PMGA of enriched soils (50%).

and observed that populations of acetotrophs and hydrogenotrophs were sufficiently abundant (10^4 – 10^5 cells g^{-1} d.w.) in a dry Italian rice soil to allow a significant CH_4 production after 100 h of submersion, with no increase of the populations. In a Camarge soil, Jouliau *et al.* (1996) also observed that straw or dry algal material addition increased populations of hydrogenotrophs and formatotrophs similarly, while four times more CH_4 was produced with straw than with algae.

Relations between populations, activities, and soil properties

Cross-correlations among the properties of 21 soils (peat not included) (table 2, area 1,11; 10,20) showed a number of positive correlations:

- PMTA with both pH and available P (highly significant) and soil N (weak);

- PMGA with (1) total soil N in both control and straw enriched soils, (2) Zn, (3) available P in straw enriched soils, and (4) organic C content (weak);
- methanotrophic and methanogenic populations with active Mn content of the soil;
- methanogenic populations with soil OM content;
- methanotrophic populations with soil N content and pH (weak);
- acetotrophs, methylotrophs (high) and methanotrophs (weak) with pH;
- acetotrophs and methylotrophs (weak) with available P.

No significant correlation was observed between soil texture and microbial populations or potential activities. Including the peat soil in the analysis rendered most correlations between population densities and OM content non significant while correlations between potential activities and OM content became significant (table 2, area 11,2; 20,4).

