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# Organic matter quality of forest floor as a driver of C and P dynamics in acacia and eucalypt plantations established on a Ferralic Arenosols, Congo

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## Abstract

**Background:** Land-use change and forest management may alter soil organic matter (SOM) and nutrient dynamics, due in part to alterations in litter input and quality. Acacia was introduced in eucalypt plantations established in the Congolese coastal plains to improve soil fertility and tree growth. Eucalypt trees were expected to benefit from N<sub>2</sub> fixed by acacia. However, some indicators suggest a perturbation in SOM and P dynamics might affect the sustainability of the system in the medium and long term. In tropical environments, most of the nutrient processes are determined by the high rates of organic matter (OM) mineralization. Therefore, SOM stability might play a crucial role in regulating soil-plant processes. In spite of this, the relationship between SOM quality, C and other nutrient dynamics are not well understood. In the present study, OM quality and P forms in forest floor and soil were investigated to get more insight on the C and P dynamics useful to sustainable management of forest plantations.

**Methods:** Thermal analysis (differential scanning calorimetry (DSC) and thermogravimetry (TGA)) and nuclear magnetic resonance (solid state <sup>13</sup>C CPMAS and NMR and <sup>31</sup>P-NMR) spectroscopy have been applied to partially decomposed forest floor and soils of pure acacia and eucalypt, and mixed-species acacia-eucalypt stands.

**Results:** Thermal analysis and <sup>13</sup>C NMR analysis revealed a more advanced stage of humification in forest floor of acacia-eucalypt stands, suggesting a greater microbial activity in its litter. SOM were related to the OM recalcitrance of the forest floor, indicating this higher microbial activity of the forest floor in this stand might be favouring the incorporation of C into the mineral soil.

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**Conclusions:** In relation with the fast mineralization in this environment, highly soluble orthophosphate was the dominant P form in both forest floor and soils. However, the mixed-species forest stands immobilized greater P in organic forms, preventing the P losses by leaching and contributing to sustain the P demand in the medium term. This shows that interactions between plants, microorganisms and soil can sustain the demand of this ecosystem. For this, the forest floor plays a key role in tightening the P cycle, minimizing the P losses.

**Keywords:** Mixed-species plantations, Soil chemical stability, Organic P, Forest ecosystems, Potential climate change mitigation

## Highlights

- High rates of organic matter mineralization determine most of the nutrient processes in the tropics
- Highly soluble orthophosphate was the dominant phosphorus (P) form in both forest floor and mineral soils
- Forest floor plays a key role in tightening P cycle and minimizing P losses
- Higher microbial activity in the forest floor of the mixed acacia and eucalypt stand favours the incorporation of C into the mineral soil

## Background

Large areas of the native savannas span on over 6 million ha in Central Africa (Schwartz and Namri 2002). This includes savannas of the Congolese coastal plains on inherently nutrient-poor soils with low potential for agriculture. These soils are mainly coarse textured Ferralsols (Mareschal et al. 2011), nutrient-poor with low soil organic matter (SOM) content, i.e., low C and N concentration and CEC (Nzila et al. 2002; Koutika et al. 2014). They are acidic with low Al and Fe oxide amounts (Mareschal et al. 2011) and moderate P availability (Laclau et al. 2010). They were ranked as low P-fixing soils according to the P availability (Koutika 2019). One management practice that was predicted to be compatible with soil constraints was afforestation with fast growing eucalypt in the 1950s (Makany 1964), to provide wood for industrial and fuel energy for the rural population, since more than 94% of population use natural wood and charcoal for energy (Shure et al. 2010). However, their productivity declines in second and subsequent rotations due to a sharp decrease in soil fertility, since low amounts or lack of fertilizers cannot compensate for nutrients exported through tree harvest (Corbeels et al. 2005; Laclau et al. 2005).

To improve soil fertility and sustain forest productivity, *Acacia mangium* has been introduced into these eucalypt plantations (Bernhard-Reversat 1993; Bouillet et al. 2013). Mixed plantations of acacia and eucalypt increased: (i) organic N stock in bulk soil (Koutika et al.

2014) and N concentration in both bulk and particulate organic matter (POM 4000–50  $\mu\text{m}$ ) fractions (Koutika et al. 2017); (ii) cumulative net production of mineral N (Tchichelle et al. 2017); and (iii) soil C stocks and concentration in both bulk soil and POM fractions in stands containing acacia at the end of 7-year first rotation and 2 years into the second rotation (Koutika et al. 2014; Koutika et al. 2017). Improved soil fertility in the mixed-species stands enhanced stand wood biomass (Epron et al. 2013), and improved both soil fertility and stand wood biomass. This demonstrates its potential to sequester C and therefore also to contribute to climate change mitigation (Rumpel et al. 2018). These benefits are involved by the increased productivity and the litter input in acacia-eucalypt stands (Forrester et al. 2013). However, they might also be due to the changes in decomposition rates of the forest floor. Some studies have found greater decomposition rates in mixed-species plantations (Forrester et al. 2005; Xiang and Bauhus 2007), which would promote higher soil nutrient availability and a greater proportion of C incorporated into the mineral soil (Chavez-Vergara et al. 2014).

In spite of these benefits, recent studies have shown that SOM quality, e.g. N and C concentration in coarse POM (4000–250  $\mu\text{m}$ ), declines in tandem with pH values and available P at 5 years relative to 2 years into the second rotation in all stands (Koutika et al. 2017; Koutika et al. 2019). Concerning P, it is also possible that the decrease in P availability might be due to the increased soil acidity (Koutika et al. 2019). This decrease is more pronounced in afforested stands containing acacia relative to pure eucalypt at the end of the 7-year first rotation (Koutika et al. 2014). This tendency was also found at 2 years (Koutika et al. 2016), but was more pronounced at 5 years into the second rotation e.g., less than 2 mg P per kg (Koutika et al. 2019).

This decrease can be explained by the high P demand in supplying symbiotic root nodules and  $\text{N}_2$  fixation processes of acacia, as a nitrogen-fixing species (NFS) (Binkley 1992; Inagaki et al. 2011), and SOM quality (e.g., the effects of new organic residues rich in N), edaphic/climate conditions and the length of rotation (Derrien et al. 2014; Koutika et al. 2019). P availability in the

medium term is determined by organic compounds of fast (diester) or moderate (monoesters) lability (Campo and Merino 2016). It is also possible that changes in organic residues amount and quality affect P dynamics, promoting the degradation of the organic molecules, or the contrary, favouring the immobilization of P in microbial biomass (Ayaga et al. 2006). All these factors may therefore greatly affect P dynamics since logging residues are rich in nutrients and may be of important amounts. Even though there were no significant difference in P concentration of leaves, bark and litterfall, Koutika et al. (2016) reported higher leaves and litterfall biomass in mixed-species stands, i.e., 0.67 and 0.50 kg DM·m<sup>-2</sup> nearby acacia tree and 0.42 and 0.17 kg DM·m<sup>-2</sup> nearby eucalypt tree at year 2 into the second rotation. Both changes in SOM and P status occurred at 5 years into the second rotation may indicate a creation of more labile SOM with an enhanced SOM mineralisation.

Soil organic matter (SOM) chemical stability may be evaluated using the thermal analysis (differential scanning calorimetry (DSC) and thermogravimetry (TGA)). These techniques relied on the exothermic decomposition of organic substances to supply information on biogeochemical stability via complement information obtained by the spectroscopic techniques (Merino et al. 2014; Campo and Merino 2016). The results of these types of analysis may be used as a proxy for SOM composition and microbial activity in soils (Harvey et al. 2012; Siewert et al. 2012; Merino et al. 2014, 2015; Campo and Merino 2016). Schmidt et al. (2011) and Lehmann and Kleber (2015) reported that C sequestration greatly relies on the amount of unprotected free light organic matter or unlinked to soil minerals, while organic matter dynamics is mainly controlled by chemical recalcitrance and the environmental conditions influenced by the microbial activity. This mechanism was considered as a short-term organic C stabilization, since SOM pool and the sink C capacity may be very sensitive to climatic changes (Marschner et al. 2008).

