Can leguminous trees increase soil phosphorus availability? A link between the P and N cycles in tropical forests and agroforests of Brazil

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Introduction

Understanding the role of leguminous trees for phosphorus (P) and nitrogen (N) cycling in tropical soils is relevant for conservation of natural forests, as well as the sustainable management of agroforests with low P input in the Atlantic Forest region of Brazil. In this context, we are use the NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy to determine the amounts and chemical nature of organic and inorganic P species, and thus answer the following questions: (1) Does N-fixing forest legumes affect fractions and P species differently in soil? (2) Would there be increase of the reserve of labile Pi with decrease of labile Po, with the other fractions and species of P not being affected? (3) Would the total soil P reserve remain constant or would there be a decrease? (4) Would mineral fertilization of N provide the same effect of leguminous trees on soil P availability? The answer to these questions will improve our understanding of how P availability is influenced by the use of leguminous trees in forest systems on highly weathered tropical soils.

Materials and Methods

Study areas are located in the Rio de Janeiro State, Atlantic Forest region, Brazil. This study was performed at the “Santo Antônio da Aliança Farm" in the municipality of Valença (22°22'22"S, 43°47'23"W); at the “Carrapeta Farm” in the municipality of Conceição de Macabú (22°05'04"S, 41° 52'07"W) and in the experimental area of the Brazilian Agricultural Research Corporation (Embrapa), at the National Agrobiology Research Center (CNPAB) in the municipality of Seropédica (22º44'29"S, 43º42'19"W). We assessed the selected sites: (1) 0% leguminous trees N_2 mix cover; (4) 75% leguminous trees N_2 mix cover; (5) acácia (*Acacia auriculiformis*); (6) sabiá (*Mimosa caesalpiniifolia*); (7) secondary forest; (8) pasture; (10) gliricídia (*Gliricidia sepium*); (12) low input of nitrogen fertilizer; (13) spontaneous vegetation without nitrogen fertilizer. The soil at all of sites was classified as Red-Yellow Argissol (Ultisol) and Red Latossol (Oxisol).

Soil P (0-10 cm) was extracted by shaking soil (4.0 g) with 32 ml of a solution containing 0.25 M NaOH + 50 mM Na_2EDTA for 10 h. After this step, each extract was centrifuged and filtered to remove any precipitates or material that did not solubilized, frozen with liquid nitrogen, lyophilized and stored. The ^{31}P nuclear magnetic resonance spectroscopy was carried out solubilizing 200 mg each lyophilized extract with solution containing 0.25 M NaOH + 50 mM Na_2EDTA and 0.1 ml of deuterium oxide, each extract was centrifuged and then transferred to a 5mm NMR tube.

Solution ^{31}P NMR spectra were acquired with proton decoupling operating at 202.446 MHz using a Bruker Avance DRX 500 MHz (Bruker, Germany), using a 6.0 µs pulse (30°), an acquisition time of 0.4 s, an delay time of 0.5 s operating at 21 °C. Approximately 30,000 scans were acquired for each sample. Chemical shifts of signals (δ) were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. The concentration of the P species was calculated by multiplying their contribution relative to the total NMR signal by the concentration of MDPA as internal reference and concentration standard (17.56 δ ppm). Signals were assigned to phosphorus compounds based on literature reports of model compounds spiked in NaOH–EDTA soil extracts. All spectral processing was done using Delta NMR Software (Jeol Resonance Inc., Tokyo, JP).

Results and Discussion

Table 1 Inorganic and organic phosphorus determined by solution ^{31}P NMR in NaOH-EDTA extracts of soils

Treatment	$^{31}P_{i\ RMN}$ total (mg kg^{-1})		$^{31}P_{o\ RMN}$ total (mg kg^{-1})		
			P-monoester	P-diester	
	ortho-P	pyrophosphate		DNA	M/D
1	151.3	32.5	77.7	6.2	12.6
4	185.2	31.4	160.3	9.9	16.2
5	192.4	38.5	191.9	8.5	22.7
6	190.3	29.6	172.1	7.2	23.7
7	153.4	19.5	74.0	4.1	17.9
8	124.4	18.8	52.6	4.6	11.5
10	208.4	36.1	142.9	13.5	10.6
12	237.6	37.6	176.8	17.0	10.4
13	170.1	21.6	67.3	5.2	12.9

P-monoester: sum of all detected peaks in the Mono1, Mono2 and Mono3 regions; M/D: ratio of monoesters to diesters.

