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Functional diversity of soil microbial community, rock phosphate dissolution and growth of *Acacia seyal* as influenced by grass-, litter- and soil-feeding termite nest structure amendments

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Abstract

We tested termite mound materials belonging to different feeding groups: *Cubitermes* (soil-feeder), *Trinervitermes* (grass-feeder) and *Macrotermes* (litter-feeder), as natural microbial inoculum to promote plant growth and increase nutrient supplies from soil organic matter and inorganic amendments (rock phosphate), through their effects on soil microorganisms (functional diversity of soil microflora, arbuscular mycorrhizal fungi, rhizobia, fluorescent pseudomonads, actinomycetes and saprophytic fungi). Experiments were made in a pot experiment with *Acacia seyal*, a leguminous tree abundant in West Africa, with a sandy soil amended or not with rock phosphate. Results indicated a stimulation of plant growth with *Cubitermes* and *Trinervitermes* mound powder (plant height and shoot biomass), similar to what was obtained with rock phosphate alone. Leaf content in N was also increased in the termite treatments (except in *Macrotermes* soil), whereas mycorrhizal colonization was inhibited as compared to the control. The development of saprophytic fungi was significantly higher in the soils amended with rock phosphate and this effect was hypothesized to be related to the production of large quantities of oxalic acid by fungal populations. The fluorescent pseudomonad populations notably increased in the soils dually amended with mound powders and rock phosphate, and this could be due to the fact that some species of this bacterial group are able to dissolve rock phosphate. The organic and inorganic amendments decreased the soil catabolic evenness in all the mound powder treatments. Among the mound materials tested, *Cubitermes* mound powder had the most promising effect, especially on SIR response to oxalate. It is concluded that soils amended both with rock phosphate and

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Cubitermes mound soil could promote the development of microbial communities, which could help to metabolize this compound and consequently enhance plant growth.

Keywords: Termites; Plant growth; Microbial community; Rock phosphate

1. Introduction

Most of the semi-arid and arid areas of West Africa are characterized by long dry periods and very infertile soils. The main limitation to plant growth is the low soil organic matter content, particularly during the establishment phase (Parry et al., 1987; Caravaca et al., 2002). Consequently, natural vegetation remains scarce and is unable to perform its natural functions such as soil protection against rain splash (Lopez Bermudez and Albaladejo, 1990) or organic matter input to the soil. Decomposition of plant residues results from three processes: comminution, catabolism (microbial and animal enzymatic activities) and production of water-soluble materials (Swift et al., 1981). Organic amendments rich in readily decomposable carbon compounds promote microbial activity (Lax and Garcia-Orenes, 1993; Diaz et al., 1994), which, in turn, maintain soil processes (Giller et al., 1997).

It has been demonstrated that pedogenesis, organic matter decomposition and nutrient cycling are highly influenced by macrofauna (Lavelle et al., 1994). Among the invertebrate macrofauna, termites, as ecosystem engineers (Jones et al., 1994), have large impacts on soil physical, chemical and biological properties. Three major termite feeding groups are usually distinguished in savannas, in terms of biomass and activity: the soil-, litter- and grass-feeders (Bignell and Eggleton, 2000), with different impacts on soil properties (Lee and Wood, 1971; Black and Okwakol, 1997; Holt and Lepage, 2000).

We studied three different species belonging to the feeding groups as outlined above. *Cubitermes* sp. (Termitidae, Termitinae), a soil-feeder, is feeding on mineral soil particles mixed with organic matter. It incorporates faeces in its mound, which is usually richer in C and N than the adjacent top-soil (Lobry de Bruyn and Conacher, 1990; Black and Okwakol, 1997). *Macrotermes subhyalinus* (Termitidae, Macrotermitinae), a litter-forager, foraging on leaves and woody items on the soil surface is cementing finer particles with saliva to built its mound, whose C and N contents are similar or even lower than the adjacent top-soil. *Trinervitermes* sp. (Termitidae, Nasutitermitinae), a grass-feeder, foraging for dead dry standing grass, incorporates also faeces in its mound structure, which exhibits higher carbon content and very high CEC as compared to the adjacent top-soil (Ndiaye et al., 2003).

The epigeal mounds built by these feeding groups (grass-, litter- and soil-feeding termites) could be considered as islands of higher chemical fertility in tropical areas (Spain and Okello-Oloya, 1985). Moreover, soil-feeding termites significantly modify the soil microbial diversity and activity (Fall et al., 1999; Brauman, 2000; Brauman et al., 2000) and the plant symbiotic microflora (NDiaye et al., in press). In contrast, our knowledge is still scarce about the potential effects of the other termite feeding groups on soil microbial diversity and activity, known to play a key role in ecological processes involved with arbuscular mycorrhizal fungi (Abbott and Robson, 1991; Hooker and Black, 1995; Van der Hejden et al., 1998), rhizospheric bacteria, actinomycetes or saprophytic fungi (Garbaye, 1991).