To better estimate both changes in SOM status and P forms, soil chemical stability could be combined to <sup>31</sup>P-NMR spectroscopy. <sup>31</sup>P-NMR has emerged as a precise technique to identify, simplify and quantify soil organic P forms and transformations. It provides a better understanding through soil P dynamics changes that occurred in SOM status in response to changes in rainfall regime such as annual precipitation (Campo and Merino 2016, 2019). Diester forms of P are often the main substrate in P-limited ecosystems such as mixed acacia and eucalypt plantations established on inherently nutrient-poor and sandy soils in the Congolese coastal plains. The organic P is the long-term reserve of P, which is not fixed to Fe

and Al as commonly found in weathered soils (Sanchez and Uehara 1980). Land-use change and forest management practices may decrease/increase the proportion organic P (monoesters and diester) to P inorganic (orthophosphate and pyrophosphate). Both OM content in mineral soil, the composition of roots and leaves are very important. Their distribution depends on the species types, i.e., vegetation cover. Overall, SOM dynamics, including soil organic carbon (SOC) sequestration and soil fertility, are closely linked to P dynamics (Lal 2014). Evaluation of SOM stability via chemical recalcitrance and organic forms of P in the different stands, i.e., pure acacia (100A) and eucalypt (100E) and mixed-species acacia and eucalypt stands (50A50E), are crucial to estimate the potential of this forest management to improve soil fertility, sustain forest productivity and mitigate climate change.

Therefore, the main objective of this study is to provide information about the SOM quality and its relation with the P fractions determining the soil P availability, i.e., how the forest management carried out in Congo to efficiently use these sandy soils to affect SOM/P dynamics. This paper answers to two main questions: (1) how previously reported decline in SOM status (N and C concentration in POM fractions) may be linked to SOM chemical recalcitrance? (2) how reported decline in soil available P will be related to P organic forms and SOM quality? We addressed these questions by testing the three following hypotheses: (i) SOM chemical recalcitrance is more likely lower in stands containing acacia, i.e., the pure acacia (100A) and the mixed-species (50A50E), since previous studies have reported high N mineralization and cumulative net N mineral production; (ii) higher proportion of organic P (monoesters and diester) to inorganic P (orthophosphate and pyrophosphate) will be observed in the soil beneath the stands containing acacia relative to pure eucalypt (100E) due to the greater microbial immobilization and higher demand for available inorganic P; (iii) both SOM chemical recalcitrance and P organic forms are linked to the decline in N and C concentration in POM fractions, previously reported at 5 years into the second rotation.

## Materials and methods

### Site description, trial establishment and sampling

The experimental site is located on a plateau close to Tchissoko village in the Republic of the Congo, 35 km from Pointe-Noire (4°44'41'' S & 12°1'51'' E, 100 m Alt.) on a deep Ferralic Arenosol laying on a geological bedrock composed of thick detritic layers of continental origin dated from plio-pleistocene. These soils are characterized by a low CEC (< 0.5 cmol<sub>c</sub>·kg<sup>-1</sup>), a high sand content (> 90% of the mineral soil), very low clay and silt content (6% and 2%, respectively) and low iron oxide

content (< 1.5% of the bulk soil, Mareschal et al. 2011). They are acidic with low C and N content (Nzila et al. 2002; Koutika et al. 2014); while they may be ranked as low P fixing soils (Koutika 2019). P availability of the studied soils ranged from 1.5 to 11 mg P·kg<sup>-1</sup> (Koutika et al. 2014, 2019). The climate in the area is subequatorial with high mean annual air humidity and air temperature (85% and 25 °C, respectively) and low seasonal variation (about 2% and 5 °C, respectively). Annual precipitation averages 1200 mm over 10 years, with a dry season extending from June to September. The original vegetation was a native tropical savanna dominated by the C<sub>4</sub> Poaceae *Loudetia arundinacea* (Hochst.) Steud, which was afforested in 1984 with eucalypt hybrids.

An experimental trial was installed in May 2004. The first rotation of 7 years ended in 2011, then the second rotation started in March 2012 which included pure stands of *Acacia mangium* (100A) and of *Eucalyptus urophylla* × *E. grandis* (100E) and mixed-species stands with 50% of acacia and 50% of eucalypt (50A50E) in five replicates (randomized block design) at a density of 800 trees·ha<sup>-1</sup> (Koutika and Mareschal 2017). Each stand covered 1250 m<sup>2</sup>, i.e., 37.5 m × 33.3 m, comprised an inner plot of 36 trees (6 × 6), with two buffer rows on all sides. The two species were planted alternately along each row in the mixed-species 50A50E stand. The rows were spaced between by 3.75 m, with 3.33 m between the trees in a row. These densities are optimal and commonly used in commercial plantations with regard to stem wood production in eucalypt monocultures in the area, i.e., 800 trees·ha<sup>-1</sup> (Epron et al. 2013). This first rotation was harvested in January 2012 at the age of 7 years with the removal of the debarked commercial-sized boles, while all remaining residues, i.e. branches, bark and leaves were left behind and evenly distributed on the soil.

The soil samples have been collected using 5 cm × 5 cm sampling cylinders. The composite soil sample has been made of 9 replicates for each stand (pure acacia and eucalypt) and of 18 for the mixed-species stands (50 acacia and 50 eucalypt) in April 2017, i.e., at 5 years into the second rotation. Therefore, one composite sample has been obtained for each stand in each block, i.e., one pure acacia (100), one eucalypt (100E) and one mixed-species stand (50A50E). Using a square metallic frame (50 cm × 50 cm), the forest floor litter was sampled on 4–6 locations within each stand in May 2017. Collected samples were oven-dried at 65 °C during 72 h and weighed.

#### Chemical analyses of litter and soil samples

N and C concentrations on 36 composite soil samples were evaluated using a Macro VARIO Cube Elemental Analyser (Germany). Measurements were performed with 3 technical replicas for each sample. Available P was determined using anion exchange resins. Two

anion-exchange resin strips (BDH#551642S), each 20 mm × 60 mm, were added to 0.5 g (soil) or 0.1–0.2 g (POM fraction) and suspended in 30 mL distilled water. Phosphate adsorbed by the anion-exchange resin was recovered in 20 mL of 0.5 mol·L<sup>-1</sup> HCl after shaking for 16 h (100 r·min<sup>-1</sup>) according to the method of Tiessen and Moir (2008). Malachite reactive P was determined at 630 nm with a JENWAY 6305 Spectrophotometer (UK). P availability has been performed with 3 technical replicas for each sample.

#### Characterization of organic matter (OM) thermal stability

Thermal analysis (differential scanning calorimetry (DSC) and thermogravimetry (TGA)) and <sup>31</sup>P nuclear magnetic resonance (<sup>31</sup>P-NMR) have been used for OM quality and P forms, respectively. These techniques have been applied in a long-term experiment, including pure stands of acacia (*Acacia mangium*) and eucalypts (*Eucalyptus urophylla* × *E. grandis*) and mixed acacia and eucalypt stands at 5 years into the second rotation. Differential scanning calorimetry and thermogravimetry (TGA/DSC, Mettler-Toledo Intl. Inc., New Castle, DE, USA) were carried out on the 9 composite samples of mineral soil, 9 coarse (F) and 9 fine (FI) forest floor according to Campo and Merino (2016). Around 2 g of each 18 samples were submitted to increase temperatures to obtain peaks in loss of matter (TGA) or the release of energy in the form of heat (DSC). Samples were placed in open aluminium pans under dry air (flow rate, 50 mL·min<sup>-1</sup>) with a scanning rate of 10 °C·min<sup>-1</sup> between 50 °C to 600 °C. To calibrate the calorimeter, samples of Indium (mp: 156.6 °C) were used, while baseline correction and determination of different thermal indices were made using the STARe software (Mettler-Toledo). The heat of combustion (Q, in J per g) was evaluated by integration of DSC curves (in W·g<sup>-1</sup>) over the exothermic region (150–600 °C). Weight losses and energy changes associated with moisture loss were excluded since data recorded at < 150 °C were not considered. Mass loss (both measured between 150 °C and 550 °C) in each measurement allowed to determine Q values. Three groups representing different degrees of resistance to thermal oxidation were obtained from DSC curves (Merino et al. 2014): (i) Q1 or labile organic matter, mainly carbohydrates and other aliphatic compounds (200 °C–375 °C); (ii) Q2 or recalcitrant organic matter, i.e., lignin or other polyphenols (375 °C–475 °C); and (iii) Q3 or highly recalcitrant organic matter, such as polycondensed aromatic forms (475 °C–550 °C). T50 DSC and T50 TG, i.e., the temperatures at which 50% of energy (DSC) and 50% mass loss (TGA) of SOM are released under the given conditions, respectively, and were also evaluated, while ash content was obtained from the decrease in weight between 150 °C and 550 °C.

