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1 **Increasing plant diversity promotes ecosystem functions in rainfed rice based short rotations in**
2 **Malagasy highlands**

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11

12 **Abstract**

13 Plant diversification is one of the main ways to ecologically intensify agroecosystems to improve their
14 sustainability and resilience. Rotations and/or a mixture of crops can mitigate pest and weed
15 infestation, reduce diseases, and improve soil fertility and crop productivity. However, rainfed rice
16 yields in the Malagasy highlands remain low despite the frequent use of cropping systems including
17 crop rotations and mixtures. In this study, we compared three rainfed rice based short rotations with
18 rainfed rice monocropping to quantify the benefits of plant diversification on different ecosystem
19 functions such as weed and nematode control, soil fertility, soil macrofauna abundance and diversity,
20 and rice yield over four cropping seasons. The three rotations were based on rice in rotation with one
21 legume, groundnut (RG), a cereal-legume mixture, sorghum and cowpea (RSC), or a mixture of
22 legumes, velvet bean and crotalaria (RVC). Rice growth, N content and yield, soil N content, weed
23 biomass, nematofauna and macrofauna were assessed and a profitability analysis was performed at
24 rotation scale. The legume mixture had a significant and positive effect on rice growth, N content and
25 yield, soil N content, and weed and nematode control due to high biomass production in the
26 cropping cycle including legume mixture, by limiting weed growth and leaving a large quantity of N-
27 rich residues to enrich the soil for the following rice crop. The nematicide properties of the legume
28 mixture may reduce the infestation of plant-feeding nematodes. The RG and RSC rotations produced

29 intermediate results. While rice yields were higher in these rotations than when rainfed rice was
30 grown alone, weed biomass remained high due to minimal competition with weeds during the crop
31 rotation cycle especially with groundnut. For RSC, nematode control was limited as both sorghum
32 and cowpea are host plants for nematodes. Despite a year with no crop income with the RVC
33 rotation, profitability was higher mainly due to the increased rice yield and reduced field
34 management costs. The choice of species is thus crucial to optimize ecosystem functions adapted to
35 farmers' context and objectives.

36 **Keywords:** Agroecology, cover crop, diversification, ecological intensification, ecosystem services,
37 legume, rotation

38

39 **Introduction**

40 The negative externalities of conventional agriculture are widely recognised (Altieri, 1999; Tilman et
41 al., 2001). Increasing plant diversity has become one of the main ways to improve agroecosystem
42 productivity and sustainability. Plant diversity can enhance different ecosystem services including
43 primary production, nutrient cycling, and pest, disease, and weed control (Beillouin et al., 2019;
44 Bommarco et al., 2013; Ratnadass et al., 2012) while improving farming system resilience (Lin, 2011).
45 Increasing plant diversity affects different ecological functions in the agroecosystem, enabling the
46 intensification of ecological processes, which can replace external inputs such as fertilizers or
47 pesticides (Isbell et al., 2017; Kremen et al., 2012; Tamburini et al., 2020). The main challenge is
48 finding the right synergies and trade-offs between the services expected from agroecosystems whose
49 links are highly complex (Garcia et al., 2018; Rapidel et al., 2015). Iverson et al. (2014) reported
50 possible synergies between production and biocontrol in diversified cropping systems but results
51 depend on the cropping system design and the crops used. Gaba et al. (2020) showed that
52 multifunctionality was enhanced by increasing weed diversity thanks to a positive impact on
53 pollination, pest control and soil fertility, and a neutral effect on productivity.

54 Plant diversification can be incorporated in agroecosystems in different ways, such as crop rotations
55 and/or crop mixtures (Malézieux et al., 2009). The advantages of crop rotation are that it interrupts
56 pest, disease, and weed cycles, enables better exploration of soil resources in space and over time
57 and favors soil biological activity (Hooper and Vitousek, 1998; Liebman and Dyck, 1993; Ratnadass et
58 al., 2012; Tiemann et al., 2015). These benefits are related to the number of species and to their
59 specific identity (Finney and Kaye, 2017; Hooper, 1998; Ranaldo et al., 2020). Smith et al. (2008)
60 showed that the positive rotation effect on crop production was mainly due to the number of the
61 crops in the rotation, but this positive effect varied with the crop studied and increased with the
62 presence of legumes.