In this study, we hypothesize that termite mound material of different feeding groups could be used as natural microbial inoculum to promote plant growth and increase nutrient supplies from organic or inorganic amendments (i.e. organic matter, rock phosphates), through their effects on soil microorganisms. In order to verify this hypothesis, we investigated the influence of three main feeding groups mound material on the functional diversity of soil microflora, focusing on several microbial groups development (arbuscular mycorrhizal fungi, rhizobia, fluorescent pseudomonads, actinomycetes, saprophytic fungi) in a pot experiment with *Acacia seyal*, a leguminous tree abundant in West Africa, with a sandy soil amended or not with a rock phosphate.

2. Materials and methods

2.1. Epigeal mounds sampling

Termite mounds (five per termite feeding group) were sampled in a shrubby savanna, 50 km north of Ouagadougou (Burkina Faso), near the small village of Yaktenga. Soils are shallow and rich in gravels above a hard-pan level. The landscape is characterized with large hydromorphic spots (so-called «bowé»), interwined with more deeper soils. Mushroom-shaped *Cubitermes* mounds will prefer shallowed soils, while *Macrotermes* and *Trinervitermes* establish preferentially on deeper soils. Termite mounds (about 5 kg) were crushed and passed through a 2-mm sieve.

2.2. Chemical and microbiological analysis of the epigeal mounds

The NH_4^+ and NO_3^- contents were determined according to the method of Bremner (1965). Available P was analysed with the method of Olsen et al. (1954). The Grant and West (1986) method was used to estimate the content of ergosterol, in order to quantify the fungal biomass of epigeal mounds. Two grams of mound powder were placed in 100-ml glass bottles, and 20 ml methanol, 2 g KOH, 5 ml ethanol were added. The mixture was boiled for 30 min at 70 °C using a condenser. The ergosterol was extracted by 1 min hand shaking with 2×30 ml *n*-hexane. The water from hexane extracts was eliminated by addition of ammonium sulfate, followed by rotary evaporation to dryness. Ergosterol was resuspended with 2 ml methanol and its amount was determined by HPLC with an UV-detector at 282 nm using a 150-mm Biorad RP column (4.6 mm inner diameter) packed with Bio-Sil C18 HL 90-3S (3-μm particle size) and a 3-cm Biorad guard column. The mobile phase was 95% methanol and the flow rate was 1 ml min^{-1}. Two measurements were made per sample.

Each subsample of mound powder was carefully mixed and 20 g of powder were sampled to determine the microbial biomass using the fumigation–extraction method (Amato and Ladd, 1988). The results were expressed as μg C g^{-1} of dry soil.

Enumerations of colony forming units (CFU) were performed on King's B agar medium for the fluorescent pseudomonads and an isolation medium for the actinomycetes (Difco™ Actinomycete Isolation Agar). Ten grams fw per sample were vigorously suspended in 100 ml of sterile 0.1M $MgSO_4 \cdot 7H_2O$ solution for 30 min. Serial dilutions of homogenized suspensions were plated on nutrient media and incubated at 25 °C for 48 h to detect and count fluorescent pseudomonads (King et al., 1954) and for 1 week for the actinomycetes as described by the manufacturer. The King's B plates were examined under UV light to count and to select the fluorescent colonies.

2.3. Glasshouse experiment

Seeds of *A. seyal*, originating in Lery (Burkina Faso), were surface-sterilized with concentrated 36 N sulfuric acid for 30 min. The acid solution was then decanted off and the seeds were rinsed and imbibed for 12 h in four rinses of sterile distilled water. Seeds were then transferred aseptically to Petri dishes filled with 1% (w/v) water agar medium. These plates were incubated for 3 days at 25 °C in the dark. The germinating seeds were used when rootlets were 1–2 cm long.

The sandy soil used in this experiment was sampled from a millet field near Ouagadougou (Burkina Faso). Before use, the soil was crushed and passed through a 2-mm sieve. Its chemical and physical characteristics were as follows: pH (H_2O) 5.6, clay 4.6 (%), fine silt 0.0 (%), coarse silt 0.8 (%), fine sand 25.5 (%), coarse sand 69.1 (%), carbon 0.204 (%), nitrogen 0.04 (%), C/N 5.1, soluble P 4.3 ppm, total P 116 (ppm). This soil was mixed with 10% (v/v) of each mound powder. The soils were further amended with Kodjari Rock Phosphate (KRP) (0.1% w/v; insoluble rock phosphate powder) or it remained unamended (controls). Kodjari Rock Phosphate (Burkina Faso) (Pourtal, 1973) was ground with a pestle and mortar and passed through a 90-μm sieve. Its chemical characteristics are indicated in Table 1.

Plastic bags (1 dm^3) filled with soil mixtures were planted with one pre-germinated seed each of *A. seyal*. Seedlings were kept in a glasshouse (35 °C day, 25 °C night, daylength approximately 12 h) and daily watered without fertilizer. Eight replicates per treatment were arranged in a completely randomized design.