### Analysis of functional C groups by solid-state $^{13}\text{C}$ CPMAS NMR

Samples of forest floor taken in the three stands under study were analysed by solid-state  $^{13}\text{C}$  NMR spectroscopy. In the mineral soil, the SOM contents were too low to get proper spectra. The equipment (Agilent Varian VNMRS-500-WB spectrometer) had a zirconia rotor of volume 160  $\mu\text{L}$  and operated at a proton resonance frequency of 500 MHz. Carbon chemical shifts were referenced to the carbon methylene signal of solid adamantane at 28.92 ppm. Cross-Polarization Magic Angle Spinning (1D CPMAS) analysis was carried out under the following conditions: contact time of 1 ms, inter-scan delay of 1 s (a proton T1 experiment has been performed to check the suitability of that interval), and MAS rate of 12 kHz. The NMR spectra were processed and the MestReNova software 8.1.0 (Mestrelab Research Inc.) was used to quantify the area of the signals. For integration, the spectra were divided into four regions representing different chemical environments of a  $^{13}\text{C}$  nucleus: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–210 ppm). Using MestReNova-7.0.3 (Mestrelab Research S.L., Santiago de Compostela, Spain), the contributions of the various C groups to total C were determined. The integral regions were corrected for spinning sidebands (SSB) when they appeared in the spectra.

### Analysis of P forms by $^{31}\text{P}$ NMR spectroscopy

Nine composite samples were made from the 36 previously obtained for the N and C analyses (paragraph above). On the 9 composite soil samples the  $^{31}\text{P}$ -NMR was performed as described in Campo and Merino (2019). In each of the 9 forest samples (one replicate for each stand type, i.e., pure acacia (100A), eucalypt (100E) and mixed-species stands (50A50E) in 3 out of 5 blocks of the experimentation), a composite sample of each component (forest floor and mineral soils) were created. Therefore, for both components, 9 composite samples (e.g. 1 samples of forest floor for 100A, 100E and 50A50E) were used. These 9 samples were extracted by the method proposed by Cheesman et al. (2010) as modified by Noack et al. (2012). Briefly, 1-g forest floor or soil sample was placed in Falcon centrifuge tubes with 30 mL of 0.25 mol·L<sup>-1</sup> NaOH and 50 mmol·L<sup>-1</sup> EDTA. The tubes were shaken for 16 h at room temperature. The extracts were centrifuged at 6500 rpm for 10 min and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 4300 DW). One mL of 50 mg·L<sup>-1</sup> methylene diphosphonic acid (MDPA) solution was added as an internal standard to 20 mL of each extract, frozen at -80 °C, and lyophilized. Three-hundred mg of each lyophilized extract was then re-dissolved in 0.3 mL of deuterium oxide

(D<sub>2</sub>O) and 2.7 mL of a solution containing 1.0 mol·L<sup>-1</sup> NaOH and 0.1 mol·L<sup>-1</sup> EDTA. This solution was placed in a 5-mm NMR tube for analysis. Spectra were acquired at 25 °C in a Varian VNMRS-500-WB NMR spectrometer at a  $^{31}\text{P}$  frequency of 202.296 MHz.

The recovery delay (0.5 s) was set to both optimize and reduce the time of experiments, while the other conditions were as follows: 90° pulse of 6  $\mu\text{s}$ , acquisition time, 0.2 s, and broadband 1H decoupling. For each sample, 100,000 scans were acquired. The spectra obtained have a line broadening of 2 Hz. Spectroscopic signals were assigned to the different P compounds following Newman and Tate (1980) and Turner et al. (2003a): orthophosphate (around 5.3 ppm), orthophosphate monoesters (3–5.1 ppm), pyrophosphates (-5.5 ppm) and orthophosphate diesters (-2 to 0 ppm). The following monoesters were detected: phosphatidic acid (4.3 ppm),  $\beta$ -glycerophosphate (3.9 ppm), scyllo-inositol (3.2 ppm) and myo-inositol (5, 4.1, 3.8 to 3.4 ppm). The MDPA internal standard appeared at 16.5 ppm. Peak signal areas were distinguished by integration of spectra. All spectral processing was carried out with MestReNova software, version 8.1.0 (Mestrelab Research Inc., Santiago de Compostela, Spain).

### Statistical analyses

The one-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between treatments for measured parameters. Data were assessed for homogeneity and normality. When significant ( $p < 0.05$ ) differences were indicated, Tukey's HSD test was used to assess differences in mean values. Pearson correlations were used to explore different relationships between TA parameters, SOM and P fractions. All analyses were performed with SPSS software (version 20, SPSS Inc., Chicago, IL, USA).

## Results

### Concentrations of N, C and P in forest floor and mineral soils

Table 1 shows the data of C, N and P in mineral soil and forest floor for the three types of stands studied: pure stands of acacia (100A), of eucalypt (100E) and the mixed of acacia and eucalypt (50A50E). The forest floor of the 100A stand shows higher N and P concentrations and therefore lower values in the C/N and C/P ratios. However, this trend could not be identified in the mineral soil, where the three stands show similar values.

### Organic matter characterization of forest floor and mineral soils

The forest floor samples were made up by litter in different degree of decomposition. Since it was collected in April, at the end of the humid period (October–May), it

**Table 1** Concentration of nitrogen (N), carbon (C) and C/N ratio in the forest floor and mineral soil (0–5 cm) in the three types of stands studied: pure acacia (100A), and eucalypt (100E) and mixed-species (50A50E). Mean values with standard error in brackets are SE

	Mass (g·m <sup>-2</sup> )	C (%)	N (%)	C/N	P* (mg·kg <sup>-1</sup> )	C/P*	P** (mg·kg <sup>-1</sup> )
<b>Forest floor</b>							
100A	2.50	25.62 (± 2.88)	1.19 (± 0.12)	22.16 (± 2.55)	0.052	492.7	3.83 (± 0.06)
50A50E	1.10	37.72 (± 1.80)	1.15 (± 0.07)	39.81 (± 3.57)	0.043	877.2	3.65 (± 0.11)
100E	1.25	31.68 (± 2.77)	1.18 (± 0.01)	28.92 (± 3.07)	0.040	792.0	3.80 (± 0.03)
<b>Mineral soil</b>							
100A		1.47 (± 0.110)	0.11 (± 0.009)	14.2 (± 1.08)	0.011	112.7	1.47 (± 0.11)
50A50E		1.70 (± 0.136)	0.16 (± 0.017)	11.4 (± 0.74)	0.012	146.6	1.46 (± 0.00)
100E		1.57 (± 0.183)	0.14 (± 0.019)	12.1 (± 1.58)	0.015	102.2	1.46 (± 0.01)