Table 2 Inorganic and organic phosphorus determined by solution ^{31}P NMR in NaOH-EDTA extracts of soils

Treatment	NaOH-EDTA extraction (mg kg^{-1})			
	$^{31}P_{i\ RMN}$ total	$^{31}P_{o\ RMN}$ total	$^{31}P_{RMN}$ total	$P_{NaOH-EDTA}$ total
1	183.9 (38)	83.9 (17)	267.7 (55)	293.0 (60)
4	216.6 (41)	170.2 (32)	286.8 (73)	423.4 (80)
5	230.9 (44)	200.4 (39)	431.3 (83)	442.0 (85)
6	219.9 (40)	176.9 (32)	396.9(73)	434.4 (80)
7	172.8 (29)	78.1 (13)	251.0 (42)	272.2 (46)
8	143.2 (27)	49.9 (10)	193.1 (37)	211.4 (40)
10	244.5 (47)	156.5 (30)	401.0 (76)	438.9 (84)
12	275.3 (41)	192.2 (28)	467.4 (69)	558.1 (83)
13	170.1 (43)	72.5 (16)	264.1 (59)	289.1 (65)

Values in parentheses are the proportion (%) of the total soil phosphorus, and element ratios are mass based; $^{31}P_{i\ RMN}$ total: sum of or ortho-phosphate and pyrophosphate; $^{31}P_{o\ RMN}$ total: sum of P-monoesters and P-diesters (DNA); $^{31}P_{RMN}$ total: sum of $^{31}P_{i\ RMN}$ total and $^{31}P_{o\ RMN}$ total

