Introducing N_2-fixing trees (*Acacia mangium*) in eucalypt plantations rapidly modifies the pools of organic P and low molecular weight organic acids in tropical soils.

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Abstract

Many studies have shown that introducing N_2-fixing trees (e.g. *Acacia mangium*) in eucalypt plantations can increase soil N availability as a result of biological N_2 fixation and faster N cycling. Some studies have also shown improved eucalypt P nutrition. However, the effects of N_2-fixing trees on P cycling in tropical soils remain poorly understood and site-dependent. Our study aimed to assess the effects of planting *A. mangium* trees in areas managed over several decades with eucalypt plantations on soil organic P (Po) forms and low molecular weight organic acids (LMWOAs). Soil samples were collected from two tropical sites, one in Brazil and one in the Congo. Five different treatments were sampled at each site: monospecific acacia, monospecific eucalypt, below acacias in mixed-species, below eucalypts in mixed-species as well as native vegetation. Po forms and LMWOAs were identified in sodium hydroxide soil extracts using ion chromatography and relationships between these data and available P were determined. At both sites, the concentrations of most Po forms and LMWOAs were different between native ecosystems and monospecific eucalypt and acacia plots. Also, patterns of Po and LMWOAs were clearly separated, with glucose-6-P found mainly under acacia and phytate and oxalate mainly under eucalypt. Despite the strongest changes occurred at site with a higher N_2 fixation and root development, acacia introduction was able to change the profile of organic P and LMWOAs in less than 10 years. The variations between available Pi, Po and LMWOA forms showed that P cycling was dominated by different processes at each site, that are rather physicochemical (via Pi desorption after LMWOAs release) at Itatinga and biological (via organic P mineralization) at Kissoko. Specific patterns of Po and LMWOAs forms found in soil sampled under acacia or eucalypt would therefore explain the effect of acacia introduction in both sites.

Keywords: Mixed-species plantation, P cycling, Ion chromatography, Ferralsol, Brazil, Congo

1. Introduction

Eucalyptus plantations cover about 25 million ha worldwide (Borralho et al., 2018). *Eucalyptus* species have been planted in many tropical countries to face the growing demand of firewood and pulp (Booth, 2013) as in Brazil where the area cultivated with eucalypts is about 7.5 million ha (IBGE, 2019). Large amounts of N and P mineral fertilizers are required in tropical eucalypt plantations to sustain high yields (Gonçalves et al., 2013). The introduction of N_2-fixing tree species (NFT), such as *Acacia mangium* (Willd) into eucalypt plantations could provide an alternative to mineral fertilizers (Forrester et al., 2006; Laclau et al., 2008). *A. mangium* is a fast-growing nitrogen fixing tree species largely planted in the tropics (Yamashita et al., 2008), as in Indonesia where the area of *A. mangium* plantations is about 1.6 million ha (Hardie et al., 2018). Mixed-species plantations associating acacias with eucalypts have been established in several tropical countries and have made it possible to increase the total aboveground biomass compared to monospecific eucalypt stands in Australia (Forrester et al., 2004), Brazil (Santos et al., 2016), Hawaii (Binkley et al., 2003) and the Congo (Bouillet et al., 2013), although a similar benefit was not observed in several other experiments in Brazil (Bouillet et al., 2013; Santos et al., 2016). The effects of acacias on N cycling in mixed-species plantations have been intensively studied. Whatever the effect on the growth of the other trees, introducing acacias into mixed plantations with eucalypts significantly increased soil N mineralization and N budget compared to eucalypt monocultures (see for example Koutika and Mareschal (2017) in the Congo and Voigtlaender et al. (2019) in Brazil).

Remarkably, available P measured in soils under acacias or in mixed plantations was often lower than under eucalypts (Koutika et al., 2014; Koutika et al., 2016; Koutika, 2019). The same effect was reported in plantations with *E. saligna* and the NFT *Albizia facaltaria* (Binkley et al., 2000). Furthermore there is a greater accumulation of P in biomass of the NFT than in the non-NFT when grown as monocultures (Binkley et al., 2000; Le Cadre et al., 2018), suggesting that the NFT may be able to modify the P cycle in soil to increase plant P uptake and accumulation. The factors that could explain the increase in P bioavailability under acacia are still poorly understood. One of the most likely mechanism could be related to the enhanced phosphatase release from acacia roots, which would increase Po mineralization (Zou et al., 1995; Khanna, 1997). We can also assume that indirect effects related to N input, such as the stimulation of soil organic matter (SOM) decomposition (Forrester et al., 2005)

90 and the modifications of microbial communities (Santos et al., 2017; Pereira et al., 2019)
91 could also impact P cycling and P bioavailability, even if direct links need to be shown.
92 The main source of P for plant uptake is considered to be mineral P as free
93 orthophosphate ions (Pi) in the soil solution (Hinsinger, 2001). These orthophosphate ions can
94 be adsorbed onto soil constituents such as clay, oxides and organic matter (OM), and can form
95 numerous complexes with cations, becoming unavailable for plant uptake. An efficient way to
96 desorb Pi is to release low molecular weight organic acids (LMWOAs) such as citrate, oxalate
97 and malate. Thanks to their carboxyl groups on their structure (Jones, 1998; Plassard and
98 Fransson, 2009), these compounds are able to mobilize soil P through three processes: mineral
99 solubilization, ligand exchange and complexation with cations (Jones, 1998; Wang and
100 Lambers, 2020). Several studies have demonstrated the efficiency of those organic anions,
101 especially citrate bearing three carboxylic groups, to release soluble P from Fe or Ca
102 (Gypser and Freese, 2020; reviewed by Wang and Lambers, 2020). Also, Casarin et al.
103 (2004) found a linear relationship between bicarbonate-extractable P concentrations and
104 oxalate concentrations in samples of cambisol collected under ectomycorrhizal hyphae. In
105 addition, to sustain the release of orthophosphate ions to the soil solution, LMWOAs could
106 also increase the release of organic P forms complexed with cations or metals and substrate
107 availability to phosphatase enzymes, thus accelerating P cycling in soil.
108 This effect of LMWOAs could be very important because organic P (Po) can represent
109 more than 50% of total P in the topsoil, especially in forest ecosystems (Vitousek and
110 Sanford, 1986; Achat et al., 2010; Vincent et al., 2010). This organic P fraction originates
111 from living cells that have metabolized Pi into various components such as nucleic acids
112 (DNA, RNA), phospholipids or metabolic compounds such as those used for energy storage
113 (adenosine triphosphate ATP, adenosine diphosphate ADP and adenosine monophosphate
114 AMP) and cellular metabolism (glucose-6-phosphate G6P or fructose-1,6-bisphosphate Fruc
115 bisP). Depending on the nature of phosphorus bond, Po is can be classified into 3 classes:
116 phosphate esters (P-O-C), phosphoric acid anhydrides (P-O-P) and phosphonates (P-C)
117 (Turner et al., 2005; Darch et al., 2014). Phosphate esters can be divided into 2 sub-classes:
118 phosphate monoesters (P monoesters) containing a single C chain (R) linked to the P group
119 (R-O-P), such as glucose-6-phosphate (G6P), mononucleotides or inositol hexakisphosphate
120 (phytate), and phosphate diesters (P diesters) containing two C chains linked to the same P
121 group (R_1-O-P-O-R_2), such as nucleic acids and phospholipids. Some phosphoric acid
122 anhydrides, such as ATP, are used to store energy but they are also found in inorganic P

123 compounds such as linear polyphosphates and pyrophosphate. Phosphonates are present in
124 living cells in compounds such as 2-aminoethyl phosphonic acid (Turner et al., 2005). The
125 main Po forms detected in the topsoil of various ecosystems are P monoesters, as reported for
126 forest soils (Turrión et al., 2001; Turner and Engelbrecht, 2011), for pasture soil (Doolette et
127 al., 2009) and for cultivated soils (Bünemann et al., 2008a; Ahlgren et al., 2013). The P forms
128 in plants and bacteria are predominantly P monoesters and diesters while P monoesters,
129 pyrophosphates and polyphosphates are the most common in fungi (Makarov et al., 2005).

130 The Po forms extracted from soil with NaOH solution have been commonly analyzed
131 using ^{31}P NMR spectroscopy (Turner et al., 2003; Smernik and Dougherty, 2007; Zhang et al.,
132 2012; Cade-Menun and Liu, 2014; George et al., 2018). This method has been used
133 successfully to determine the concentrations of P diesters and P monoesters in the topsoil of
134 tropical forests (Vincent et al., 2010; Aleixo et al., 2019; Aleixo et al., 2020), and determine
135 the recalcitrance of some compounds such as phytate in soils with high iron and aluminum
136 contents (Vincent et al., 2012). Recently, the Po composition of NaOH extracts from tropical
137 soil has been determined using ion chromatography (IC) (Waithaisong et al., 2015). Although
138 IC less capable than ^{31}P NMR because only P monoesters are separated and quantified in
139 NaOH extracts and not P diesters, this method has the advantage of separating LMWOAs and
140 P forms (P-monoesters and phosphoric acid anhydrides, whether organic or inorganic) in a
141 single run (Waithaisong et al., 2015).