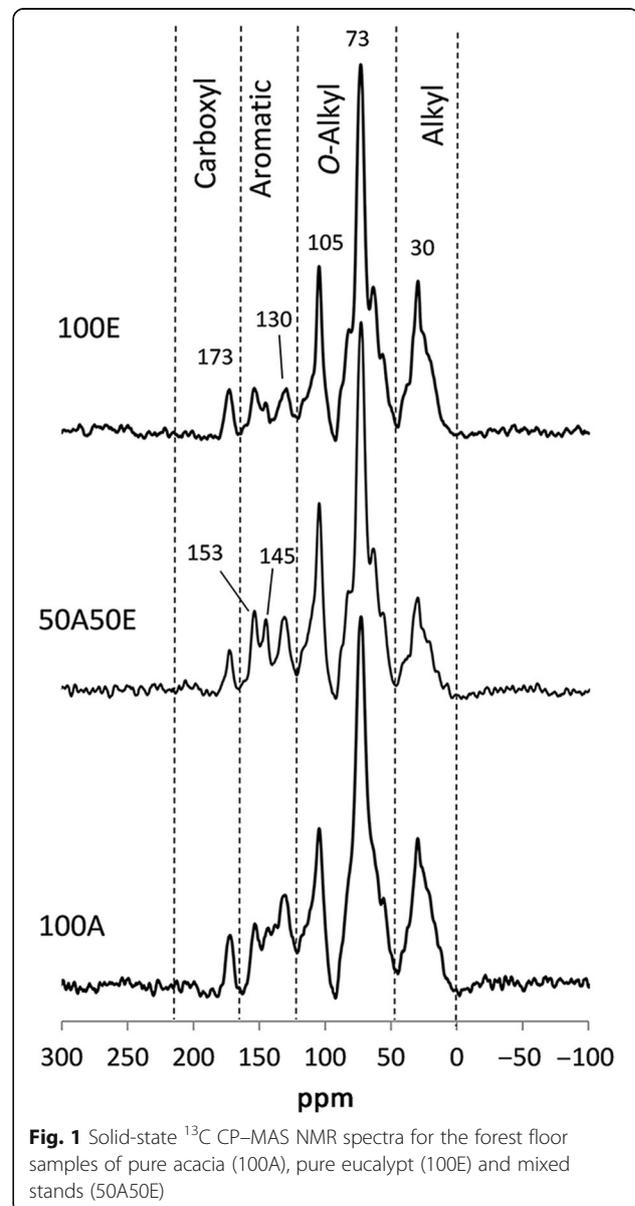
\*NaOH+EDTA extractable P

\*\*P extracted with the anion-exchange resin method

is expected a rather higher amount of decomposed litter. Solid-state <sup>13</sup>C NMR spectra of forest floor samples are shown in Fig. 1, whereas the relative C distribution among the different functional groups is in Table 2. In the forest floor from the three types of stands, the major C components were O-alkyl C (57%–63% of total intensity) and alkyl C (13% and 22% of total intensity) structures. Two prominent signals, generated by cellulose and hemicelluloses, appeared at 73 and 105 ppm. The carbonyl (173 ppm) signals were strongest in 100A, possibly because of the greater contribution of amide C. The alkyl region was mostly dominated by the peak at 30 ppm, derived from long chain aliphatic molecules (lipids, suberin). The signal at 20 ppm may be due to short chain aliphatic structures. In the chemical-shift region of 110–160 ppm (aromatic and phenolic C), the peaks at 153 and 145 ppm are attributed to tannins and lignin (Preston et al. 1997).

The most important differences in the two pure stands are the higher proportion of carbohydrates (O-alkyl C) in 100E and the higher proportion of alkyl groups in 100A. This last stand also showed a prominent peak at 153 ppm, which suggests higher tannin content. The forest floor of the mixed stand showed an intermediate composition in these compounds, and also unveiled a lower proportion of aromatic ones. Different indexes can be associated with the degree of decomposition/humification of the organic matter. The ratio of alkyl/O-alkyl was higher in the stands including acacia (100A and 50A50E), which might indicate a more advanced stage of decomposition/humification of this OM. In line with this, the O-alkyl:aromatic C ratio was lower in these same stands, especially in the mixed one, which might also indicate a more advanced stage of humification (Chavez-Vergara et al. 2014).

The thermal analysis applied to forest floor OM produces two types of information: the energy released (Differential Scanning Calorimetry, DSC) and the weight loss

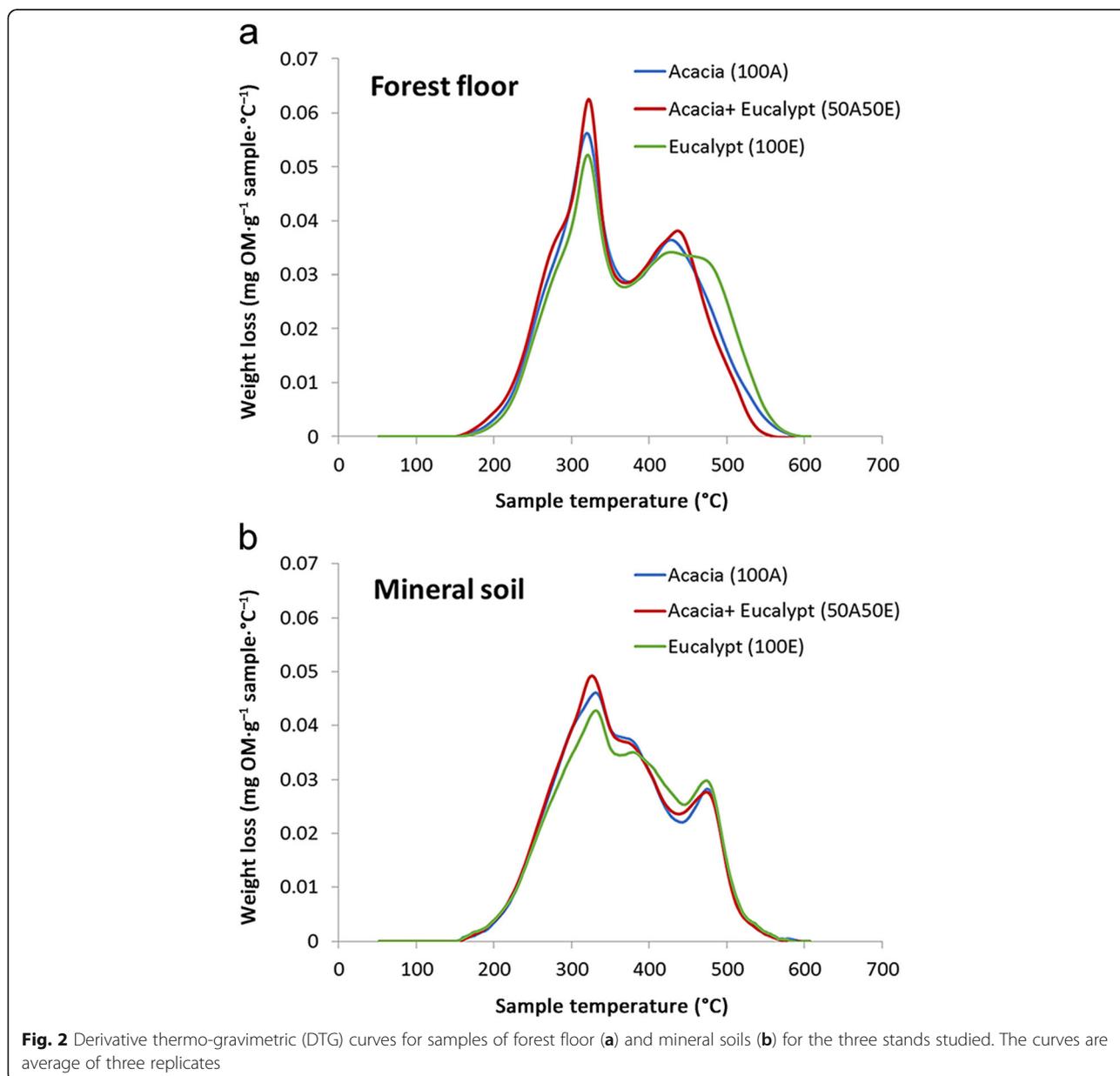
**Fig. 1** Solid-state <sup>13</sup>C CP-MAS NMR spectra for the forest floor samples of pure acacia (100A), pure eucalypt (100E) and mixed stands (50A50E)

**Table 2** Integrated peak areas from <sup>13</sup>C CP-MAS NMR spectra (%) of the forest floor of the three types of stands studied: pure acacia (100A), pure eucalypt (100E) and mixed stands (50A50E)

	Alkyl C (0–45 ppm)	O-alkyl (45–110 ppm)	Aromatic C (110–160 ppm)	Carboxyl C (160–200 ppm)	Alkyl/O-alkyl	O-alkyl:aromatic C	HB/Hi	Aromaticity
100A	20	57	21	1	0.35	2.71	0.71	0.27
50A50E	22	63	13	2	0.35	4.85	0.54	0.15
100E	13	62	22	2	0.21	2.8	0.55	0.29

(Thermogravimetry, TG), which are related. Figure 2 shows the derivative thermogravimetric analysis (DTG) curves for forest floor and mineral soils of the three stands studied. The curves of the forest floor samples were characterized by a bi-modal shape with two peaks

corresponding to the thermal degradation of different organic compounds. The first peak, related with the most labile organic compounds, was located at 315–330 °C. The second one, at 425–435 °C, was less prominent which corresponds to the recalcitrant OM compounds.