After 4-month culture, plants were uprooted and the root systems gently washed. Root nodules induced by indigenous rhizobia were counted. The dry weight

Table 1
Chemical characteristics of the insoluble natural rock phosphate (Truong Binh et al., 1983)

	Kodjari Rock Phosphate
Ca (%)	32.0
CO_2 (%)	1.0
K (%)	0.119
Na (%)	0.605
Mg (%)	1.060
Fe (%)	0.375
Al (%)	0.488
S (%)	0.025
Cl (%)	0.043
F (%)	3.2
Total P (%)	13.16
Water soluble P (%)	0.032

(1 week at 65 °C) of the shoot was measured. Ground samples of leaves were ashed (500 °C), digested in 2 ml HCl 6N and 10 ml HNO_3 N and then analysed by colorimetry for P (John, 1970). Another subsample of leaf tissue was ground and digested in 15 ml H_2SO_4 36N containing 50 g l^{-1} salicylic acid for N (Kjeldal) determination. The root systems were cut into 1-cm root pieces and mixed. The internal colonization of arbuscular mycorrhizal fungus along the root systems was quantified by clearing and staining the roots according to the method of Phillips and Hayman (1970). The root pieces were placed on a slide for microscopic observation under 250× magnification (Brundrett et al., 1985). About fifty 1-cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed in terms of fraction of root length with mycorrhizal internal structures (vesicles or hyphae): (length of root fragments colonized/total length of root fragments)×100. Then, the dry weight of roots was measured (60 °C, 1 week).

The soil from each pot was mixed and kept at 4 °C. The NH_4^+ and NO_3^- contents, microbial biomass, CFU enumeration of fluorescent pseudomonads and actinomycetes were performed as described before. Saprophytic fungi were counted using Dichloran-Rose Bengal-chloramphenicol (DRBC) agar medium.

2.4. Measurement of the catabolic diversity of microbial communities in soil treatments

Microbial functional diversity in soil treatments was assessed by measurement of the patterns of in situ catabolic potential (ISCP) of microbial communities (Degens and Harris, 1997). Twenty-one substrates, comprising a range of amino acids, carbohydrates, organic acids and amides, were screened for differences in SIR responsiveness between soil treatments (Table 2). The substrate concentrations providing optimum SIR responses are indicated in Table 2 (Degens and Harris, 1997). Substrates suspended in 2-ml sterile distilled water were added to 1 g equivalent dry weight soil (West and Sparling, 1986) in 10-ml bottles. CO_2 production from basal respiratory activity in the soil samples was also determined by adding 2-ml sterile distilled water to 1 g equivalent dry weight of soil. After the addition of the substrate solutions to soil samples, bottles were immediately closed and kept at 28 °C for 4 h. CO_2 fluxes from the soils were assessed using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger™) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were expressed as μg CO_2 g^{-1} soil h^{-1}. Catabolic diversity was measured by catabolic richness and catabolic eveness. Catabolic richness, R, was the number of substrates used by microorganisms in each soil treatment. Catabolic eveness, E (variability of substrate used among the range of substrates tested), was calculated using the Simpson-Yule Index, $E=1/\sum p_i^2$ with p_i=respiration response to individual substrates/total respiration activity induced by all substrates for a soil treatment (Magurran, 1988).

Table 2
Organic compounds and their concentrations used to assess patterns of ISCP of soil treatments

Organic substrates	Organic substrates
Amino acids (15 mM)	*Carboxylic acids* (100 mM)
L-Phenylalanine	Ascorbic acid
L-Glutamine	Na-citrate
L-Serine	α-Ketoglutaric acid
	Malonic acid
Carbohydrates (75 mM)	Succinic acid
D-Glucose	Tartaric acid
D-Mannose	Uric acid
Sucrose	Oxalic acid
	Na-formate
Amides (15 mM)	Gallic acid
D-Glucosamine	Malic acid
	Tri-citrate
	DL-α-Hydroxybutyric acid
	α-Ketobutyric acid

2.5. Statistical analysis

Data were treated with one-way analysis of variance. Means were compared using PLSD Fisher test ($p<0.05$). The percentages of mycorrhization were arcsin(sqrt) transformed before statistical analysis. Co-inertia analysis (CIA) was performed for the plant and soil microbial characteristics and SIR responses. CIA (Chessel and Mercier, 1993; Dolédec and Chessel, 1994) is a multivariate analysis technique that describes the relationships between two data tables. Numerous methods have been suggested for this (e.g., canonical analysis, Gittins, 1985; canonical correspondence analysis, Ter Braak, 1986; PLS regression, Höskuldsson, 1988), but one of the simplest, from the theoretical point of view, is co-inertia analysis.

CIA has already been used in various domains, including chemometry (Devillers and Chessel, 1995), phytopathology (Lamouroux et al., 1995), hydrobiology (Castella and Speight, 1996), limnology (Verneaux et al., 1995) or phytoecology (Bornette et al., 1994). The geometrical interpretation of CIA is simple. Classical methods like principal components analysis (PCA), correspondence analysis or multiple correspondence analysis, aim at summarizing a table by searching orthogonal axes on which the projection of the sampling points (rows of the table) have the highest possible variance. This characteristic ensures that the associated graphs (factor maps) will best represent the initial results. To extract information common to both tables, canonical analysis searches successive pairs of axes with a maximum correlation. By using the covariance instead of the correlation, CIA maximizes the product of the correlation by the projected variances on each axis in the environmental and floro-faunistic spaces. This ensures that CIA axes will have both a good correlation and a real meaning for each of the two tables.