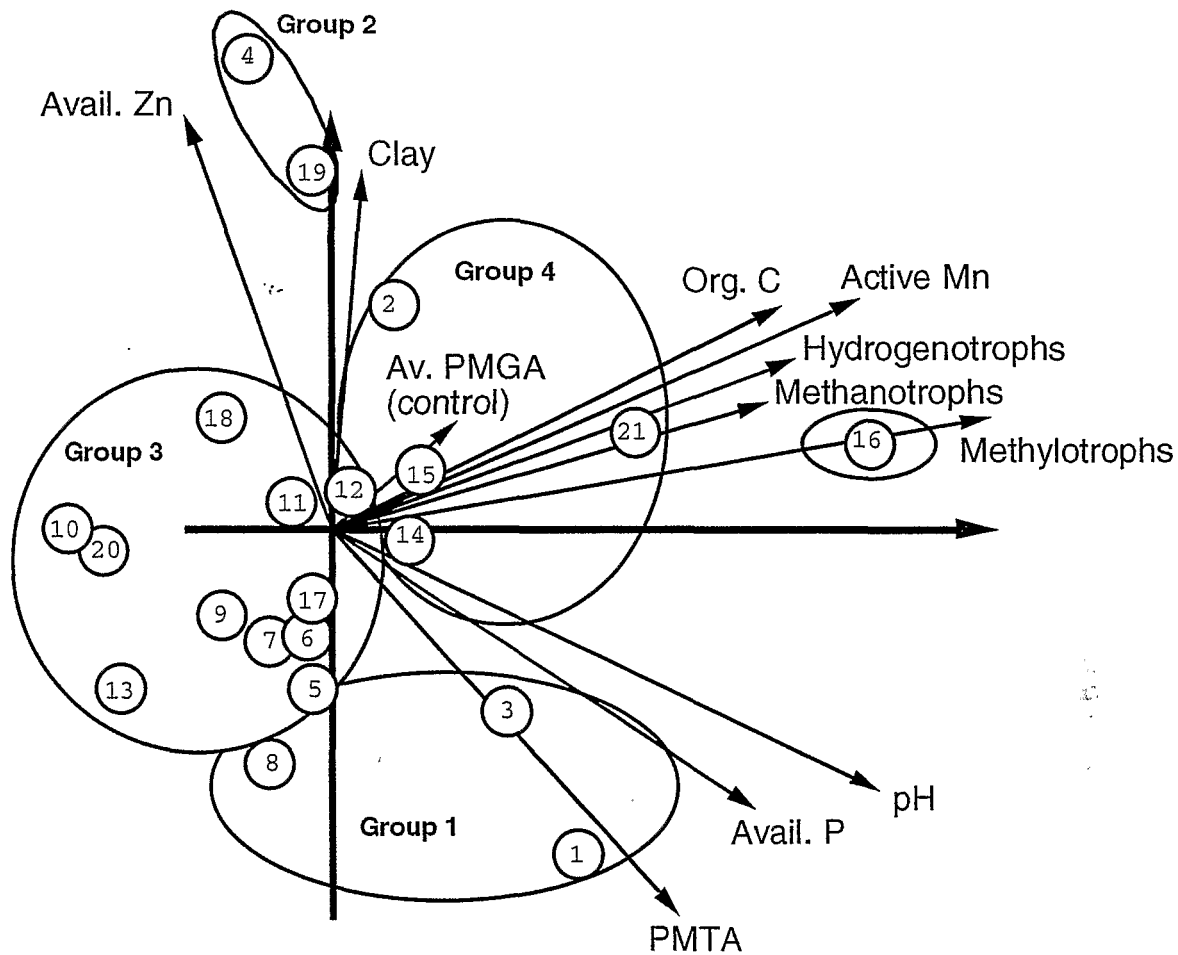


Figure 3. – Principal component analysis of the physico-chemical and microbiological properties of the 21 rice field soils (peat soil not included). The first axis was positively loaded on methylotrophs (88%), Mn and pH (>70%), and hydrogenotrophs, methanotrophs, C and available P (55–65%). The second axis was positively loaded on Zn (72%) and clay (62%) and negatively on PMTA (–68%).

Principal component analysis of the microbiological and physico-chemical properties of the 21 soils (peat not included) was performed with a reduced number of variables to avoid redundancy and to simplify the interpretation (*fig. 3*). Among correlated variables of a same kind, only one was used. For example, among the four PMGA estimates, which were all highly correlated, only average PMGA in control soils was used. The three first axis represented 68% of the total variance. Five soil groups were distinguished. Group 1 (three soils) and soil 16 were discriminated from other soils by their high PMTA and high available P content (c.t.n.: 52%). Soil 16 was distinct from Group 1 by its highest methanotrophic population and C content (c.t.n.: 55%). Group 2 was composed of two acidic soils with high Zn content (c.t.n.: 47%), and high PMGA (c.t.n.: 24%).

Groups 3 (11 soils) and 4 (four soils) were discriminated from each other by the higher Mn content (c.t.n.: 29%) and methanotrophic populations (c.t.n.: 20%) in group 4. Including the peat soil in

the analysis confirmed its unusual characteristics but caused a decrease in the level of discrimination among other soils (data not shown).

To our knowledge, only few data relating soil properties with methanotrophic populations in rice soils are currently available for comparison. Nesbit & Breitenbeck (1992) observed a positive correlation between soil available N or total N and methane uptake by upland soils. We also observed a weak correlation between total N and PMTA. Hütsch *et al.* (1994) reported a decrease of methane oxidation in an upland soil when pH was decreasing. In agreement with this result we observed a positive correlation between soil pH and PMTA.

The only available data relating soil properties with methanogenic activities and populations are those by Garcia *et al.* (1974) who studied 29 acidic soils (average pH 4.95) in Senegal, among which more than half were saline soils rich in sulphate, originating from mangrove areas. Wang *et al.* (1993a) studied the

relationships between CH₄ production and physico-chemical properties in 16 rice field soils.

Garcia *et al.* (1974) reported a positive correlation between OM content and methanogenic activities in non saline soils and no correlation between OM content and methanogenic populations. Wang *et al.* (1993a) found a significant correlation between soil OM content and CH₄ production when considering selectively the soils exhibiting a significant methanogenic activity (>24 ng g⁻¹ dry soil in 10 d). We also found a weak positive correlation between average PMGA and soil OM content. In contrast to Garcia *et al.* (1974), we found significant correlations between methanogenic populations and OM content, which may result from the much broader ranges of methanogenic densities observed in our collection of soils.