**Fig. 2** Derivative thermo-gravimetric (DTG) curves for samples of forest floor (a) and mineral soils (b) for the three stands studied. The curves are average of three replicates

Figure 3 shows the most relevant parameters for the DTG analysis, T50w (the temperatures at which 50% of mass loss of SOM is released) and the three groups of OM with different degrees of resistance to thermal oxidation (W1, W2 and W3). In the forest floor, the most labile OM compounds (200 °C–375 °C) made up 54%–56% of the OM, whereas the most recalcitrant (> 475 °C, W3) varied among 10% and 14%. Although the variability is important, the forest floor of 100A and 50A50E stands showed the lowest OM recalcitrance, which might be related to the higher alkyl/*O*-alkyl ratio and the lower aromaticity (Table 2) found by NMR.

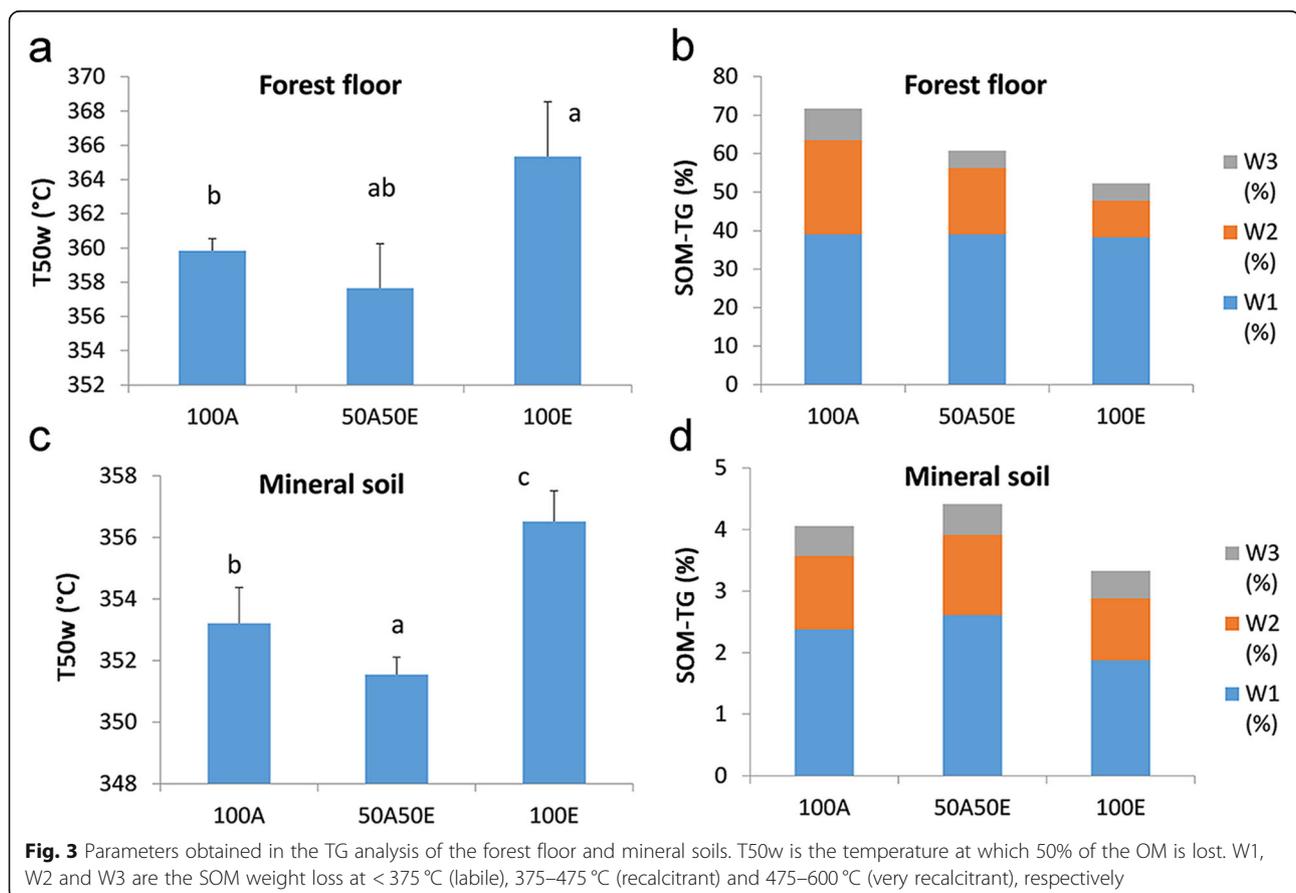
In the mineral soil, the thermolabile SOM made up the 56%–58% of the SOM in the three stands. The SOM of the mixed-species stand showed higher percentage of thermolabile compounds (W2, Fig. 3), and, in accordance with it, a lower recalcitrance (lower T50w). A significant relationship between T50w of the forest floor and mineral soil were found ( $r = 0.55$ ,  $p < 0.05$ ; Fig. 4). The SOM content in the mineral soil was related to the T50w values ( $r = -0.67$ ,  $p < 0.05$ ).

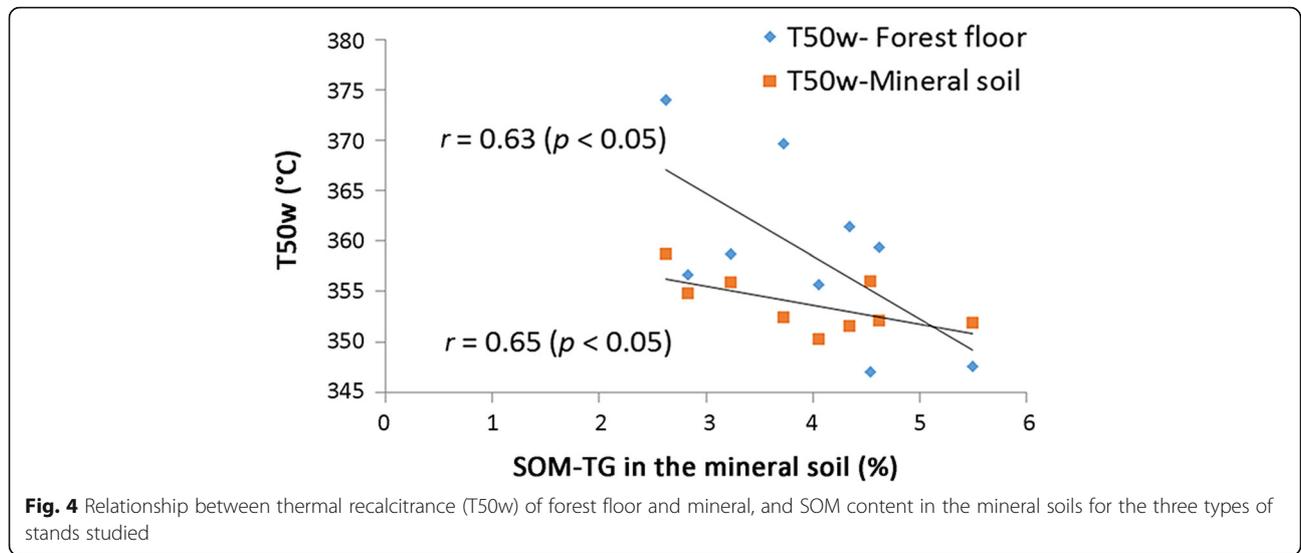
#### Extractable P and P fractions in forest floor and mineral soils

The concentration of NaOH+EDTA extractable P in the forest floor increased with the presence of acacia in the

stands (Fig. 5). The Fig. 6 shows the  $^{31}\text{P}$  NMR spectra of the extractable P in these samples, and the Fig. 7, the distribution of P forms. In the forest floor of the three stands, the main P form was found as orthophosphate ( $243.5 \pm 0.113 \text{ mg P}\cdot\text{kg}^{-1}$ ) (Fig. 6). Inorganic P (Orthophosphate-P and Pyrophosphate, this latter almost negligible) made up  $55\% \pm 3.3\%$  of the extractable P. The second P form was monoesters-P, which represented the  $37.4\% \pm 3.0\%$  of the extractable P. Diester-P made up the  $7.7\% \pm 0.5\%$  of the extractable P. It is important to point out that the forest floor of the mixed stands showed the highest proportion of organic P ( $51\% \pm 2\%$ ). In these stands both monoesters and diester P were higher than in the pure stands (100A and 100E). In the three cases, the two main monoester-P forms were myo-inositol and  $\beta$ -glycerophosphate, which made up the 88% of the monoesters in the three stands (Fig. 7b). The forest floor of the mixed stand showed a rather different distribution of monoesters with respect to the pure stands: lower myo-inositol and higher concentrations of phosphatidic acid and  $\beta$ -glycerophosphate.