142 So far, no data have been reported in the literature on the effects of fast-growing tree
143 species such as eucalypt and acacia, either planted in monoculture or in mixed plots, on the
144 composition of soil Po and LMWOAs. A recent study using NMR has shown that associating
145 NFT with non-NFT increases the availability of soil P by exploiting different P sources, with
146 a strong increase of the stocks of soil P forms (P monoesters, DNA, pyrophosphate and
147 orthophosphate) (Aleixo et al., 2020). Although the relationships between N and P cycling in
148 forest ecosystems have been investigated in many studies (Lu et al., 2013; Huang et al., 2014;
149 Yang and Zhu, 2015), the effect of increasing N on soil P bioavailability is not clear. In some
150 cases, increasing N has been found to alter microbial community composition by decreasing
151 microbial biomass (Li et al., 2014; Zang et al., 2017) and enzymatic activities (Turner and
152 Wright, 2014), suggesting a negative effect on the P cycle. However, the bioavailability of
153 soil P is important for the N cycle as nitrogen-fixing symbiosis requires high amounts of P
154 (Ribet and Drevon, 1996) that can be satisfied by the production of extracellular phosphatase
155 enzymes. These are rich in N, and thus demanding in N (Treseder and Vitousek, 2001).
156 Several examples highlight the ability of NFTs to regulate the biomass, diversity and

157 functioning of soil microbes by modifying soil extracellular enzyme activities (Rachid et al.,
158 2013; Huang et al., 2014; Rachid et al., 2015; Santos et al., 2017; Bini et al., 2018; Pereira et
159 al., 2018; Pereira et al., 2019). The introduction of acacia trees in eucalypt plantations also
160 modifies mycorrhizal symbiosis with both arbuscular (Bini et al., 2018; Pereira et al., 2018)
161 and ectomycorrhizal fungi (Rachid et al., 2015).

162 As suggested from previous research, we hypothesized that the introduction of acacia
163 trees in eucalypt plots could modify P cycling leading to different patterns of organic P forms
164 and/or LMWOAs in the soil. In order to check this hypothesis, we asked the following
165 questions (1) does planting exotic fast-growing species modify Po and LMWOA pools
166 relative to native vegetation? (2) do the Po and LMWOA pools differ among eucalypt or
167 acacia monospecific plantations? (3) is acacia introduction in eucalypt plots able to modify Po
168 and LMWOA pools relative to monospecific plots? (4) could we use the variations between
169 available Pi, Po and LMWOA forms to understand P cycling? We addressed these questions
170 in two tropical experiments, one in Brazil and the other in the Congo with blocks of the same
171 treatments: monospecific *Acacia mangium*, monospecific *Eucalyptus* and mixed-species plots
172 with 50% of each species as well as the nearby native vegetation on the same type of soil.
173 Although the sites have comparable characteristics, with nutrient poor, acidic, sandy soils, N_2
174 fixation by the acacias was the highest at the Congo site (Tchichelle et al., 2017; Paula et al.,
175 2018). In addition, acacias grew better than eucalypts only at the Congo site (Bouillet et al.,
176 2013). After measurement of available P with bicarbonate, and total P with NaOH, we
177 separated and quantified the Po and LMWOA forms using ion chromatography.

178

179 **2. Materials and methods**

180

181 **2.1. Site description**

182 The study was conducted at two sites: one in Brazil, in the São Paulo state (Itatinga
183 site), and the other in the Congo, on the Atlantic coast of Pointe Noire (Kissoko site). The
184 main characteristics of the two sites are shown in supplementary table 1. Annual rainfalls
185 were close at the two sites while the mean annual temperature was about 5°C lower at Itatinga
186 (20°C) than at Kissoko (25°C). The native ecosystems were replaced by *Eucalyptus*
187 plantations in 1940 at Itatinga and in 1984 at Kissoko. The native ecosystems present before
188 afforestation were tropical savannas dominated by trees and shrubs at Itatinga (Maquere,
189 2008) while grasses were dominant at Kissoko (Laclau et al., 2002).

190 *A. mangium* was introduced in May 2003 at Itatinga and in May 2004 at Kissoko in
191 order to compare the wood production under different silviculture practices (Bouillet et al.,
192 2013). *Eucalyptus grandis* was planted at Itatinga and a hybrid between *Eucalyptus grandis*
193 and *Eucalyptus urophylla* (*E. urophylla x grandis)* was planted at Kissoko. The effects of
194 introducing acacias into the eucalyptus plantations were different between the two sites and
195 are summarized in the supplementary table 1. The N_2 fixation in acacia plots was much higher
196 at Kissoko than at Itatinga (Tchichelle et al., 2017; Paula et al., 2018; Voigtlaender et al.,
197 2019). At the end of the rotation, the acacia trees in monospecific plots produced more above
198 total biomass than the eucalypts in the Congo but not in Brazil (Bouillet et al., 2013). When
199 grown in mixed-species plots, the ratio of acacia to eucalypt biomass decreased less at
200 Kissoko than at Itatinga. Eucalypts planted with acacia were more productive than eucalypt
201 monoculture at Kissoko, but less productive at Itatinga (Epron et al., 2013). At Itatinga, all
202 trees were harvested in May 2009 and the second rotation was established in November 2009
203 with the same treatment at the same position. At planting, the eucalypts and acacias were
204 fertilized with P (40 kg ha^{-1}) at Itatinga and N (43 kg ha^{-1}) at Kissoko, within a radius of 50
205 cm around the tree (Bouillet et al., 2013).
206 The soil characteristics of the two sites are presented in table 1. The soils were
207 Ferralsols at Itatinga and Ferralic arenosols at Kissoko (FAO-UNESCO, 1989). These acidic
208 sandy soils are low in exchangeable elements, with a low CEC (Cation Exchange Capacity).
209
210 **2.2. Experimental design and soil sampling**
211 At each site, there were three blocks for each treatment: monospecific *A. mangium*
212 (Ac), monospecific *Eucalyptus* (Euc) and mixed-species with 50% of each species (50:50), as
213 well as nearby native vegetation on the same soil type (Nat). In addition, for the 50:50
214 treatment, we distinguished two zones, one close to acacias (noted Ac-AE) and one close to
215 eucalypts (noted E-AE), giving five treatments in total. For Ac, Euc and 50:50, each block
216 had 10 x 10 trees (6 x 6 inner rows), planted at 3 m by 3 m at Itatinga and 3.33 m by 3.75 m at
217 Kissoko. In these blocks, the 0-10 cm soil layer was sampled for the zones around three pairs
218 of trees along a diagonal near the center of the plot (Fig. 1A-C) using a cylindrical steel soil
219 corer with an internal diameter of 5 cm driven into the soil by a sledgehammer. For each tree,
220 5 soil cores were taken inside a quarter of the Voronoï square always located at the right side
221 of the tree to allow for spatial variability. Three composite samples (R1, R2, R3) were made
222 from the 10 soil cores collected near the pair of trees. For the native ecosystem, we chose
223 three nearby locations (equivalent to the three blocks of each treatment). In each native

224 location, we sampled 10 soil cores from the 0-10 cm soil layer every meter along each of
225 three transects, to give three replicated composite samples (Fig. 1 D). The soil was sampled at
226 the end of rainy season at both sites (in February 2012 at Itatinga and May 2009 at Kissoko).
227 The soil samples were air dried, sieved at 2 mm and stored at room temperature until analysis.
228

229 **2.3. Soil extraction and colorimetric P determination**

230 Two extractions were carried out on air-dried soil samples. First, labile P was
231 extracted with 0.5 M $NaHCO_3$, pH 8.5 according to Olsen et al. (1954) procedure. Briefly, the
232 mixture soil – bicarbonate solution (1/10, w/v) was shaken end-over-end for 30 min at room
233 temperature. After centrifugation (2683 rcf, 10 min) the supernatant was filtered through a
234 0.22 µm cellulose membrane filter before measuring free Pi, with or without mineralization of
235 the solution (see below). Then, P and LMWOAs were extracted with 0.5 N NaOH (1:1, w/v)
236 as described in Waithaisong et al. (2015). The soil mixture was shaken end-over-end for 16h
237 at room temperature and centrifuged as for labile P. The supernatant was acidified with 6 N
238 HCl (1/300, v/v) and left at room temperature for 3 h to precipitate the humic acids. The
239 solution was then centrifuged again (2683 rcf, 20 min) and the supernatant was used as the
240 soil extract for assaying free orthophosphate ions, before or after mineralization, and for ion
241 chromatography (IC). A part of the soil extract was immediately stored at -20°C before IC.
242 The total P of bicarbonate and NaOH extracts was obtained by digestion with 12 N HCl (v/v)
243 at 110 °C for 16 h (Ali et al., 2009). The free phosphate ion (Pi) concentrations were
244 measured using the malachite green method (Ohno and Zibilske, 1991). The organic P was
245 estimated by subtracting the free Pi from the total P.
246

247 **2.4. Organic P and LMWOA analyses**

248 Following Waithaisong et al. (2015), five Po forms (glucose-6-phosphate, fructose-
249 1,6-bisphosphate, AMP, ATP and phytate), inorganic phosphate, pyrophosphate, and four
250 LMWOAs (malate, malonate, oxalate and citrate) were determined by IC. However, to reduce
251 analysis time, we used only three of the nine composite samples for each treatment. To select
252 these 3 replicate samples, the average of Po concentration was calculated for the nine
253 composite samples. The sample with the Po concentration closest to this average was selected
254 from the three replicate samples in each block. The soil extracts were then prepared for
255 injection by eliminating chloride ions using $AgNO_3$-cartridges (Dionex OnGuard II-AG
256 cartridge, Thermo Scientific). One ml of sample was then mixed with 0.22 ml of ultrapure
257 water or with a standard solution containing 11 different anions to spike the sample. For a

given treatment, the first sample was injected 6 times. The three first injections were carried out with the soil extract mixed with water. This allowed us to check the precision of each peak area and the retention time. The three other injections were performed with the soil extract spiked with all standards together at three different concentrations to allow for any interaction between the soil solution and the anions. The two other soil samples were injected three times with water. Peak identification and calculations were carried out following Waithaisong et al. (2015). As it was not possible to separate glucose-6-P from sulphate in the chromatogram, glucose-6-P was also assayed by enzyme assay using a Glucose-6-phosphate kit (Sigma, Ref MAK014) as described by Waithaisong et al. (2015).