Garcia *et al.* (1974) found no correlation between soil aerobic pH (measured before anaerobic incubation) and methanogenic populations or activities. Wang *et al.* (1993a) also observed no correlation between soil aerobic pH and CH₄ produced. Our results, similarly, showed no correlation between pH and activities in control soils. Apparently, significant correlations between soil pH and methane production were only observed with pH measured after 7–10 days of submersion in acidic soils (Garcia *et al.*, 1974; Wang *et al.*, 1993a). As we did not measure soil pH after submersion, we have no data available for comparison. In contrast with Garcia *et al.* (1974), we observed a positive correlation between soil pH and methanogenic populations enumerated on methanol and acetate (sarcinae), while correlations with hydrogenotrophs and formatotrophs (rods) were not significant. Garcia *et al.* (1974) enumerated methanogens on a non-selective rich medium after 8–10 days of growth, which was favourable to hydrogenotrophs but unfavourable to slow growing sarcinae.

CONCLUSIONS

Methanotrophs were more abundant than methanogens in most soils. The duration of the dry fallow seemed to increase the methanotrophs/methanogens ratio.

Rice straw incorporation strongly increased PMGA. A much higher variability of PMGA among control soils than among soils enriched with straw indicated that methanogenesis depended more upon substrate availability than upon the density of methanogens. PMTA was higher than PMGA in most control. In soils with straw added, PMTA was equal to or higher than PMGA in half of the soils tested.

The correlations between populations and their activities were not significant in most cases, indicating that the density of methanogens or methanotrophs present in a dry soil did not reflect its potential

to produce or oxidize CH₄, and that activities were probably mostly substrate dependent. Positive correlations were observed between populations of methanotrophs and methanogens but not between PMGA and PMTA. This confirms that activities were mostly substrate dependent. In agreement with this observation, a weak correlation was obtained between PMGA in straw enriched soils and PMTA.

The potential for methanotrophy was higher than that for methanogenesis in most soils. However, the interpretation and extrapolation of these results must take into account that potential activities were measured under conditions that rarely occur simultaneously in a rice field (anaerobiosis vs. field capacity).

Soil aerobic pH was positively correlated with the abundance of methanotrophs and PMTA but showed no clear effect on methanogens and PMGA. Methane production is known to be favoured by neutrality (Lindau *et al.*, 1993; Wang *et al.*, 1993b; Garcia, 1990), therefore a polynomial relationship can be expected. Characterizing relationships between pH and methane production may require (1) pH measurements performed after submersion, (2) a broad range of pH and (3) a number of samples sufficiently high to demonstrate such a relation.

Soil OM content had no significant effect on methanotrophs and PMTA. It was better correlated with the abundance of methanogens than with their activity.

Methanotrophs possess intra-cytoplasmic membranes rich in phospholipids whose density strongly increases with methane mono-oxygenase activity (Dalton, 1992), which might explain the positive correlation between soil available P and methanotrophy.

Active Mn was positively correlated with methanogenic and methanotrophic populations. Active Mn is involved in early reduction processes in submerged soils and its concentration in dry soils is positively correlated with that in submerged soils (De Datta, 1981), but the reason for observed correlations is unclear.

No correlation was observed between soil texture and populations or potential activities.

Results showed that the ratio between potential methanotrophy and potential methanogenesis was mostly governed by methanotrophy, and that soils prone to methanotrophy were above neutrality, rich in available P and with lower clay content.

From an applied aspect, P application could help reducing CH₄ emission from rice fields but P fertilizer is expensive (about three times more than N fertilizer in Asia) and its use is limited by economics. Therefore, introducing drainage periods during the crop cycle appears to be the most promising management to favour methanotrophy and reduce methanogenesis. Such a practice has other advantageous effects such as controlling some rice pests and disease vectors (Roger, 1996).

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