Unlike the forest floor, in the mineral soil, the pure eucalypt stand (100E) showed a lightly higher extractable P (Fig. 5). In the current study, higher extractable concentration of P was found in forest floor of pure acacia





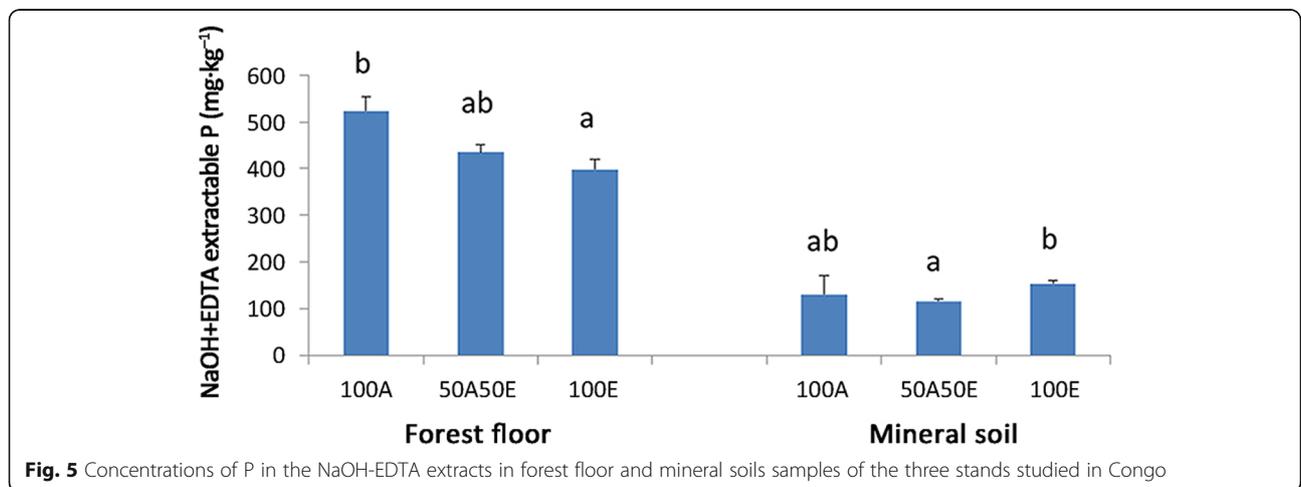
**Fig. 4** Relationship between thermal recalcitrance (T50w) of forest floor and mineral, and SOM content in the mineral soils for the three types of stands studied

(100A, Fig. 5). Like in the forest floor, soils orthophosphate P was the predominant P form in the mineral soils of the three stands ( $66.5\% \pm 0.35\%$ ) (Fig. 7c). Pyrophosphate, the other inorganic P compound, was negligible in the three soils. With respect to the organic P forms, monoesters were the only organic P compounds with significant concentrations in the tree soils, and represented the 67%–68% of the extractable P. Diester-P was found in very low concentrations in the three stands (<2%) (Fig. 7c). Although the three stands show similar distribution in extractable P forms, the pure eucalypt stand reveals a lightly higher percentage of organic P (33%). The concentration of monoester-P compounds followed the following decreasing trend: myo-inositol > scyllo-inositol >  $\beta$ -glycerophosphate ~ phosphatidic acid (Fig. 7d). Nevertheless, the concentrations of the 4 monoesters were higher in this stand, reflecting different dynamics.

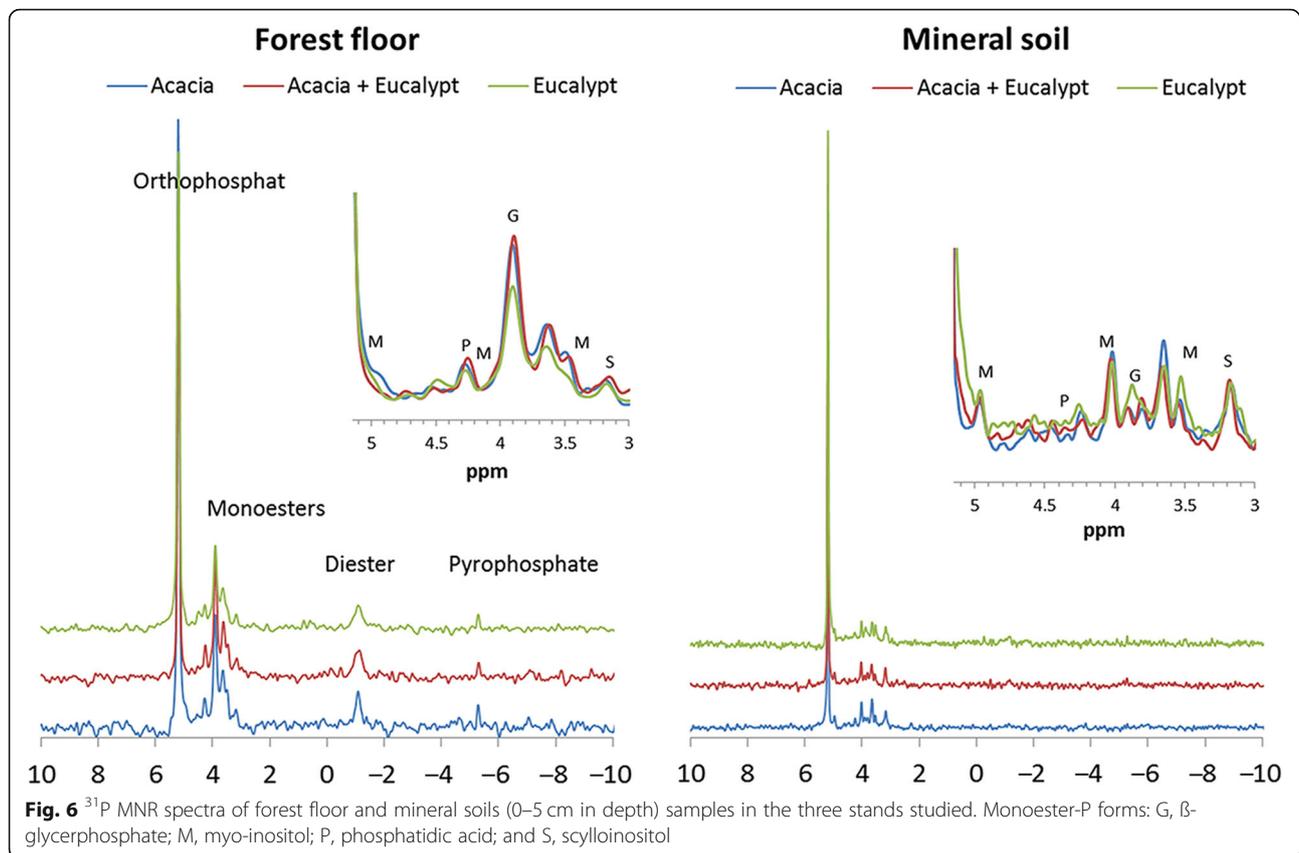
**Discussion**

**Effects of tree species mixture on chemical composition and stability of organic matter in forest floor and mineral soils**