Monte-Carlo tests can be used to check the significance of the relationship between the two tables. This method consists in performing many times a random permutation of the rows of one (or both) table, followed by the re-computation of the total co-inertia. By comparing the total co-inertia obtained in the normal analysis with the co-inertia obtained after randomization, we get an estimation of the probability to encounter a situation similar to the observed situation without relationship between the two tables (i.e., a significance test of the relationship). Computations and graphical displays were made with the ADE-4 software (Thioulouse et al., 1997).

3. Results

NH_4^+ contents were significantly higher in *Cubitermes* sp. and *Trinervitermes* sp. mound powders than in those of *Macrotermes* sp. (Table 3). In contrast, NO_3^- content was significantly higher in *Macrotermes* sp. than in the other mound powders. Available P was significantly higher in *Trinervitermes* sp. (Table 3).

For the microbiological characteristics, the population of fluorescent pseudomonads was larger in *Macrotermes* sp. mound powder whereas the ergosterol content was lower than those measured in the others. The lowest actinomycetes population was

Table 3
Biological and chemical characteristics of mound powders

	Cubitermes sp.	*M. subhyalinus*	*Trinervitermes* sp.
NH_4^+ (μg N g^{-1} of dry mound powder)	40.9 b*	9.4 a	37.1 b
NO_3^- (μg N g^{-1} of dry mound powder)	206.9 a	3408.9 b	42.3 a
Available P (μg g^{-1} of dry mound powder)	7.7 b	3.5 a	10.2 c
Microbial biomass (μg C g^{-1} of dry mound powder)	17.0 a	22.5 ab	36.5 b
Fluorescent pseudomonads ($\times 10^2$ CFU g^{-1} per dry mound powder)	< 1 a	79.3 b	< 1 a
Actinomycetes ($\times 10^2$ CFU g^{-1} per dry mound powder)	37.3 b	39.5 b	22.5 a
Ergosterol (μg g^{-1} of dry mound powder)	1.381 b	0.316 a	1.717 b

* Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).

Table 4
Growth response of *A. seyal* seedlings in soil amended with mound powders and/or rock phosphates after 4-month culture

Treatments	Height (cm)	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)
Control	18.1 a[a]	295.2 a	38.7 a
+KRP[b]	29.7 c	640.6 b	131.2 b
+*Cubitermes* sp.	27.1 bc	619.3 b	70.1 a
+*Cubitermes* sp.+KRP	23.5 ab	528.4 ab	76.7 a
Control	18.1 a	295.2 a	38.7 a
+KRP	29.7 b	640.6 c	131.2 c
+*Macrotermes* sp.	19.7 a	314.6 ab	47.7 a
+*Macrotermes* sp.+KRP	24.9 ab	489.1 bc	87.6 b
Control	18.1 a	295.2 a	38.7 a
+KRP	29.7 b	640.6 b	131.2 b
+*Trinervitermes* sp.	28.2 b	567.8 b	78.2 a
+*Trinervitermes* sp.+KRP	21.3 a	369.1 a	76.3 a

[a] For each mound powder origin, data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP: Kodjari Rock Phosphate.

recorded in *Trinervitermes* sp. mound powder. The microbial biomasses were ranked according to the mound origins as follow: *Trinervitermes* sp.>*Macro-Macrotermes* sp.>*Cubitermes* sp. (Table 3).

Rock phosphate added alone to the soil significantly increased height, shoot biomass and root biomass of *A. seyal* seedlings compared to the control (Table 4). *Cubitermes* sp. and *Trinervitermes* sp. powders significantly stimulated the height and shoot biomass but had no effect on the root biomass. No significant effect of *Macrotermes* sp. mound soil was recorded on height, shoot and root biomass (Table 4). The effects on height and shoot biomass of dual amendments (mound powder+rock phosphate) were not significantly different from those obtained with the powder alone for *Macrotermes* and *Cubitermes* (Table 4). In contrast, for *Trinervitermes* sp., a negative effect of the dual amendment has been recorded for height and shoot biomass, as compared to the *Trinervitermes* powder alone (Table 4). Root biomass was significantly enhanced with *Macrotermes*+rock phosphate compared to the control.

Mycorrhizal colonization was significantly lower in all the mound treatments than in the non inoculated soil (control), with or without rock phosphate, except for *Cubitermes* mound powder with rock phosphate treatment (Table 5). The number of nodules per plant was not significantly modified in the soils amended with *Macrotermes* or *Trinervitermes* mound powders (Table 5). However, it was significantly higher than in the control for the *Cubitermes*+rock phosphate (Table 5). Highest nitrogen leaf contents were recorded in the treatments with rock phosphates for *Cubitermes* and *Trinervitermes* mound powders (Table 5). Values found with KRP+*Macrotermes* sp. mound powder were significantly higher than in the control, while *Macrotermes* mound powder alone was not. Compared to the not inoculated treatment (control), highest P leaf contents were recorded for the treatments with KRP (Table 5), excepted with the soil only amended with *Trinervitermes* mound powder.