2.5. Data analysis

Unless stated, data are presented as average values and the variability is shown as the standard error of mean (SEM). For each site, the normality of the distribution of data was verified before any statistical analysis using the Shapiro-Wilk test. The effect of the treatment on Pi, Po (bicarbonate or NaOH-extractable) and LMWOA concentrations at each site was tested with one-way ANOVA, ($p < 0.05$) after verifying the homogeneity of variance (Bartlett test). The Duncan method was used for multiple comparisons to identify differences among treatments. As the data between the two sites were very different, the homogeneity of the variances was not verified and the inter-site comparison was made with a Linear Mixed Model by using the lmer and emmeans functions to tests the site effect for each treatment through pairwise comparisons ($p<0.05$). For each site, we carried out a PCA on centered and reduced variables in four treatments (Ac, Euc, Ac-AE, E-AE) to get the correlation circles between Olsen data, Po and LMWOA forms. We carried out also a between-class analysis (BCA) on a matrix of Po forms and LMWOA concentrations determined for each treatment to assess the effect of the treatment on the profile of Po forms and LMWOAs. BCA maximizes the differences between the centroids of the classes of samples because its classification method is based upon the ordination of classes of samples rather than of samples. The statistical significance was assessed by permutation (Monte-Carlo test, 1000 permutations, $p<0.05$). These functions are available in the ade4 package (Chessel et al., 2004). All statistical analyses were performed with R software version 3.6.2 (R Core Team, 2014).

3. Results

3.1. Bicarbonate and NaOH extractable inorganic phosphate and organic P

292 Bicarbonate-extractable Pi concentrations did not vary among treatments, at each site.
293 However, the values were significantly higher at Kissoko than at Itatinga by a factor of 5 to
294 10. As for Pi, bicarbonate-extractable Po did not vary among treatments. Except in native
295 ecosystems, Po concentrations were slightly higher at Kissoko than at Itatinga, by a factor < 2
296 (Table 2).

297 At both sites, extractable NaOH-Pi concentrations were significantly different between
298 the native and planted treatments (Table 2). However, while, at Itatinga, the native treatment
299 had a higher concentration of Pi than the planted treatments, at Kissoko the concentration was
300 lower. NaOH-Pi concentrations were not significantly different between the various planted
301 treatments at either site. However, the values were very different ($p<0.001$) between the sites,
302 with the concentrations in soils from Kissoko being about 20 times greater than those from
303 Itatinga.

304 For both sites, NaOH-Po concentrations were generally lower in the planted treatments
305 than in the native ones, except under acacia at Kissoko. NaOH-Po concentrations were not
306 significantly different between the various planted plots at either site, except under acacia at
307 Kissoko. The differences between sites were much less pronounced than for NaOH-Pi
308 concentrations, with a factor of about 1.5 between them.

309 Taken as a whole, the Itatinga site was dominated by NaOH-Po which accounted for
310 85% of the total NaOH-extractable P in the planted plots, whereas the Kissoko site was
311 dominated by NaOH-Pi which accounted for 60% of the total NaOH-extractable P in the
312 planted ecosystems.

313

314 **3.2. Soil organic P composition and concentration**

315 Six individual P compounds were identified by ion chromatography (IC) (Fig. 2). For
316 all soil samples, the concentration of Po calculated from the sum of all P compounds
317 identified was very close to that of total NaOH-Po (mean values ranging from 29 to 41 mg P
318 kg^{-1} at Itatinga and from 54 to 76 mg P kg^{-1} at Kissoko, see Table 2). Furthermore, the
319 recovery rate of total Po measured by IC was close to the concentrations of total Po assayed
320 by colorimetry for the same extracts (yields of 100.45 ± 14.4 % for Itatinga and 102.6 ± 18.8
321 % for Kissoko). AMP and G6P were the main phosphate monoesters as they accounted for 64
322 to 85% of the total Po at Itatinga and 56 to 96% of the total Po at Kissoko. The other
323 monoesters were Fruc bisP and phytate, accounting for 6 to 28% of the total Po at Itatinga and
324 6 to 31% of the total Po at Kissoko. Phosphoric acid anhydride (ATP) and inorganic

pyrophosphate (PrP) were also found in the soils at low concentrations (≤11% at both sites, except in the native areas at Kissoko where they accounted for up to 39% of the total Po).

Changing the land use from native ecosystems to plantations modified glucose-6-P concentrations at both sites, and AMP and PrP only at Itatinga. For AMP and PrP, the concentrations were higher for Nat whereas glucose-6-P concentrations were higher for Ac (Fig. 2). There were no significant differences between planted ecosystems for any Po compound, except for significantly higher glucose-6-P concentrations for Ac at Itatinga. The main differences between sites were for AMP and phytate concentrations, which were always higher at Kissoko than at Itatinga. However, the effect depended on the tree species, with the soils collected under acacias dominated by AMP while soils collected under eucalypts were dominated by phytate.

3.3. LMWOA composition and concentration

Malate, oxalate and malonate were the main LMWOA forms at both sites (Fig. 3). Citrate concentrations were very low compared to these. However, citrate concentrations were the most affected by the land use change, being significantly lower in the planted plots than in Nat. At Itatinga, malate concentrations were also lower in the planted plots, except under eucalypt. There were no significant differences in LMWOA concentrations between the planted plots, except for Ac at Kissoko where the malate and oxalate concentrations were the lowest. The most significant differences between sites were for oxalate concentrations which, for Ac, were lower by a factor of about 4 at Kissoko compared to Itatinga.

3.4. Relationships between organic P forms, LMWOAs and bicarbonate-extractable Pi and Po

The correlation circles given by PCA carried out on data from afforested plots differed strongly between the two sites (Fig. 4A). At Itatinga, bicarbonate-extractable Pi correlated strongly with oxalate and to a lesser extent with malonate and ATP, and was opposite to PrP. In contrast, bicarbonate-extractable Po did not correlate with any of the variables. AMP, phytate, fructose-bisP, malate and citrate were strongly correlated between them and opposite to glucose-6-P. At Kissoko (Fig. 4B), bicarbonate-extractable Pi and Po co-varied and correlated with AMP and G6P. The other compounds (oxalate, phytate, PrP, malate, citrate and fructose-bisP) were not correlated with bicarbonate extractable Pi and Po.

3.5. Ordination of organic P and LMWOA forms among treatments

BCA of the complete dataset showed that the native areas were very different from the planted plots and this masked the effects of the various planted treatments on the ordination of P forms and LMWOAs (data not shown). We, therefore, chose to present only the BCA maps of the data for the planted treatments (Fig. 5). The separation between treatments was highly significant at both sites (Monte-Carlo test, p=0.003 and 0.002 respectively at Itatinga and Kissoko). In total, the ordination explained about 80% (Itatinga) and 93 % (Kissoko) of variance (Fig. 5).

At Itatinga, the soils from Ac and Euc were clearly separated (Fig. 5B). As shown in Figures 5A and 5B, Ac had high concentrations of glucose-6-P and ATP, and low concentrations of AMP, phytate, Fruc bisP, malonate and malate. Euc had high concentrations of AMP, phytate, Fruc bisP, malate and malonate indicating that eucalypts tended to accumulate these compounds in the topsoil. Neither Ac nor Euc accumulated citrate and PrP in the topsoil. The concentrations in E-AE were close to Euc leading to overlapping on the BCA map (Fig. 5B). However, the concentrations in Ac-AE were very different from Ac. Ac-AE had high PrP and citrate concentrations and low oxalate concentrations (Fig. 5A, 5B).

At Kissoko, the soils from Ac and Euc were also clearly separated (Fig. 5D). As shown in Figures 5C and 5D, Ac had higher concentrations of glucose-6-P and AMP than the other treatments, while the concentrations of all other Po forms and LMWOAs were lower. In contrast, the soils from Euc accumulated all Po forms (except glucose-6-P and AMP) and all LMWOA forms (Fig. 5C). Soils from Ac-AE and E-AE were intermediate between Euc and Ac (Fig. 5D), with intermediate concentrations of Po and LMWOA forms (Fig. 5C).