63 Including legumes in cropping systems, in rotation or/and in intercropping, is common in family
64 farms in Africa (Waggoner, 1996), particularly for (i) their ability to fix atmospheric nitrogen (N), (ii)
65 their potential to enrich soil mineral fertility via their N-rich residues, (iii) their facilitating effect on
66 non-legume crops leading to a better exploration of soil resources, and (iv) their ability to suppress
67 weeds. These benefits generally increase yields and reduce the use of external inputs (Chikowo et al.,
68 2007; Namatsheve et al., 2020; Snapp et al., 2019). Consequently, they also play a major role in
69 enhancing ecosystem functions. However, plant species have to be carefully chosen based on their
70 functional traits related to the expected services (Blesh, 2017; Tribouillois et al., 2015) and to
71 optimize their complementarity with non-legume species in space and over time to explore soil
72 resources, and to compete for light (Bedoussac et al., 2015; Garcia et al., 2020; Vandermeer et al.,
73 1998). Rice is the main staple food crop grown in Madagascar and is mainly cultivated in lowland
74 areas. However, given population growth and the current need to import rice, increasing crop
75 productivity is one of the main objectives of agricultural development, especially since average yields
76 of irrigated rice (around 3 t.ha⁻¹, Naudin *et al.*, 2019) are well below the potential (yield gap of
77 around 2.1 t.ha⁻¹; FAO, 2004). Rainfed upland rice, and more generally rainfed crops, are widely
78 grown to satisfy the food needs of the growing population and also because lowlands are already
79 saturated. In mid-western Vakinankaratra, one of the most productive areas of the country, rainfed

80 rice accounts for around 15% of the area cultivated by family farms (Razafimahatratra et al., 2017).
81 The constraints smallholder farmers have to face include numerous pests, weeds and diseases, poor
82 soil fertility, and poor quality manure, while they lack access to external inputs (fertilizers,
83 pesticides), and depend to a great extent on manual labour (Raboin et al., 2014; Raminoarison et al.,
84 2020). Currently, farmers grow legumes, tubers, and other cereals in pure or crop mixtures in
85 rotation with rainfed rice both to compensate for their limited access to exogenous inputs and to
86 diversify their sources of income. Yet, given the many constraints encountered in this region, average
87 rice yields are extremely low ($1.6 \text{ t}\cdot\text{ha}^{-1}$, Razafimahatratra *et al.*, 2017).

88 To better understand and quantify the potential gain of plant diversification in fragile and poor
89 environments with limited ecosystem functions (rice production, control of white grubs, nematodes,
90 and weeds, N soil fertility and soil macrofauna activity), we compared different short-term rotations
91 based on rainfed rice with rice monocropping systems over a period four years. These diversified
92 rotations included legumes, alone or mixed with cereals. The specific aims of this study were to (i)
93 quantify the effect of plant diversification on the above-mentioned ecosystem functions, (ii) assess
94 possible links between them, and (iii) compare the different rotations and the rice monoculture
95 based on their ecosystem functions and on a profitability analysis.

96

97 **Material and methods**

98 *2.1. Study site*

99 This study was carried out at Ivory station, located in mid-western Vakinankaratra region
100 ($19^{\circ}33'18.90''$ lat. S, $46^{\circ}24'53.83''$ long. E, 930 m a.s.l.) over four cropping seasons 2015/2016,
101 2016/2017, 2017/2018 and 2018/2019, hereafter referred to as Year 1, Year 2, Year 3, and Year 4.
102 Cropping season corresponds to the rainy season, which lasts from November to the end of
103 March/beginning of April in the study region. An automatic weather station (CIMEL, Electronique,
104 Paris France) located near the experimental field recorded daily weather data. Average temperature

105 during the four cropping seasons was 24.7 ± 0.6 °C and annual rainfall was 1225 ± 84 mm, with
106 monthly variations between seasons (Figure 1).

107 At the start of the experiment in 2015, soil samples were collected at six randomly selected points in
108 the experimental field in the 0-10, 10-20, 20-40 cm soil layers to determine selected physical-
109 chemical soil properties (Table 1). The soil type at the experimental site was a sandy-clay-loamy
110 Ferralsol (FAO classification) with 32-18-50% clay-silt-sand composition in the 0-40 cm soil layer. Soil
111 pH (H₂O) was measured using a glass electrode (Kalra, 1995). Available phosphorus (P) was
112 determined using the Olsen method (Olsen et al., 1954), cation exchange capacity (CEC) using the
113 cobaltihexamine chloride method (Fallavier et al., 1985) and total carbon (C) and N by dry
114 combustion using a Carbon Hydrogen Nitrogen (CHN) microanalyzer (ThermoFisher Flash 2000, USA).

115

116 2.2. Experimental design and crop management

117 The field experiment was set up in November 2015 in a field cropped in the previous year with maize
118 and cassava. Three two-year rotations including legumes alone or in a crop mixture, namely (i) rice
119 after groundnut (RG), (ii) rice after sorghum-cowpea (*Vigna unguiculata*) intercropping (RSC) and (iii)
120 rice after velvet bean (*Mucuna pruriens*)-crotalaria (*Crotalaria spectabilis*) intercropping (RVC) were
121 compared with rainfed rice monocropping (RR) in a factorial randomized block design with four
122 replications. Rice was considered as the main crop. In each year of the experiment, each crop or crop
123 mixture in the rotation was grown in an individual 45.9 m² plot. The cultivars used for the experiment
124 were the rice cultivar Nerica 4, the groundnut cultivar Marabe, the sorghum cultivar IS 2787, the
125 cowpea cultivar Farimaso (Malagasy cultivar) and the velvet bean cultivar utilis. The three rotations
126 were selected for different purposes based on expert knowledge: RG was selected to provide a cash
127 crop and green manure made of groundnut residues, RSC to provide sorghum (grain and vegetative
128 biomass) as forage for livestock, and grain for food and green manure with cowpea, and RVC for its
129 potential to produce large quantities of green manure thanks to the combination of an erect and a
130 climbing plant, and to control plant-feeder nematodes.