No significant differences were recorded for NH_4^+ contents among all the treatments (Table 6). The addition of KRP into the soil significantly inhibited NO_3^- contents in all the treatments. Compared to the

Table 5
Effect of soils amended with mound powders and/or rock phosphates on mycorrhizal colonization, number of nodules per plant and leaf mineral (N, P) contents of *A. seyal* seedlings after 4-month culture

Treatments	Mycorrhizal colonization (%)	Number of nodules per plant	N (mg)	P (mg)
Control	47.5 b[a]	0.75 ab	9.4 a	630.9 a
+KRP[b]	22.5 a	4.25 bc	17.9 b	1693.3 b
+*Cubitermes* sp.	10.0 a	0.25 a	20.6 b	1149.8 ab
+*Cubitermes* sp.+KRP	65.0 b	6.5 c	14.1 ab	1387.1 b
Control	47.5 b	0.75 a	9.4 a	630.9 a
+KRP	22.5 a	4.25 a	17.9 c	1693.3 b
+*Macrotermes* sp.	26.2 a	2.25 a	11.2 ab	698.5 a
+*Macrotermes* sp.+KRP	31.2 a	3.00 a	15.9 bc	1515.2 b
Control	47.5 b	0.75 a	9.4 a	630.9 a
+KRP	22.5 a	4.25 a	17.9 bc	1693.3 b
+*Trinervitermes* sp.	23.7 a	1.75 a	19.0 c	1258.1 b
+*Trinervitermes* sp.+KRP	12.5 a	2.5 a	12.6 ab	1301.7 b

[a] For each mound powder origin, data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP: Kodjari Rock Phosphate.

Table 6
Effect of soils amended with mound powders and/or rock phosphates on soil nitrogen content and on the microbial biomass after 4-month culture

Treatments	NH_4^+ (μg g^{-1} of soil)	NO_3^- (μg g^{-1} of soil)	Microbial biomass (μg C g^{-1} of soil)
Control	0.0 a[a]	26.3 b	20.3 a
+KRP[b]	0.0 a	11.4 a	26.0 ab
+*Cubitermes* sp.	0.05 a	31.2 b	30.8 b
+*Cubitermes* sp.+KRP	0.07 a	15.1 a	27.8 b
Control	0.0 a	26.3 b	20.3 a
+KRP	0.0 a	11.4 a	26.0 ab
+*Macrotermes* sp.	0.02 a	33.4 c	28.3 b
+*Macrotermes* sp.+KRP	0.17 a	12.1 a	29.3 b
Control	0.0 a	26.3 c	20.2 a
+KRP	0.0 a	11.4 a	26.0 ab
+*Trinervitermes* sp.	0.02 a	16.5 b	33.8 b
+*Trinervitermes* sp.+KRP	0.05 a	14.6 ab	29.3 b

[a] For each mound powder origin, data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP: Kodjari Rock Phosphate.

Table 7
Effect of soils amended with mound powders and/or rock phosphates on the number of fluorescent pseudomonads, actinomycetes and saprophytic fungi after 4-month culture

Treatments	Fluorescent pseudomonads (CFU g^{-1} of soil)	Actinomycetes (CFU g^{-1} of soil)	Saprophytic fungi (CFU g^{-1} of soil)
Control	25 a[a]	1000 a	50 a
+KRP[b]	75 a	825 a	125 a
+*Cubitermes* sp.	725 b	925 a	75 a
+*Cubitermes* sp.+KRP	425 b	1050 a	1675 b
Control	25 a	1000 a	50 a
+KRP	75 a	825 a	125 a
+*Macrotermes* sp.	50 a	825 a	25 a
+*Macrotermes* sp.+KRP	300 b	950 a	1700 b
Control	25 a	1000 a	50 a
+KRP	75 a	825 a	125 a
+*Trinervitermes* sp.	75 a	975 a	75 a
+*Trinervitermes* sp.+KRP	150 b	1425 a	1425 b

[a] For each mound powder origin, data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP: Kodjari Rock Phosphate.

control, a higher NO_3^- content was found in the *Macrotermes* sp. treatment (Table 6). Microbial biomass was significantly stimulated after mound powder amendment whereas KRP had no significant effect (Table 6).

Populations of actinomycetes were not significantly modified by mound powders and/or KRP amendments (Table 7). In contrast, when mound powders and KRP were dually added, fluorescent pseudomonads and saprophytic fungal populations were significantly larger than in the control. *Cubitermes* sp. amendment alone significantly increased the number of fluorescent pseudomonads per gram of soil (Table 7).

Compared to the control treatments, microbial catabolic richness was significantly lower in the *Cubitermes* sp. mound treatment and, in contrast, higher in the *Trinervitermes* sp.+KRP (Table 8). Microbial catabolic eveness was generally lower with the amendments, excepted for the *Macrotermes* sp. treatment (Table 8), where it was similar to the control.