4. Discussion

4.1. P fractions of the two sites

The two sites had low total P concentrations, typical of tropical, highly weathered soils (Fujii et al., 2018) and they were of the same order of magnitude (Table 1). However, the concentrations of bicarbonate-extracted Pi, considered as plant available P, and those of NaOH-extracted Pi, considered to be mostly adsorbed onto the surfaces of Fe and Al oxides, and moderately available for plants (Aleixo et al., 2017), were very different between the two sites. The Itatinga soils had a low Pi concentration which could be a result of the iron and aluminum contents being higher at Itatinga than at Kissoko (Maquère et al. 2008, Mareschal et al., 2011). Surprisingly, the values of NaOH-Pi were only two times higher than the values of bicarbonate-Pi, suggesting that NaOH extraction was not strong enough to release Pi from

393 soil constituents. At Kissoko, values of bicarbonate-Pi were high, indicating that this site has a
394 good Pi availability. This may explain the high N_2 fixation rates recorded for acacia by
395 (Tchichelle et al., 2017), as this process is highly demanding in P (Houlton et al., 2008; Nasto
396 et al., 2014; Nasto et al., 2017; Png et al., 2017).
397 In contrast to Pi, the concentrations of bicarbonate- or NaOH-extracted Po were of the
398 same order of magnitude at both sites. Interestingly, NaOH extracted always more Po than
399 bicarbonate, suggesting microbial P release during NaOH extraction. Also, soils from Itatinga
400 had a higher ratio between NaOH- and bicarbonate-Po than soils from Kissoko, suggesting
401 that Itatinga soils immobilized more P in their microbial biomass as organic P compounds
402 (Bünemann et al., 2008c). This also suggests that the low Pi concentration at Itatinga did not
403 hamper the microbial development. This hypothesis is supported by recent results in
404 temperate forest soils showing that labile P was rapidly incorporated into microbial biomass
405 when available P was low (Pistocchi et al., 2018).
406
407 **4.2. Impact of afforestation with eucalypts and acacias on soil Po and LMWOA forms**
408
409 The determination of individual P compounds at Itatinga showed that the difference in
410 total NaOH-Po between Ac and Euc treatments and Nat could be explained by both AMP and
411 pyrophosphate concentrations that were significantly lower in Ac and Euc than in Nat. These
412 two compounds could originate from the hydrolysis of microbial ATP in the microbial
413 biomass. This hydrolysis could be mediated by fungal enzymes such as endopolyphosphatases,
414 able to release PrP and AMP from ATP *in vitro* (Andreeva et al., 2019). At Kissoko, the
415 positive effect of acacia trees on total Po in the topsoil could be a result of a higher
416 concentration of glucose-6-P than in the native ecosystem. This P form is the first step of
417 carbohydrate oxidation in all organisms and its high abundance indicates active microbial
418 populations.
419 At both sites, the same four main LMWOAs were identified (Fig. 3). The greatest
420 differences between the planted and the native treatments at both sites were for citrate whose
421 concentration was much lower under Euc and Ac than in Nat. The release of carboxylates by
422 plants has been studied in numerous species over the last decades, showing considerable
423 variation and rather lower rates for many crop species than for fungal and bacterial
424 populations (Hinsinger et al., 2015; Wang and Lambers, 2020). Legumes appear to release
425 more carboxylates than other plant species, especially the cluster roots of white lupin
426 (Lambers et al., 2013). A high level of citrate would, therefore, have been expected in the

427 topsoil under acacias. However, *A. mangium* does not seem to form cluster roots (Robin A.,
428 unpublished data), which might help to explain the low level of citrate in the soil under the
429 acacias. The same hypothesis could explain the lower levels of malate (at both sites) and
430 oxalate (at Kissoko) in Ac than in Euc and Nat.
431
432 **4.3. Effects of Acacia and Eucalypt monocultures on P forms**
433 At both sites, the ordination plots (Fig. 5) showed that acacias and eucalypts induced a
434 very clear separation of Po forms. Ac soils always had higher levels of glucose-6-P than Euc
435 soils. Glucose-6-P was detected in soil leachates (Espinosa et al., 1999) and in soil cultivated
436 with corn (He et al., 2011) but has not yet been identified in forest soils (Turrión et al., 2001;
437 Turner, 2008; Vincent et al., 2010; Turner and Engelbrecht, 2011; Vincent et al., 2012) or
438 other terrestrial ecosystems (Bünemann et al., 2008a; Doolette et al., 2009). However, this
439 discrepancy between the literature and our results, where glucose-6-P was quantified
440 specifically using an enzyme test, could be explained because glucose-6-P probably belongs
441 to the pool of P monoesters identified by ^{31}P NMR used for all those previous studies. The
442 phosphorylation of glucose into glucose-6-P is the first step of glycolysis in living organisms,
443 and, in our soil extracts, the origin was probably mainly living bacterial or fungal populations
444 present in the soil samples. This suggests that the N_2-fixing acacias stimulated the growth of
445 bacterial or fungal populations. However, PrP concentrations were the lowest in Ac. As PrP
446 concentrations are correlated with soil fungi (Makarov et al., 2005; Bünemann et al., 2008b),
447 this suggests that acacias stimulated the bacterial population more than the fungal population.
448 Generally speaking, we found low phytate concentrations in our soil samples, as
449 previously observed in tropical soils where phytate was even reported to be absent (Vincent et
450 al., 2010; Turner and Engelbrecht, 2011) or strongly stabilized with iron and aluminum when
451 the concentration was higher (Vincent et al., 2012), as in our soils. However, Ac soils were
452 always associated with the lowest phytate concentrations. These results could be explained by
453 the acacias having a greater phytate mineralization ability than eucalypts by selecting more
454 phytate-mineralizing bacterial populations as shown in the rhizosphere of N_2-fixing common
455 beans (Maougal et al., 2014). Conversely, we cannot exclude that phytate accumulation in
456 soils under monospecific eucalypt plots was a consequence of higher inputs of phytate since
457 we did not measure phytate production of the trees in our study.
458 On average, the main Po compound at both sites was the nucleotide AMP but its
459 concentration in Ac compared to Euc depended on the site. At Itatinga, AMP concentrations
460 were higher in Euc whereas at Kissoko they were higher in Ac. In addition, at Itatinga, AMP

was covariant with Fruc bisP and in opposition to ATP whereas, at Kissoko, AMP was in opposition to both to Fruc bisP and ATP. These different patterns for AMP, Fruc bisP and ATP strongly suggest that AMP could have a different origin at Itatinga and Kissoko. AMP can originate from three main pools in soil. Firstly, it could arise from a pool adsorbed on soil constituents and recalcitrant to mineralization, leading to its accumulation in the soil. Secondly, it could result from the degradation of nucleic acids occurring during alkaline soil extraction, as suggested by several authors (Turner et al., 2003; Cade-Menun et al., 2010; Vincent et al., 2010). Finally, AMP could be associated with the energy balance of the living microbial cells in the soil. In this latter case, the ratio between AMP and ATP would regulate the synthesis of ATP to provide energy to the cells. If this ratio is high, there is a lack of ATP and its synthesis will be up-regulated to provide energy to the cells through glycolysis and the phosphorylation of fructose-1-P to fruc bisP. At Itatinga, the pattern indicates that when the ATP concentration is high, the concentrations of AMP and fruc bisP are low and *vice versa*, suggesting strongly that AMP is associated with the energy balance of living cells in the microbial communities in the soils. This would mean that most of the Po is immobilized in the microbial fraction at Itatinga despite a much lower availability of Pi in these soils compared to that at Kissoko. In contrast, at Kissoko, AMP was not covariant with Fruc bisP, suggesting that the AMP probably does not originate only from the living cells but also from an adsorbed pool on soil constituents and/or nucleic acid degradation. Interestingly, AMP and ADP were shown to be taken up by the roots of *Fagus sylvatica* (Scheerer et al., 2019). Hence, at Kissoko, as AMP does not seem to be locked into the microbial biomass, it could also serve as a source of P for the trees.

4.4. Effects of *Acacia mangium* and *Eucalyptus* monocultures on LMWOAs

At each site, Euc soil accumulated more LMWOAs than Ac soil (Fig. 3). These LMWOAs have been found in soils and in rhizospheres (Fox and Comerford, 1990; Baziramakenga et al., 1995; Cawthray, 2003; Hinsinger et al., 2015) and can originate from fungal, bacterial or plant sources. However, fungal and bacterial populations have much greater capabilities for releasing LMWOAs than plant roots (Hinsinger et al., 2015). In particular, ectomycorrhizal species were shown to be able to release various LMWOAs such as oxalate, citrate, succinate (Machuca et al., 2007), although most release mainly oxalate (Plassard and Fransson, 2009; Courty et al., 2010), whereas arbuscular species did not release oxalate but acetate and formiate (Toljander et al., 2007) and low amounts of citrate and malate (Tawaraya et al., 2006). Bacterial P solubilizers are also able to release the same LMWOAs as

495 fungi plus malate (Khan et al., 2007). However, bacterial populations can also use LMWOAs
496 as a C source, as reviewed by Jones (1998). Hence, the concentrations of LMWOAs measured
497 in our soil samples resulted from the balance between their production, mainly by fungal and
498 bacterial populations, and their consumption, mainly by bacteria. We suggest that the pattern
499 of the differences in the LMWOA profiles could reflect a greater abundance of fungi in the
500 rhizosphere of eucalypts than of acacias, especially of ectomycorrhizal species. This
501 hypothesis is supported by numerous studies showing that eucalypts have abundant
502 ectomycorrhizal roots (Robin et al., 2019). Conversely, even if acacia roots are able to form
503 ectomycorrhizal roots, we observed here that they were much less abundant than for eucalypts
504 (Robin A., unpublished data). Hence, the microbial populations associated with acacia roots
505 could release lower levels of LMWOAs at both sites as it has been shown that planting *A.*
506 *mangium* trees in eucalypt plantations greatly modifies the microbial communities in the
507 rhizosphere of each tree species (Rachid et al., 2013; Huang et al., 2014; Rachid et al., 2015).
508 The *A. mangium* rhizosphere could also be enriched in bacteria able to use malate (at both
509 sites) and oxalate (at Kissoko), explaining the lower levels of these LMWOAs in Ac soils
510 relative to Euc soils.
511
512 **4.5. Effects of introducing an N_2-fixing species into a eucalypt plantation on Po and**
513 **LMWOA forms**
514 Replacing 50% of eucalyptus trees by acacia trees induced different effects on P forms
515 and LMWOAs at each site. The strongest effect was observed at Kissoko where the soils
516 sampled either around acacia (Ac-AE) or eucalypt (E-AE) trees in mixed-species plantations
517 were clearly separated from monospecific acacia or eucalypt treatments. This could be due to
518 a better growth of acacia in mixed plots at Kissoko than at Itatinga. The total aboveground
519 biomass of the acacias was 25% (Itatinga) and 70% (Kissoko) of the total aboveground
520 biomass of the eucalypts (Table S1, Bouillet et al., 2013). This implies that the root
521 development of the acacias was probably much higher at Kissoko than at Itatinga, which
522 would explain the stronger effect of acacias in mixed plantations at Kissoko than at Itatinga.
523 These results indicate that, even after a short duration (8 years at Itatinga or 7 years at
524 Kissoko), acacias were able to modify the concentrations of P compounds and LMWOAs in
525 the soil. In particular, at Kissoko, oxalate concentrations were much greater in soils collected
526 in mixed-species plantations than in monospecific acacia plots. Interestingly, *in vitro*
527 experiments showed that oxalate released six times more glucose-6-P than Pi when the same
528 concentrations of these two compounds were complexed with ferrihydrite (Goebel et al.,

529 2017). A greater release of oxalate from fungal populations associated with eucalypts
530 combined with glucose-6-P released from the microbial populations associated with acacias
531 could result in a higher substrate (glucose-6-P) availability for soil phosphatase. Such a
532 mechanism could explain the increase in P accumulation observed in the trees of the mixed
533 plantation at Kissoko (Le Cadre et al., 2018).