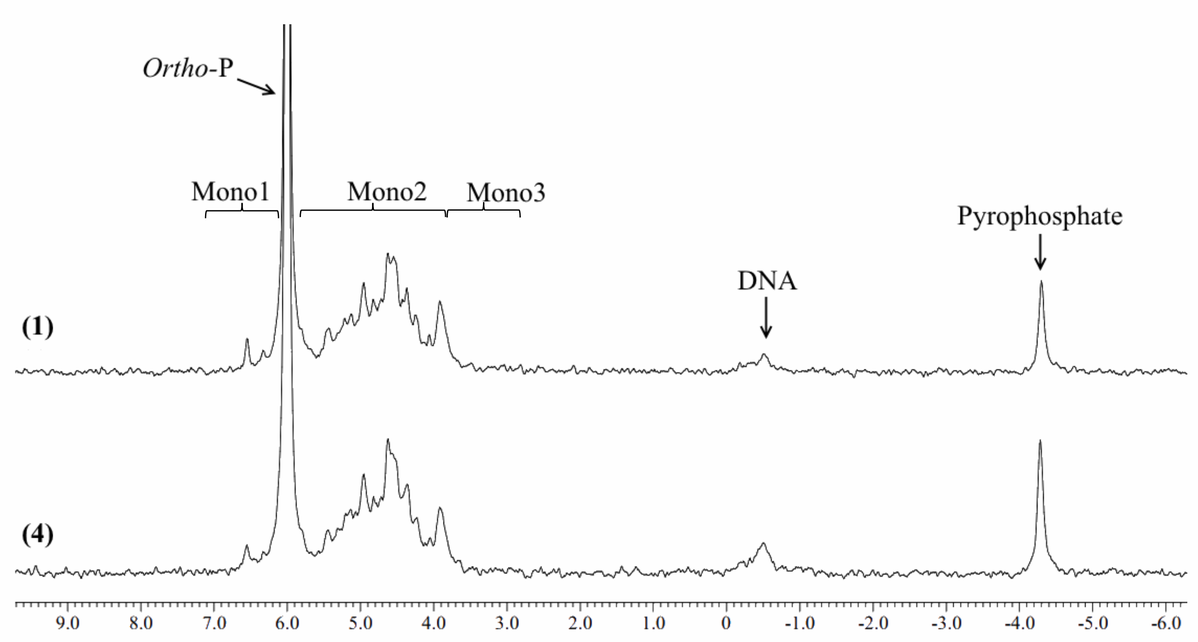

Figure 1 ^{31}P NMR spectra of NaOH-EDTA extracts of soil. Spectra are scaled to the orthophosphate peaks (6.0 δ ppm). Details in the pyrophosphate, P-monoester (Mono1, Mono2 and Mono3) and DNA regions are shown. Spectra were plotted with 5 Hz line broadening for the main spectra. Site 1: (1) 0% leguminous trees N_2 mix cover; (4) 75% leguminous trees N_2 mix cover

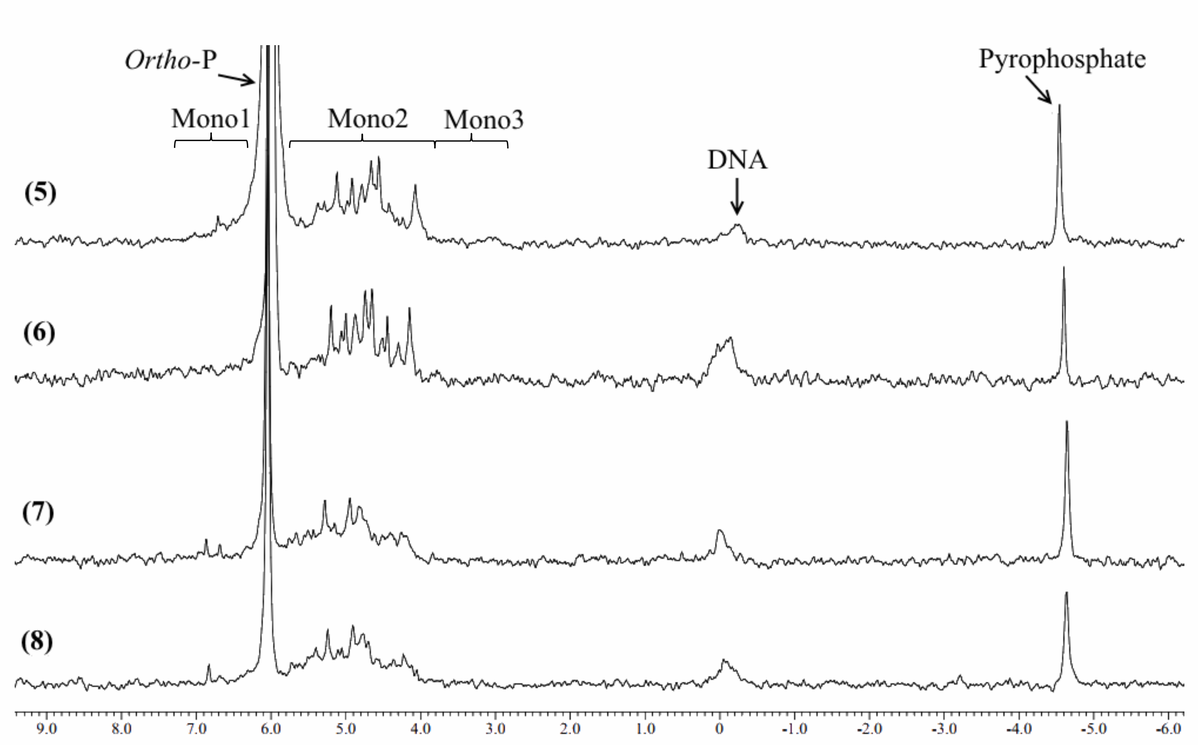

Figure 2 ^{31}P NMR spectra of NaOH-EDTA extracts of soil. Spectra are scaled to the orthophosphate peaks (6.0 δ ppm). Details in the pyrophosphate, P-monoester (Mono1, Mono2 and Mono3) and DNA regions are shown. Spectra were plotted with 5 Hz line broadening for the main spectra. Site 2: (5) acácia (*Acacia auriculiformis*); (6) sabiá (*Mimosa caesalpiniifolia*); (7) secondary forest; (8) pasture.