The factor map of the SIR responses (Fig. 1A) showed, on the first axis, uric and succinic acids as opposed to glutamine and oxalic acid. On the second axis, formic acid was opposed to serine. On the factor map of plant growth and microbial variables

Table 8
Microbial catabolic richness (R) and eveness (E) (Simpson Yule Index) of soils amended with mound powders and/or rock phosphates on the number of fluorescent pseudomonads, actinomycetes and saprophytic fungi after 4-month culture

Treatments	R	E
Control	17.5 b[a]	11.0 c
+KRP[b]	17.5 b	8.6 b
+*Cubitermes* sp.	14.0 a	5.1 a
+*Cubitermes* sp.+KRP	17.8 b	6.2 a
Control	17.5 a	11.0 b
+KRP	17.5 a	8.6 a
+*Macrotermes* sp.	18.5 a	10.8 b
+*Macrotermes* sp.+KRP	18.2 a	8.1 a
Control	17.5 a	11.0 b
+KRP	17.5 a	8.6 a
+*Trinervitermes* sp.	18.8 ab	9.1 a
+*Trinervitermes* sp.+KRP	19.8 b	7.9 a

[a] For each mound powder origin, data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($P<0.05$).
[b] KRP: Kodjari Rock Phosphate.

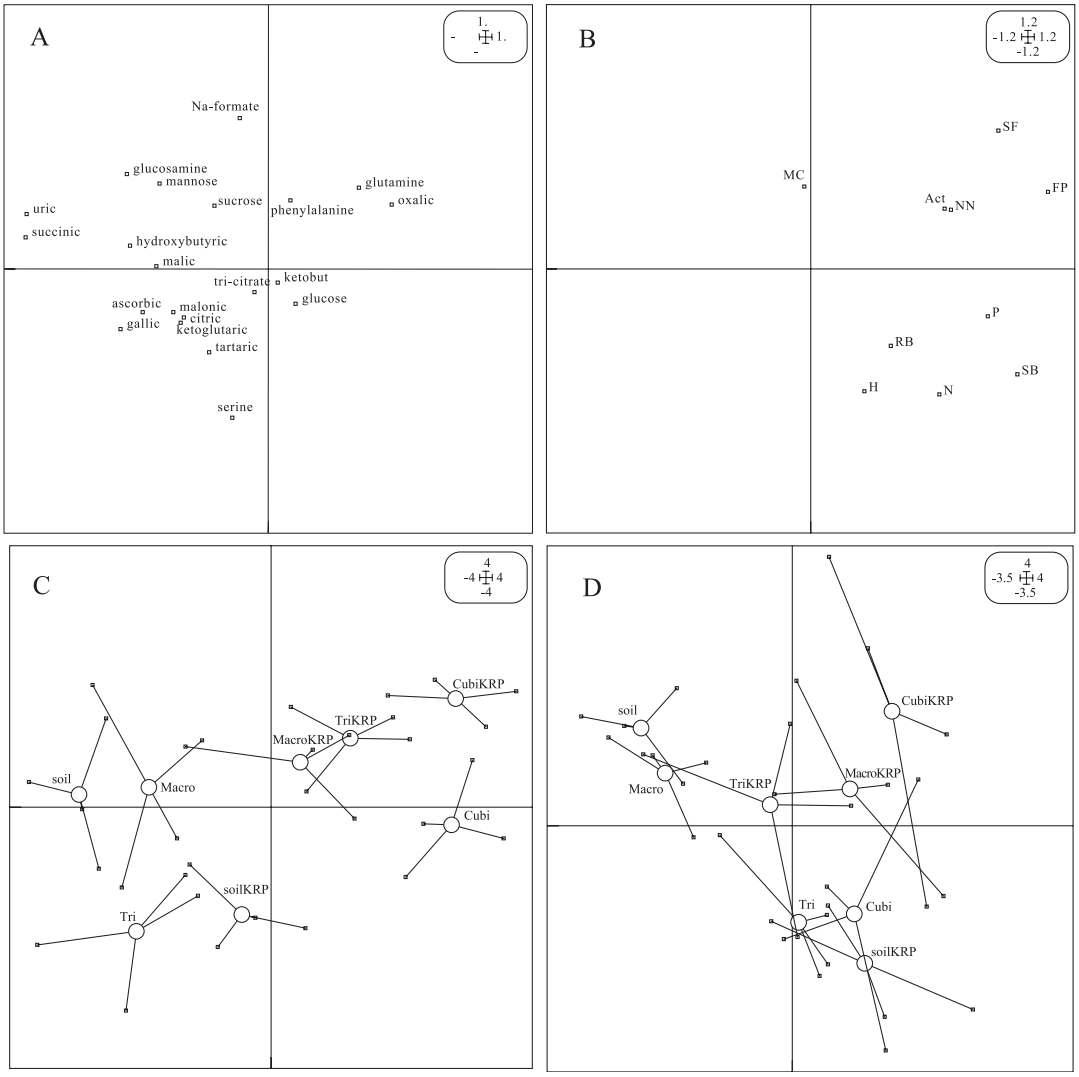

Fig. 1. Co-inertia analysis of the SIR responses of the soils amended with mound powders and plant growth and microbiological variables. A: factor map of SIR responses; B: factor map of plant growth and microbial variables (SF: Saprophytic fungi; FP: Fluorescent Pseudomonads; Act: Actinomycetes; NN: Number of Nodules per plant; RB: Root Biomass; P: P leaf content; N: Nitrogen leaf content; SB: Shoot Biomass; H: Height; MC: Mycorrhizal colonization); C: factor map of SIR responses soil samples (Cubi: *Cubitermes* mound powder amendment; CubiKRP: *Cubitermes* mound powder+Kodjari Rock Phosphate amendment; Macro: *Macrotermes* mound powder amendment; MacroKRP: *Macrotermes* mound powder+Kodjari Rock Phosphate amendment; Tri: *Trinervitermes* mound powder amendment; TriKRP: *Trinervitermes* mound powder+Kodjari Rock Phosphate amendment); D: factor map of plant growth and microbial variables soil samples (For the legend, see Fig. 1B).