534 In contrast, at Itatinga, soils sampled around the eucalypts in mixed-species treatments
535 (E-AE) displayed patterns of P compounds and LMWOAs very close to the patterns of the
536 monospecific eucalypt treatment, indicating that the eucalypts still dominated the P cycle and
537 the production of LMWOAs. However, Laclau et al. (2013), using the same experimental
538 plots, showed that, at E-AE, the eucalypt fine root biomass in the topsoil was 7 times higher
539 than the acacia fine root biomass. The soil sampled near eucalypt trees was mainly occupied
540 by eucalypt roots, which could explain why acacia trees had a low influence on soil P
541 compounds and LMWOAs. In contrast, the soil from Ac-E plots was well separated from both
542 Euc and Ac soils, with high citrate and PrP accumulations. Citrate may reflect the capacity of
543 acacia to select microbial populations able to release this LMWOA. Interestingly, it was
544 demonstrated that citrate was more effective than other LMWOAs for P mobilization (Palomo
545 et al., 2006; Oburger et al., 2011) due to the presence of three carboxyl groups. This could
546 increase the capacity of acacia roots to mobilize Pi for N_2 fixation where there is high P
547 competition between acacias and eucalypts. Pyrophosphate, which is mainly attributed to
548 fungal metabolism (Makarov et al., 2005; Bünemann et al., 2008b), could reflect an increase
549 in the fungal populations benefiting from N_2 fixation.

550

551 **4.6. Link between Po and LMWOA forms and P cycling**

552 Assaying P forms and LMWOAs in the same samples could help to assess the role of
553 these compounds in P cycling. As shown in figure 4, different trends of variation between
554 bicarbonate-Pi and bicarbonate-Po and other variables were observed. At Itatinga, the soil is
555 very poor in available Pi and was correlated with oxalate, indicating that bicarbonate-Pi
556 concentration into soil solution depends strongly upon oxalate. This result agrees with of a
557 study dealing with an ectomycorrhizal association showing a linear relationship between
558 bicarbonate-Pi and oxalate extracted from the same soil samples (Casarin et al., 2004).
559 Because bicarbonate-Pi is also correlated with malonate, this LMWOAs could also play a role
560 to determine available Pi in this soil. In contrast, malate and citrate might play a minor role in
561 Pi availability. The Po forms identified in soil extracts were very poorly correlated with
562 available Pi, suggesting that Po contributes very little to P cycling. This hypothesis is

supported by the low variation of bicarbonate-Po among other variables. Hence, at Itatinga, P cycling mainly depends on physicochemical processes with Pi desorption governed by LMWOAs, such as oxalate and malonate. As shown in BGA analysis, eucalypt treatment but not acacia was associated with LMWOAs, suggesting that Pi desorption from soil oxyhydroxydes would be more active under eucalypt than under acacia. This could partly explain that planting acacia trees in eucalypt plots did not increase the growth of eucalypt trees (Bouillet et al., 2013). At Kissoko, the reverse situation was observed, as bicarbonate-Pi and bicarbonate-Po correlated only with glucose-6-P and AMP (Fig. 4). None of the LMWOAs varied with bicarbonate-Pi or Po. These relationships suggest that P cycling is dominated by organic P mineralization, especially of glucose-6-P and AMP. As shown in BGA analysis, acacia treatment but not eucalypt was associated with glucose-6-P and AMP suggesting that bicarbonate-Pi could come from Po mineralization more active under acacia than under eucalypt. This could contribute to explaining why planting acacia trees in eucalypt plots improved strongly plant P availability (Le Cadre et al. 2018) and greatly increased the biomass of eucalypt trees (Bouillet et al. 2013).

5. Conclusion

In this study, we aimed at addressing four questions. First, we wanted to know if planting exotic tree species could induce specific changes in the patterns of P pools and LMWOAs in the topsoil compared to native ecosystems. Our results showed significant differences of Po and LMWOA concentrations between soil samples collected from native ecosystems and in monospecific eucalypt or acacia plantations at both sites. Our second question was to know if the Po and LMWOA pools differ among eucalypt or acacia monospecific plantations. At both sites, patterns of Po and LMWOAs were clearly separated, with glucose-6-P found mainly under acacia and phytate and oxalate mainly under eucalypt. Our third question aimed to know whether or not the introduction of acacia in eucalypt plots could modify Po and LMWOA pools relative to monospecific plots. This was confirmed at Kissoko as indicated by the BCA maps showing a clear separation between the monospecific treatments, with the mixed-species treatments positioned between the monospecific ones. Acacia trees changed the profiles of organic P and LMWOAs in less than 10 years at both sites. Our last question intended to improve our understanding of P cycling in monospecific and mixed-species tropical plantations. The

available Pi, Po and LMWOA forms showed that P cycling was dominated by different processes at each site, that are rather physicochemical (via Pi desorption after increase of LMWOAs) at Itatinga and biological (via organic P mineralization) at Kissoko. Our findings show that the composition of P pools and LMWOAs can change rapidly – after a first rotation lasting less than 10 years – by introducing a N_2 fixing species such as *A. mangium* into fast-growing eucalypt plantations. Further investigations are required to determine the actual effects of acacias on P bioavailability for eucalypts in tropical soils. They will help us identify the potential drivers and propose management practices that could take advantage of the beneficial effects of introducing acacias into eucalypt plantations.

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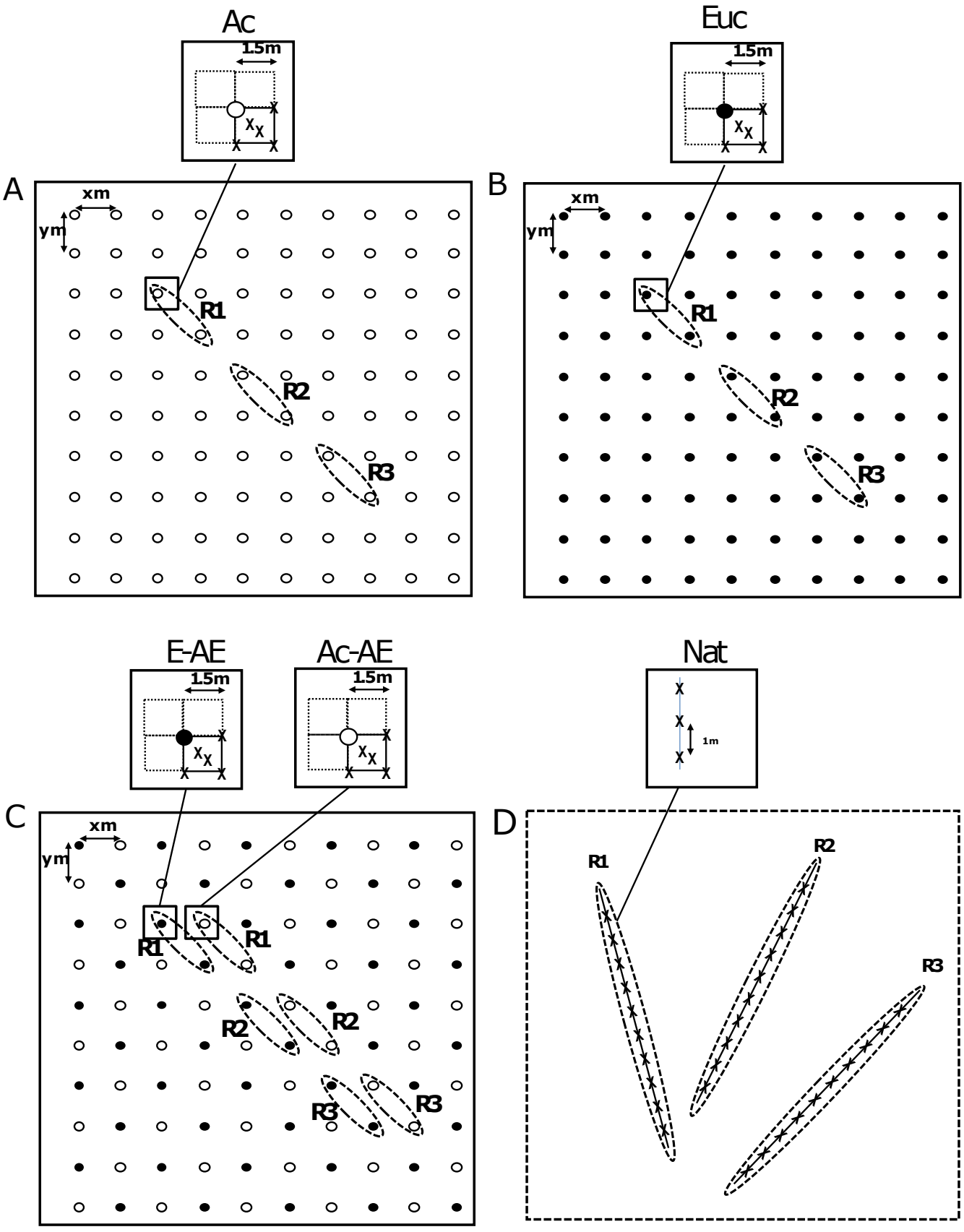
Captions to figures

Fig. 1. Schematic representation of soil sampling design used in planted plots and native ecosystems.