Under a determined environmental condition, the activity of decomposer community is highly influenced by chemical composition of the litter, which determines the great variation of litter decomposition found in the different plant species (de Toledo and de Oliveira 2008; Baldrian et al. 2012; Wang et al. 2019). Litter nutrient concentration, particularly C/N and C/P ratios, as well as the molecular composition, have been identified as main drivers of the first stage of litter decomposition on forest ecosystems (Almendros et al. 2000; Duboc et al. 2012; Freschet et al. 2012). The forest floor of the mixed-species stands showed differences in OM quality with respect to the two pure stands, which might be due to a different pattern in the decomposition/humification process. The



**Fig. 5** Concentrations of P in the NaOH-EDTA extracts in forest floor and mineral soils samples of the three stands studied in Congo

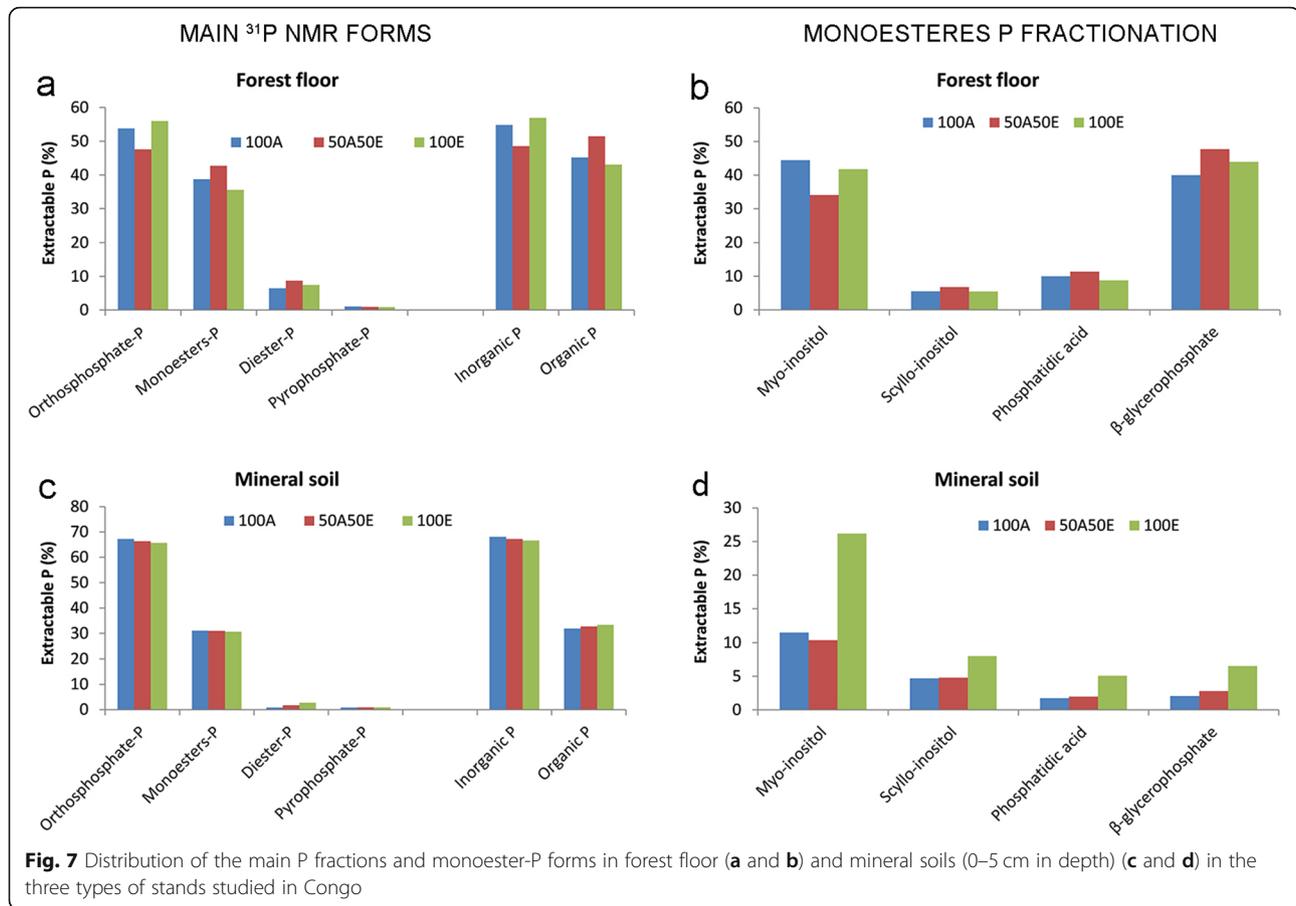


forest floor OM of this stand showed lower aromaticity ( $^{13}\text{C}$  NMR spectroscopy, Table 2) and, in relation with this, lower thermal recalcitrance (T50w values in thermal analysis, Fig. 2). These findings suggest a more advanced stage of humification of this litter with respect to the pure stands, even though the greater amount of harvest residues were reported in stands containing acacia (100A:  $11.3 \text{ Mg}\cdot\text{ha}^{-1}$ , 50A50E:  $11.6 \text{ Mg}\cdot\text{ha}^{-1}$  and 100E:  $7.7 \text{ Mg}\cdot\text{ha}^{-1}$ ; Tchichelle et al. 2017) and higher litterfall mass (100A:  $0.48 \text{ Mg}\cdot\text{ha}^{-1}$ , 50A50E:  $0.32 \text{ Mg}\cdot\text{ha}^{-1}$  and 100E:  $0.26 \text{ Mg}\cdot\text{ha}^{-1}$ ; Tchichelle et al. 2017).

Other studies e.g. Forrester et al. (2005), Xiang and Bauhus (2007), Forrester et al. (2013) have shown higher decomposition rates in mixed eucalypt-acacia litter. This leads to the greater SOM content in the mineral soil of this mixed-species stands along with enhanced SOM mineralization, observed in the present study (Table 1 and Fig. 3b). This seems to be involved by the accelerated decomposition of this litter due to enhanced dynamics of soil microbial indicators (Pereira et al. 2018). An increase in microbial activity has been also reported in eucalypt intercropped with acacia stands relative to pure or fertilized stands at 27 months after planting (Pereira et al. 2018). When mixed with eucalypt, acacia does change the soil microbial and bacterial communities, which are strongly correlated to

N, C and P contents, revealing a more effective nutrient cycling, greater stimulation of microbial activity in litter and soil (Bini et al. 2012, 2013; Pereira et al. 2017). Furthermore, arbuscular mycorrhizal fungi root colonization and the activity of acid and alkaline phosphatase increased in eucalypt intercropped with *A. mangium* with further improvement in P cycling P nutrition (Bini et al. 2018).

The negative relationship between recalcitrance of the forest floor and SOM content in the mineral soil (Fig. 4) also supports the idea of a greater gain in SOM as a consequence of the faster decomposition/humification rate. In spite of the low C/N and C/P values in the forest floor of the 100A stand, which might favour decomposition (Manzoni et al. 2010), the thermal and  $^{13}\text{C}$  NMR analysis did not reveal clear changes in SOM quality in the forest floor of the two pure stands (100A and 100E), which might indicate a different decomposition pattern. These results are in line with Bacheaga et al. (2016), reporting low decomposition rate for acacia litter even though N and P concentrations were higher. However, N concentration in forest floor litter in acacia was higher ( $1.66\% \pm 0.16\%$ ) than in eucalypt ( $0.44\% \pm 0.06\%$ ) stands, while the stock ranged from  $254 \pm 36 \text{ kg}\cdot\text{ha}^{-1}$  in acacia forest floor against  $72 \pm 6 \text{ kg}\cdot\text{ha}^{-1}$  in the eucalypt counterpart (Tchichelle et al. 2017).