(Fig. 1B), the first axis was a size factor with high values on the right, while the second axis opposed the plant variables to microbial parameters. The factor map of soil treatments for SIR responses (Fig. 1C) showed that, on the first axis, treatments with *Cubitermes* differ most predominantly from the control (not inoculated soils). Compared to Fig. 1A, these *Cubitermes* treatments (with or without KRP) were linked with the respiration response to oxalic acid and glutamine whereas control treatments were associated with the respiration response to uric and succinic acids (Fig. 1C). The interpretation of the factor map of soil treatments for plant growth and microbial variables (Fig. 1D) is similar to the

interpretation of Fig 1C. When matched with Fig. 1B, it appeared that *Cubitermes*+KRP and *Macrotermes*+KRP treatments were linked to high densities of actinomycetes, fluorescent pseudomonads, rhizobial nodules and saprophytic fungi, whereas the opposite was observed with the control and *Macrotermes* sp. treatments. *Trinervitermes* sp. and *Cubitermes* sp. treatments were both associated with high values of height, shoot and root biomass, nitrogen and phosphorus leaf contents (Fig. 1D).

The comparison between Fig. 1A and B showed that *Cubitermes* treatments (with or without KRP) exhibited high levels of actinomycetes, saprophytic fungi, rhizobial nodules and fluorescent pseudomonads.

Fig. 2 is a synthetic display of the cross correlations table analysed by co-inertia analysis. The rows corresponded to the SIR responses, and the columns to the 10 plant growth and microbial variables. Circle and square sizes are proportional to the values of the cross-correlations between SIR responses and variables. Circles stand for positive values, corresponding to positive correlations, and squares stand for negative values, corresponding to negative correlations. The position of rows and columns on this graph is given by their coordinate on the first PCA axis. It may be seen that uric and succinic acids were negatively correlated to all the plant growth and microbial variables, except for mycorrhizal colonization. Conversely, oxalic acid and glutamine were positively correlated to all the plant growth and microbial variables, except for plant N. Fluorescent pseudomonads had negative correlation with all SIR responses except with oxalic acid, tri-citrate and glutamin SIR responses. Arbuscular mycorrhizal colonization was positively correlated to glucose, phenylalanine, formic, gallic, uric and succinic acid SIR responses.

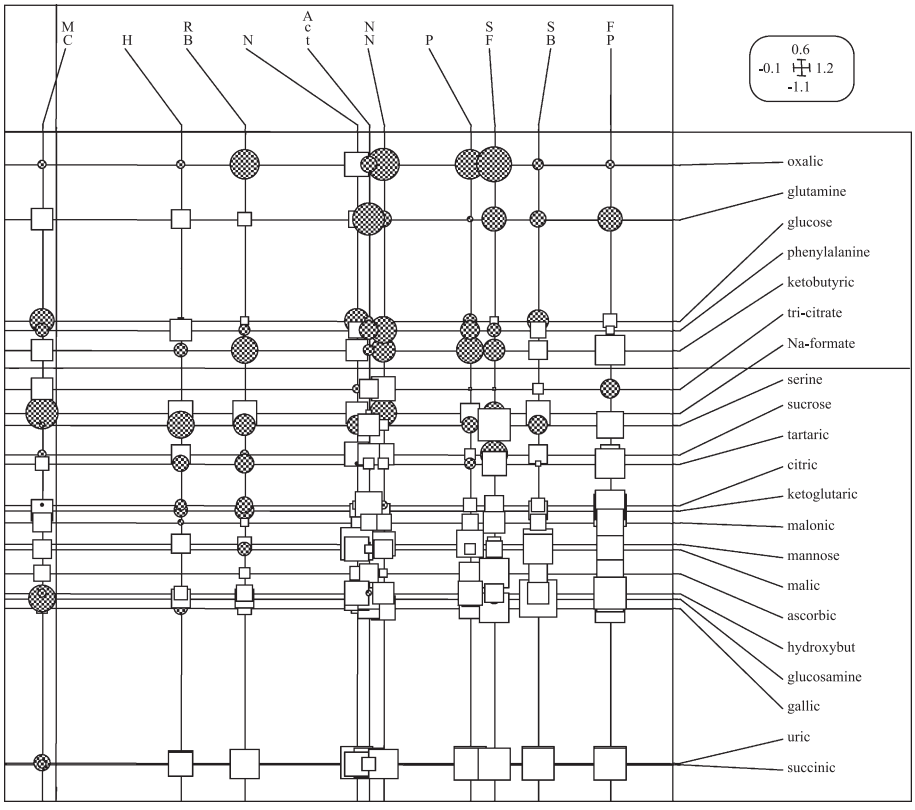

Fig. 2. Cross-correlations between SIR responses and plant growth and microbial variables (for the legend, see Fig. 1B).