A, B: monospecific plots of acacia (Ac) (open circle) and eucalypt (Euc) (black circle). C: mixed-species plots, below acacias (Ac-AE) and below eucalypts (E-AE). Each plot had 10 x 10 trees, planted at x meters * y meters with x=3 and y=3 in Brazil and x=3.33 and y = 3.75 in Congo. In each plot, 3 composite samples (R1, R2, R3) were made from 10 soil cores (0-10 cm, 5 cm diameter) from around 2 trees. For each tree, 5 soil cores (X) were sampled in a quarter of the Voronoï's square always located at the right side of the tree (detailed figure) to allow for the spatial variability. D: native ecosystem (Nat) where each area had approximately the same surface as the planted plots and 3 composite samples (R1, R2, R3) were made from 10 soil cores taken along linear transects.

961 **Fig. 2.** Composition of the Po pool in the topsoil from Itatinga and Kissoko for monospecific
962 acacia plots (Ac), below acacias in mixed-species plots (Ac-AE), below eucalypts in mixed-
963 species plots (E-AE), monospecific eucalypt plots (Euc) and native vegetation (Nat). Values
964 are means with standard error bars (*n*=3). Different letters indicate differences among
965 treatments at each site (one-way ANOVA, Duncan test, $p \leq 0.05$). Asterisks indicate site
966 effects (Linear mixed model, pairwise comparison) with the significance levels: *p<0.05,
967 **p<0.01, ***p<0.001.
968
969 **Fig. 3.** Composition of LMWOAs in the topsoil collected from Itatinga and Kissoko for
970 monospecific acacia plots (Ac), below acacias in mixed-species plots (Ac-AE), below
971 eucalypts in mixed-species plots (E-AE), monospecific eucalypt plots (Euc) and native
972 vegetation (Nat). Values are means with standard error bars (*n*=3). Different letters indicate
973 differences among treatments inside each site (one-way ANOVA, Duncan test, $p \leq 0.05$).
974 Asterisks indicate site effects (Linear mixed model, pairwise comparison) with the
975 significance levels: *p<0.05, **p<0.01, ***p<0.001.
976
977 **Fig.4.** Correlations circles between variables with bicarbonate-extractable P (from Table 2) in
978 brown, P forms (from Fig. 2) in blue, and LMWOAs (from Fig. 3) in green.
979
980 **Fig. 5.** Between class analysis (BCA) of chemical compounds for the effect of the treatments
981 in the plantations at Itatinga and Kissoko for monospecific acacia plots (Ac), below acacias in
982 mixed-species plots (Ac-AE), below eucalypts in mixed-species plots (E-AE) and
983 monospecific eucalypt plots (Euc). A and C are maps of variable responses with the
984 percentages of variance explained by the analysis given on the axes where P forms (from Fig.
985 2) are shown in blue and LMWOAs (from Fig. 3) in green. B and D are factor maps of
986 treatment responses.
987


Ac
1.5m
A
xm
ym
R1
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R3
Euc
1.5m
B
xm
ym
R1
R2
R3
E-AE
1.5m
Ac-AE
1.5m
C
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R1
R1
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R3
R3
Nat
1m
D
R1
R2
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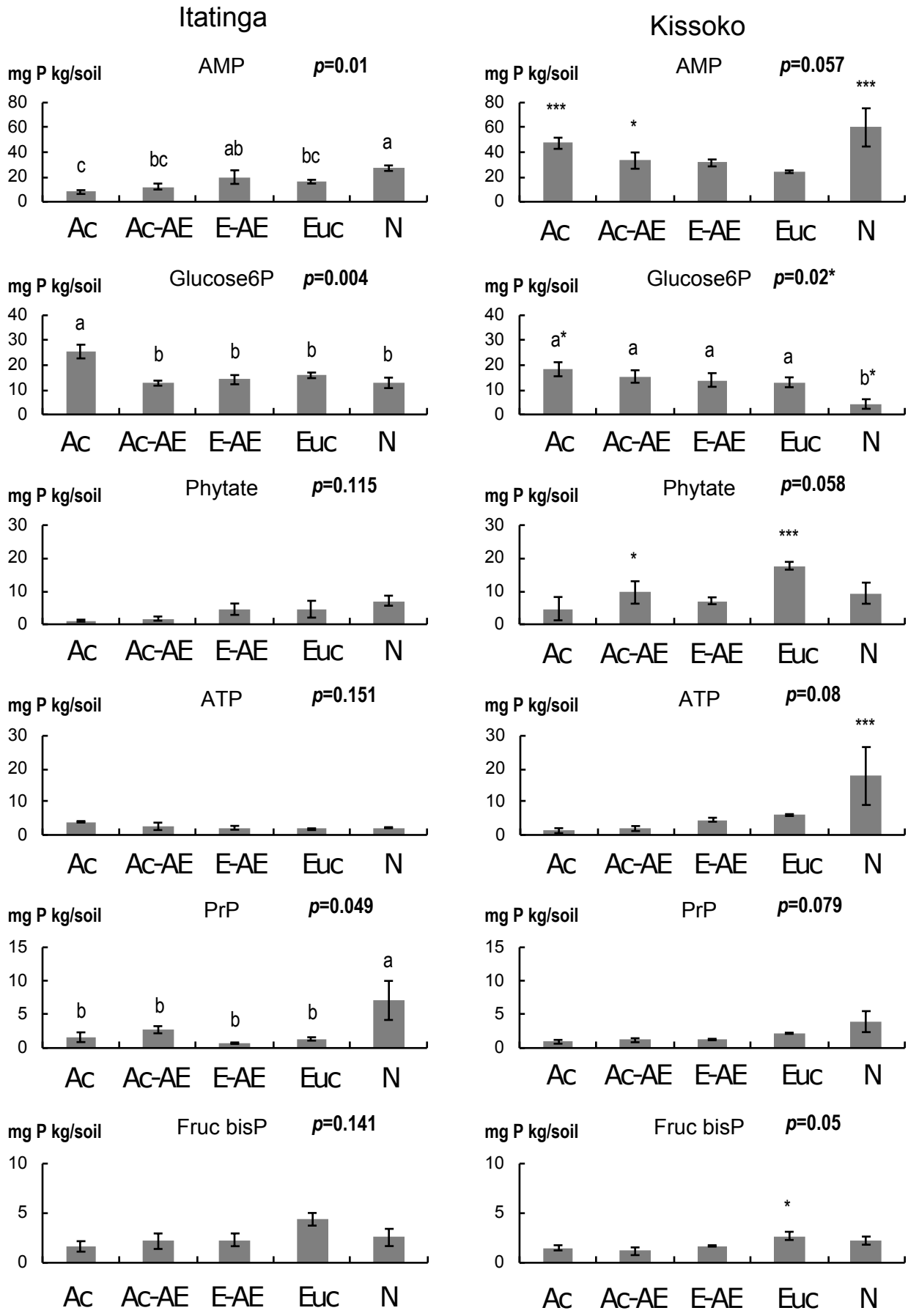
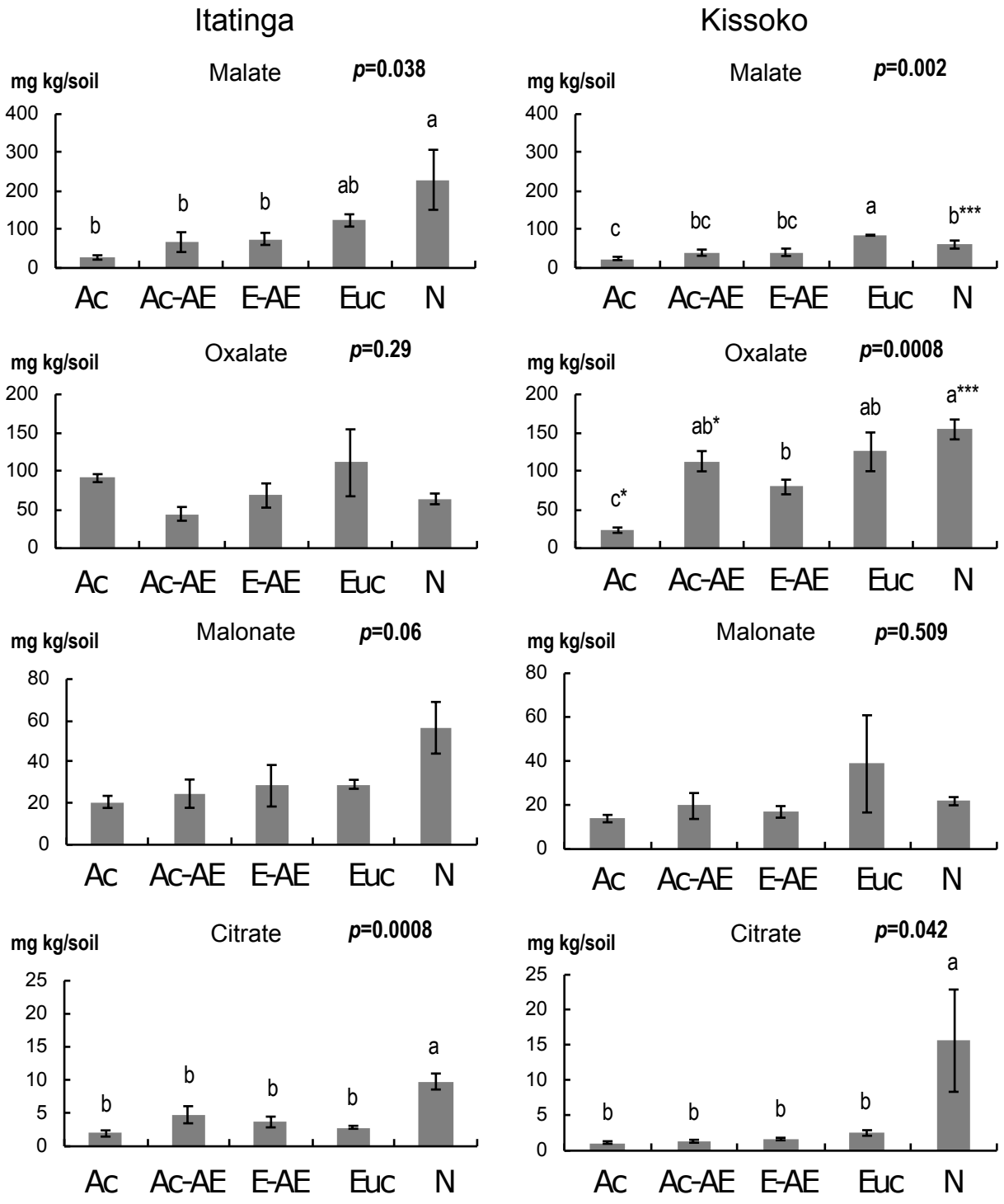
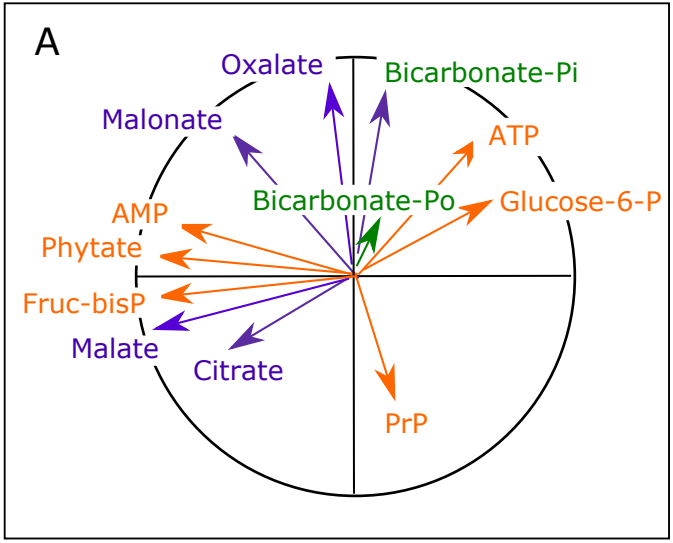