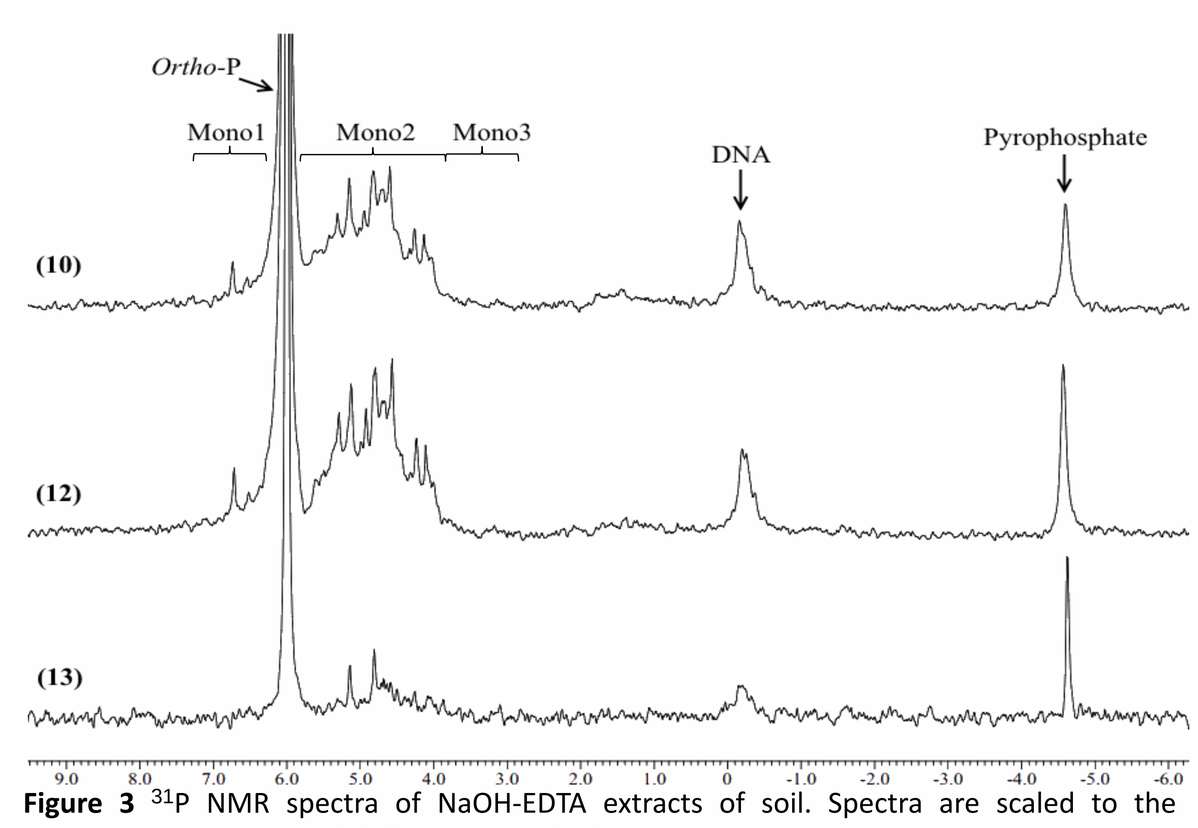

Figure 3 ^{31}P NMR spectra of NaOH-EDTA extracts of soil. Spectra are scaled to the orthophosphate peaks (6.0 δ ppm). Details in the pyrophosphate, P-monoester (Mono1, Mono2 and Mono3) and DNA regions are shown. Spectra were plotted with 5 Hz line broadening for the main spectra. Site 3: (10) gliricídia (*Gliricidia sepium*); (12) low input of nitrogen fertilizer; (13) spontaneous vegetation without nitrogen fertilizer.

Conclusions

The use of ^{31}P nuclear magnetic resonance spectroscopy in the soil showed that the leguminous trees inoculated with the diazotrophic bacteria and arbuscular mycorrhizal fungi can increase soil P concentrations by cycling and accumulation of organic and inorganic compounds of P.

The planting of leguminous trees species for recovering degraded lands may be an suitable strategy to increase the efficient of P and N cycling in highly weathered soils in the Atlantic Forest biome in Brazil.

Acknowledgments

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