4. Discussion

From this research work, four main points deserve discussion: (i) whether the Rock Phosphate amendment increased significantly plant growth but decreased mycorrhizal symbiosis; (ii) whether high levels of oxalic acid SIR responses were associated with the KRP amended soils; (iii) whether these organic and inorganic amendments decreased the catabolic eveness; and (iv) whether *Cubitermes* mound powder induced different effects on plant growth and microbial variables (i.e. mycorrhizal colonization) than the other tested mound powders.

The Rock Phosphate from Kodjari contained 0.03% of soluble P which indicates that about 30 ppm had been added to each pot. Plant growth and P leaf content were stimulated (in the treatment without mound powder), whereas mycorrhizal colonization was inhibited. It is well known that high rates of available P in soil may decrease the development of mycorrhizal symbiosis (Bolan, 1991). Moreover, the improvement of P uptake by the host plant has been reported to improve the symbiosis between rhizobia and legumes (Cornet and Diem, 1982; Sun et al., 1992). In our experiment, a significant positive correlation was found between the KRP amendment treatment and the control treatment for the number of nodules per plant (R=0.85).

Rock phosphates can be solubilized or weathered under the influence of water, acids, complexing agents and oxygen. Biological weathering or biochemical weathering is mediated by microorganisms, which excrete organic acids, phenolic compounds, protons and siderophores (Drever and Vance, 1994). Soluble organic acids affecting rock phosphate weathering in soils could be of high molecular weight (i.e. humic substances) to low molecular weight which are produced by plant roots and soil microorganisms (Ochs, 1996). These low molecular weight organic acids produced by plant roots and soil microorganisms are effective in promoting mineral dissolution. Among them, oxalate, malate and citrate are considered to be the strongest chelators of trivalent metals (i.e. Al^{3+}, Fe^{3+}) (Gadd, 1999). Oxalic acid is commonly produced by many different fungal species (Dutton and Evans, 1996). In our study, the development of saprophytic fungi was significantly higher in the soils amended with Kodjari Rock phosphate. These fungal communities probably produced large quantities of oxalic acid, which exerted a selective influence on soil microbial communities though a multiplication of oxalate catabolizing microorganisms (higher SIR response to oxalic acid). The fluorescent pseudomonad populations were also more important in the soils dually amended with mound powders and rock phosphate. It has been previously demonstrated that some strains of this bacterial group are able to dissolve rock phosphates (Premono et al., 1996; Kumar and Singh, 2001).

The organic and inorganic amendments have decreased the soil catabolic eveness in all the mound powder treatments. It has been hypothesized that soil microbial diversity is important for the maintenance of soil processes (Giller et al., 1997). Moreover, it has been established that microbial catabolic diversity in soils was highly linked with organic C pools (Degens et al., 2000). In our study, *Cubitermes* sp. and *Trinervitermes* sp. mound powders have significantly decreased the microbial catabolic eveness. Both origins contained larger fungal communities and higher NH_4^+ and P contents. Consequently, the decrease of microbial catabolic eveness could be attributable to changes in soil properties but also to changes in species composition (Degens, 1999).

The effects of *Cubitermes* mound powder were different from the others especially on its SIR response to oxalate. Important activity of soil microorganisms has been measured in mounds of *Cubitermes* sp. (Fall et al., 1999, 2001). In our study, a large population of fluorescent pseudomonads has been recorded. It is well known that this bacterial group can produce oxalate compounds and consequently, it can be concluded that the soils amended with rock phosphate and termite mound soils, promoted the development of some microbial communities, which could metabolize this organic compound.

5. Conclusion

The main purpose of this study was to test mound materials from three different termite feeding groups, as natural microbial inoculum to promote plant growth and increase nutrient supplies to the plant. Results indicated differences between termite effects

on plant growth, mycorrhizal colonization, nodulation and microbial populations (pseudomonads, actinomycetes and saprophytic fungi). The growth response of *A. seyal* seedlings was higher with rock phosphate, as well with termite powder alone from *Cubitermes* (a soil-feeder) and *Trinervitermes* (a grass-feeder). Fluorescent pseudomonad populations increased only in soil inoculated with *Cubitermes* mound powder and when the soils were dually amended with mound powders and rock phosphate for all three feeding groups. The saprophytic fungal population was much higher in the soils amended both with rock phosphate and termite mound soil (by a factor 30). We hypothesized that the enhancement of plant growth could be due to an increase of microbial numbers belonging to groups able to dissolve rock phosphates, as fluorescent pseudomonads and saprophytic fungi, producing large quantities of oxalic acid. Termite mound powder, in particular *Cubitermes* (soil-feeder) mound material appeared to be the most promising inoculum to promote the development of microbial communities, which could help to metabolize rock phosphate and consequently to enhance plant growth.

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