The lower OM recalcitrance in the mineral soil beneath acacia (100A and 50A50E stands) seems might have induced the higher N mineralisation (100A: 0.17, 50A50E: 0.15 and 100E: 0.09; Tchichelle et al. 2017). Furthermore, the authors reported significantly higher amounts of N returning to the soil through harvest residues and litterfall in acacia than eucalypt, in accordance with lower C/N ratios in the active part SOM, i.e., the particulate organic matter (POM) in 100A compared to 100E suggesting an improved soil N status in acacia stands (Koutika et al. 2017). Marschner et al. (2008) argued that SOM dynamics might be controlled by its chemical recalcitrance and the microbial activity defined by environmental conditions in mineral soil. However, the authors concluded that this should not be considered as a long-term organic C stabilization, as SOM pool, mainly its sink C capacity, are sensitive to climatic changes. Even though surrounding environment such as physicochemical and biological interactions control the rate of decomposition, C storage greatly depends on root-derived C in soil, which contains higher amount of resistant biopolymers than on aboveground inputs of leaves (Lorenz et al. 2007; Feng et al. 2008; Pisani et al. 2014). This underlines the importance of C allocation by

plants which further affect organic matter quality and quantity and its determinant impact on soil C dynamics and sequestration (Schmidt et al. 2011; Lal 2014). This is enhanced by the lack of aggregate-forming clays, i.e., low C-storing capacity of the studied sandy soil.

### Effects of tree species mixture on soil P forms and transformations

#### P in forest floor

The extractable P in forest floor is considerably lower than the data reported for natural forests from other tropical areas (Campo and Merino 2019). This might be related to the lower concentration of this element in the litterfall of the studied plantations established on the sandy soils (Koutika et al. 2016). The higher extractable P found in the forest floor of the acacia stands might be related to the higher inputs of P of the harvest residues and litterfall (Koutika et al. 2016). Stock of P in slash left after harvest at the end of the first rotation was higher in stands containing acacia following the order: 100A ( $2.9 \pm 0.3 \text{ kg} \cdot \text{ha}^{-1}$ ) > 50A50E ( $2.8 \pm 0.3 \text{ kg} \cdot \text{ha}^{-1}$ ) > 100E ( $1.6 \pm 0.02 \text{ kg} \cdot \text{ha}^{-1}$ ). Furthermore, higher total P concentration was reported in acacia wood ( $0.61 \text{ g P} \cdot \text{kg}^{-1}$  of dry mass (DM)) than in eucalypt ( $0.57 \text{ g P} \cdot \text{kg}^{-1}$  of DM) in

mixed-species stands at 2 years into the second rotation. This is confirmed by the higher extractable P concentration in acacia litterfall relative to eucalypt (Fig. 5).

The  $^{31}\text{P}$  NMR analysis carried out in the present study showed that orthophosphate was the dominant P form in the forest floor of these plantations. The high percentage of orthophosphate is in agreement with the findings of Campo and Merino (2019) for tropical forests, who reported an increased proportion in areas of long drought period. The values of organic P form are much lower than those found in SOM rich soils from temperate regions, in which they are dominant (Merino et al. 2019). The great concentration of orthophosphate could also be due to the lack of leaching during the long dry period. The main monoester compound is myo-inositol, which is derived from plant, soil microbial and mycorrhizae activity (Bunemann et al. 2008; Cade-Menun 2017). In spite of this general trend, the forest floor under the mixed plantation showed higher proportion of organic P. This higher organic P is mainly due to the higher concentration of diester,  $\beta$ -glycerophosphate and phosphatidic acid compounds (Fig. 6), which are produced by the hydrolysis of RNA of bacteria and fungi (Turner et al. 2003a, 2003b, 2005). Taking into account the higher concentrations in these compounds, and also the higher degree of humification of this litter, the increase of organic P in the mixed stand might be reflecting a process of microbial immobilization.

#### **P in mineral soils**

The NaOH-EDTA extractable P concentrations found in the mineral soils were low in comparison with observed in other tropical forest soils (Campo and Merino 2019), and those from temperate areas (Merino et al. 2019). The coarse textured soils under study are inherently poor in extractable P due to the low SOM content and pH. Most of the mineral soil P was in inorganic (70%) form. This is in accordance with the findings of Rocha et al. (2019), reporting 30% of P in organic against 70% in inorganic forms in the soil of the *Eucalyptus grandis* plantations at harvest after 12-year growth at Itatinga, São Paulo, Brazil. Our findings suggest that the lower extractable P in the mineral soil in the mixed-species stands (Fig. 5) might be due to lower P leaching from the forest floor as a consequence of the greater microbial immobilization, since a real decrease in available P (Koutika et al. 2014) would lead to a lower foliar P. As in the forest floor, the main P form in the mineral soils is orthophosphate, which is a characteristic feature of tropical SOM-poor soils with long drought period (Campo and Merino 2019). With respect to the forest floor, the mineral soils showed important decreases in all organic P compounds, but specially in the most labile compounds diester,  $\beta$ -glycerophosphate and phosphatidic acid (Fig. 7a and c), considered the most

labile organic P compounds. The decreases in myo-inositol and scyll-inositol, considered to be more stable to microbial decomposition due to their higher charges able to react with both organic and inorganic soil compounds (Turner et al. 2003a, 2003b), were lower. It can be pointed out that the decreases in all monoesters and diester P were lower in the eucalypt soil in comparison with the stands containing acacia, and might reflect their lower mineralization rates (Tchichelle et al. 2017). This may also be due to the lower OM lability i.e., higher T50w in this mineral soil. On the other hand, in spite of the light lower concentration in extractable P in mineral soil found in the mixed-species stand, a previous study (Koutika et al. 2016) revealed a higher foliar P concentration in these stands. The results of the present study are in agreement with previous studies, showing that forest floor might be playing an important role in the P dynamics in forest ecosystems (Lang et al. 2017; Rodtassana and Tanner 2018). On one hand, the P input through litter decomposition and microbial activity ensures the supply of P to the plant. In addition, in these ecosystems characterized by fast mineralization rates, the microbial immobilization generating organic P compound contributes to sustain the P demand in medium term and preventing P losses by leaching. The data show that, in these P-poor soils, the distribution of soil organic P forms is critical for the long-term supply to plants and for the sustainability of the ecosystems.

#### **Conclusions**

The forest floor of the mixed-species stands shows differences in OM quality with respect to the pure stands. The lower thermal recalcitrance (TA) and aromaticity (NMR spectroscopy) suggest a faster decomposition/humification rate. Higher microbial activity in the forest floor of the mixed acacia and eucalypt stand might be favouring the incorporation of C into the mineral soil. Most of the extractable P in both forest floor and mineral soil is found as inorganic, which might be reflecting the high mineralization rate and the lack of soluble leaching during the drought period. The higher extractable P concentrations and stock found in the forest floor of the stands containing acacia is mainly due to the higher inputs of P via harvesting residues and litterfall in these stands.

The greater biological activity in the mixed-species stands would contribute to generate more organic P than in pure stands, which would prevent the P losses to the mineral soil. The lower extractable P in mineral soil of this stand may be due to the lower P releases from the forest floor. The higher foliar P concentrations found in this stand may reveal a mechanism able to sustain the P demand in this P-poor ecosystem and a potential benefit on C sequestration and climate change mitigation.

## Abbreviations

N: Nitrogen; C: Carbon; P: Phosphorus; NFS: Nitrogen fixing species; SOM: Soil organic matter; POM: Particulate organic matter; OM: Organic matter; DM: Dry mass; CEC: Cation exchange capacity; cPOM: coarse particulate organic matter; 100A: Pure acacia or 100% acacia; 50A50E: 50% acacia and 50% eucalypt or half acacia and half eucalypt; 100E: Pure eucalypt or 100% eucalypt; R1Y7: Year 7 of the first rotation; R2Y2: Year 2 of the second rotation; R2Y5: Year 5 of the second rotation; DSC: Differential scanning calorimetry; TGA: Thermogravimetry; <sup>31</sup>P-NMR: Phosphorus-31 Nuclear magnetic resonance; <sup>13</sup>C CPMAS NMR: Solid-state <sup>13</sup>C NMR spectroscopy

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## Authors' contributions

LSK planned the project, made analyses on available P, wrote the first draft and contributed to the final stage; LC made soil analyses (C, N and S); AB contributed to the planning and supervision of soil analyses at ENEA (Italy), supervised the TWAS-ENEA International Research Fellowship and contributed to the final version. AM planned the project, supervised most analyses of the study which have been conducted in Spain, made statistical analyses, wrote and improved the manuscript by providing literature, suggestions and knowledge. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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