Itatinga
Kissoko
mg P kg/soil
AMP
p=0.01
p=0.057
Glucose6P
p=0.004
p=0.02*
Phytate
p=0.115
p=0.058
ATP
p=0.151
p=0.08
PrP
p=0.049
p=0.079
Fruc bisP
p=0.141
p=0.05
Ac
Ac-AE
E-AE
Euc
N


Itatinga
Kissoko
mg kg/soil
Malate
p=0.038
p=0.002
Oxalate
p=0.29
p=0.0008
Malonate
p=0.06
p=0.509
Citrate
p=0.0008
p=0.042
Ac
Ac-AE
E-AE
Euc
N
b
b
b
ab
a
c
bc
bc
a
b***
c*
ab*
b
ab
a***

BRAZIL



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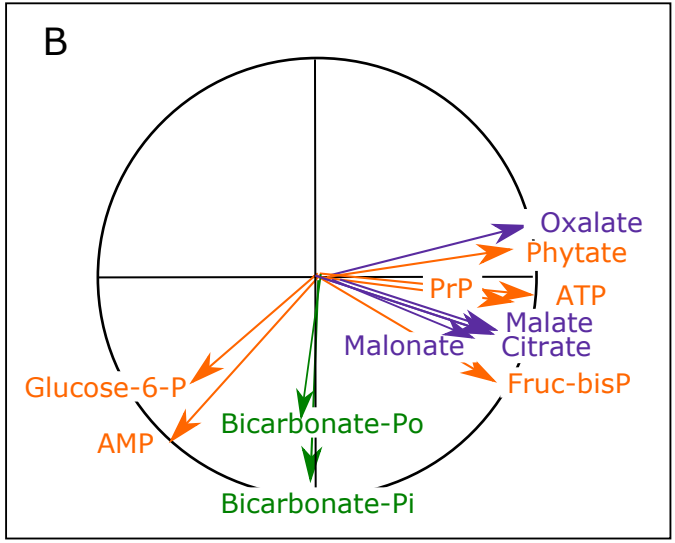
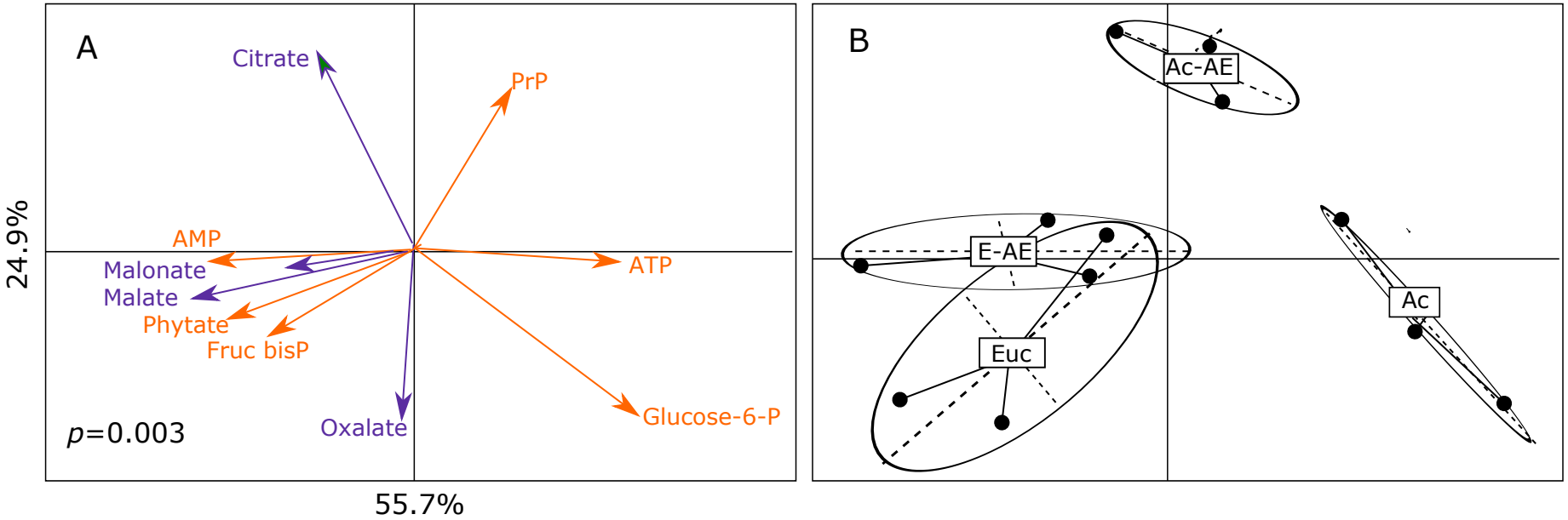


ITATINGA



KISSOKO

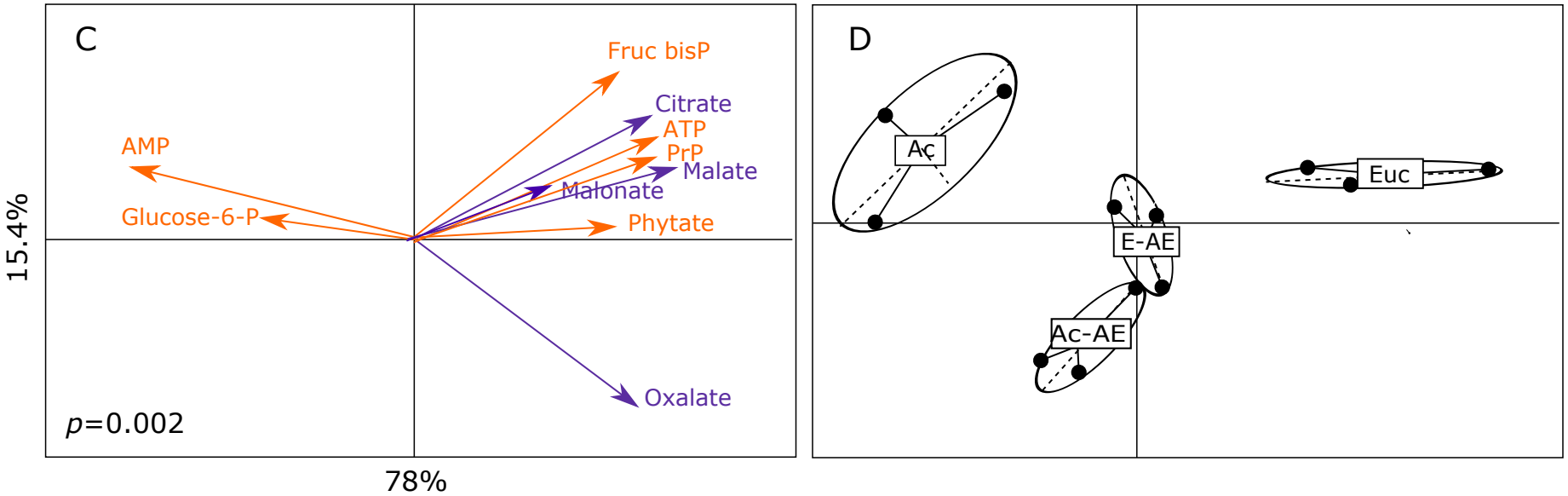
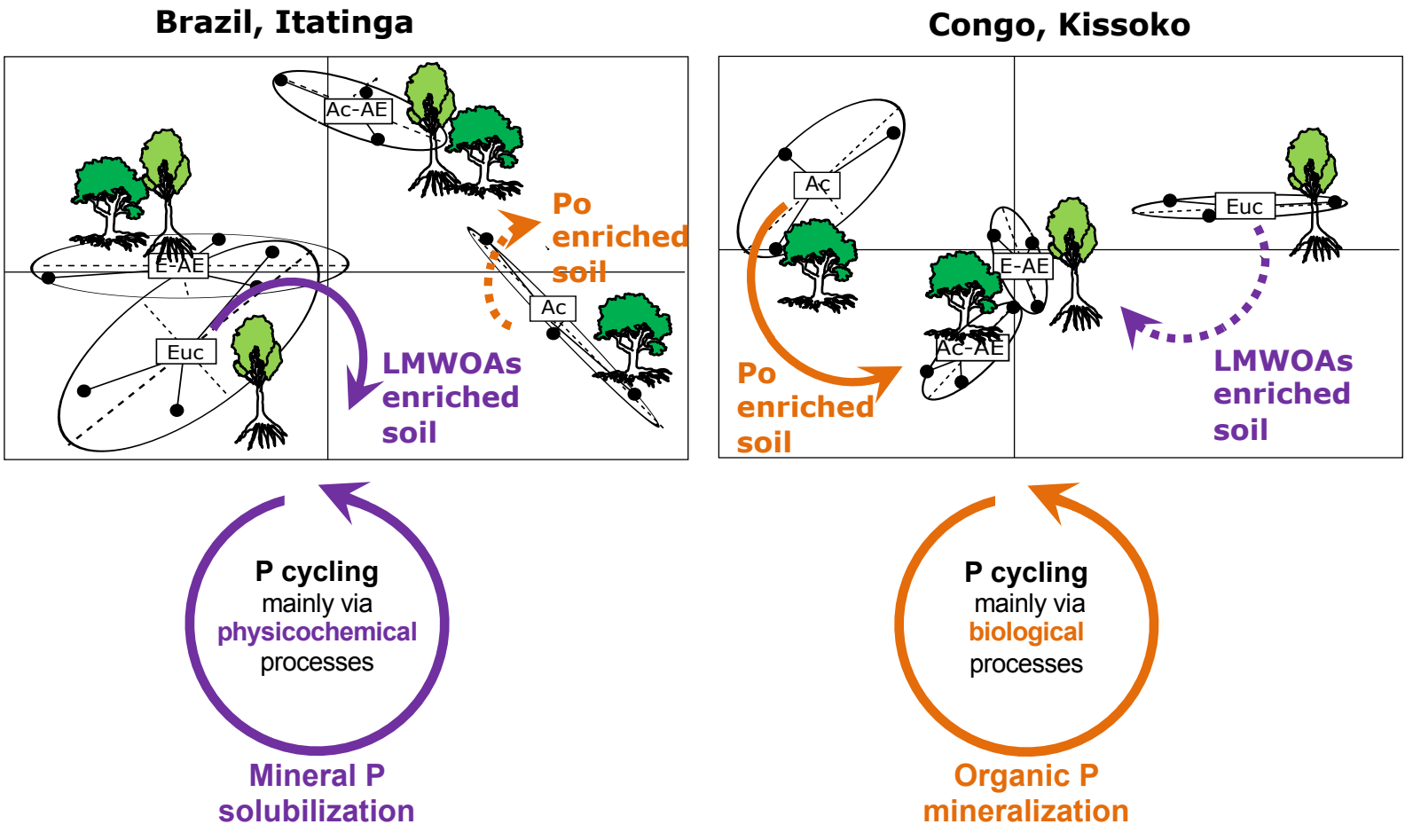


Table 1: Characteristics of the topsoil layer (0-10 cm) at the 2 sites (planted and native soils) used in the study. Values are means with standard errors given in brackets (n=12 for plantations, n=3 for native). Data indicated by a star (*) come from Voigtlaender et al. (2012) at the same site (0-5 cm, n=3). Soil texture: particle size analyzed by sedimentation. Total C and total N determined by dry combustion using a CHN micro-analyzer. P extracted with fluoro-nitro-perchloric acid. Exchangeable K, Ca, Mg, Na and CEC determined using 1N ammonium acetate at pH 7. pH measured in water.

	Itatinga (Brazil)		Kissoko (Congo)	
	Plantation	Native	Plantation	Native
Soil type (FAO)	Ferralsol	Ferralsol	Ferralic arenosol	Ferralic arenosol
Soil texture				
Sand (%)	84.5 (0.9)*	77.6 (2.3)	93.4 (0.2)	92.1 (1.0)
Silt (%)	4.1 (0.7)*	3.5 (0.2)	1.0 (0.1)	1.1 (0.2)
Clay (%)	11.4 (0.7)*	18.8 (2.3)	5.6 (0.3)	6.8 (0.9)
Chemical characteristics				
Total C (g kg^{-1})	13.83 (0.82)	15.93 (1.31)	10.79 (0.56)	6.53 (0.20)
Total N (g kg^{-1})	0.70 (0.03)	0.93 (0.07)	0.54 (0.01)	0.44 (0.01)
Total P (g kg^{-1})	0.21 (0.03)	0.21 (0.08)	0.28 (0.03)	0.28 (0.08)
C:N	19.63 (0.49)	16.98 (0.39)	19.81 (0.73)	14.74 (0.52)
Total Al (g kg^{-1})	21.78 (0.64)	27.04 (3.31)	11.83 (0.39)	12.90 (1.48)
Total Fe (g kg^{-1})	18.70 (0.42)	23.33 (8.79)	10.56 (0.29)	11.15 (0.69)
Exchangeable elements				
K ($cmol_c$ kg^{-1})	0.02 (0.004)*	0.10 (0.006)	0.02 (0.001)	0.03 (0.003)
Ca ($cmol_c$ kg^{-1})	0.46 (0.096)*	0.24 (0.024)	0.07 (0.006)	0.14 (0.068)
Mg ($cmol_c$ kg^{-1})	0.42 (0.035)*	0.17 (0.029)	0.06 (0.007)	0.08 (0.040)
Na ($cmol_c$ kg^{-1})	0.01 (0.011)*	0.03 (0.003)	0.03 (0.002)	0.03 (0.000)
CEC ($cmol_c$ kg^{-1})	1.76 (0.274)*	0.91 (0.080)	0.82 (0.035)	0.59 (0.079)
Other soil properties				
pH	5.5 (0.20)*	4.7 (0.06)	3.9 (0.02)	4.6 (0.07)

Table 2. Bicarbonate and NaOH-extractable mineral P (Pi) and organic P (Po) concentrations in the topsoil (0-10 cm) from Itatinga and Kissoko for monospecific acacia plots (Ac), below acacias in mixed-species plots (Ac-AE), below eucalypts in mixed-species plots (E-AE), monospecific eucalypt plots (Euc) and native vegetation (Nat). Values are means with standard deviation in brackets (n=9 for NaOH extractions and n=3 for bicarbonate extractions). Different letters indicate significantly differences among treatments at each site (one-way ANOVA, Duncan test, $p \leq 0.05$). For a given treatment, asterisks indicate a significant difference between sites (Linear mixed model, pairwise comparison) ** $p<0.01$, *** $p<0.001$.

		P (mg kg^{-1} dry soil)				
P fractions	Site	Ac	Ac-AE	E-AE	Euc	Nat
Bicarbonate-Pi	Itatinga	3.1 a (0.3)	3.1 a (0.5)	2.7 a (0.7)	3 a (0.9)	2.7 a (0.5)
	Kissoko	29.3 a (5.4)	19.7 a (6.3)	20.6 a (4.9)	24.4 a (2.7)	16.5 a (10.7)
	Site effect	***	***	***	***	***
Bicarbonate-Po	Itatinga	4.6 b (1)	4.4 b (0.9)	5.1 b (2.1)	4.8 b (0.5)	10.1 a (1.5)
	Kissoko	7.7 a (0.4)	7.9 a (2)	7.4 a (0.6)	7.5 a (1)	6.5 a (1)
	Site effect	***	***	**	**	***
NaOH-Pi	Itatinga	6.8 b (0.7)	7.1 b (0.4)	6.7 b (0.3)	7.7 b (0.4)	24 a (9.9)
	Kissoko	112.8 a (12.5)	99.6 a (10.1)	96.4 a (7.1)	104.4 a (7.2)	65.3 b (12.5)
	Site effect	***	***	***	***	***
NaOH-Po	Itatinga	40 b (3)	39 b (5)	44 ab (5)	40 b (6)	50 a (6)
	Kissoko	83 a (6)	53 b (4)	58 b (9)	57 b (7)	83 a (12)
	Site effect	***	**	**	**	***


Brazil, Itatinga
Ac-AE
E-AE
Euc
Ac
Po enriched soil
LMWOAs enriched soil
P cycling mainly via physicochemical processes
Mineral P solubilization
Congo, Kissoko
Ac
E-AE
Ac-AE
Euc
Po enriched soil
LMWOAs enriched soil
P cycling mainly via biological processes
Organic P mineralization