131 Tillage, sowing, weeding, and harvest were done manually. Crop management practices are detailed
132 in Table 2. For all crops or crop mixtures, the soil was tilled by hand using a traditional hand-
133 ploughing tool called 'angady', down to a depth of 15 cm, every year in October before sowing. At
134 the end of November, five to eight rice seeds were sown in a 5-cm deep hole with 20 cm × 30 cm
135 spacing between holes. The holes were dug with the angady. Manure was applied directly in the
136 holes with the rice seeds (the amounts used and nutrient contents are detailed in Table 3), no
137 mineral fertilizer was applied. All the management practices carried out on the rotation crops were
138 done some days after those carried out on rice except for harvest. Groundnut, sorghum, cowpea,
139 velvet bean and crotalaria were sown just after rice at a density of 17, 3, 8, 7 and 7 holes.m⁻²
140 respectively. They were grown without fertilizer or manure and were harvested between mid-April
141 and mid-May, depending on the crop (see Table 2). Residues were left on the soil during the dry
142 season and buried during tillage before the rice was sown, except for the sorghum straw which was
143 exported.

144

145 *2.3. Sampling and analyses*

146 *2.3.1. Rice biomass and N content*

147 In the first year of the experiment (2015/2016), rice biomass was measured at harvest whereas in the
148 three following years, it was measured in three 0.54 m² quadrats on four different dates: at each of
149 the two weedings, at flowering, and at harvest. Measurements at the second weeding in year 4 were
150 cancelled due to a cyclone. The plants were cut at ground level, oven dried at 65 °C for 72 hours and
151 weighed to obtain dry matter (DM).

152 At harvest, rice biomass was measured in a 5 m² quadrat. Aerial biomass was cut at ground level and
153 a sample of about 200 g was oven-dried at 65 °C for 72 hours and weighed to obtain DM. Rice
154 panicles were collected manually from this quadrat and hand threshed by stripping the spikelets
155 from the panicles. Unfilled spikelets were removed and filled spikelets were weighed to estimate
156 grain yield. Moisture content of filled spikelets was determined by oven drying at 65 °C for 72 hours.

157 Grain yield was adjusted to 14% moisture content on an oven-dry basis. Using the same method,
158 yield components were assessed from nine sowing holes defined at the beginning of the experiment
159 and located in the same place in the 5m² quadrat in each rice field. The number of panicles and
160 weight of 1,000 dried filled grains were first assessed, then used to calculate the number of dried
161 filled grains per panicle.

162 N content in the rice biomass and grains was measured with near-infrared spectrometry (Labspec 4
163 spectrometer; ASD Inc., Malvern Panalytical, UK) calibrated with a Dumas procedure using a Leco-N-
164 analyzer (FP528; Leco Inc., St Joseph, USA) as described in Rakotoson et al. (2017).

165

166 *2.3.2. Weed biomass*

167 Weed biomass was measured in rice plots on each of the two weeding occasions in each cropping
168 season. In Year 1, weed biomass was hand weeded on the entire plot, weighed, and 200 g samples
169 were collected. In the three following cropping seasons, weed biomass was cut at ground level in the
170 same 0.54 m² quadrats used for rice measurements. Each year, weed samples were weighed and
171 oven-dried at 65 °C for 72 hours to obtain dry matter.

172

173 *2.3.3. N content in legume residues*

174 In all four years of the experiment, fresh legume biomass was measured at harvest (April-May) in two
175 1 m² quadrats. Sub-samples of about 200 g of fresh biomass were oven dried at 65 °C for 72 hours to
176 obtain DM. N content in legume residues was determined on dry samples using the dry combustion
177 method with a CHN auto-analyzer (ThermoFisher Flash 2000, USA).

178

179 *2.3.4. Soil inorganic N content*

180 N measurements were made in the rice crop in the RR and RVC rotations, which were the two most
181 contrasted rotations in terms of biomass production. In the RG and RSC rotations, N provided by
182 groundnut, sorghum and cowpea was assessed based on the amount of residues left on the field and

183 their N content. Soil sampling was carried out in Years 2 and 3 on four different occasions (at sowing,
184 first weeding, flowering and harvest); in year 4, soil samples were only collected at sowing and
185 flowering. Considering that the effect of rotation on the rice crop would not be noticeable in the year
186 the experiment was established, no soil samples were collected in Year 1. Soil was sampled in the 0-
187 10, 10-20, 20-40, 40-60 and 60-80 cm soil layers in three locations in the plot (the same locations as
188 for rice, weed and pest measurements, except at sowing). Samples from the three locations were
189 pooled and stored in a freezer at -4 °C. Soil inorganic N (ammonium and nitrate) was extracted with 2
190 N KCl solutions by shaking the suspension (30 g soil per 100 ml of solution) for 1 h. Samples were left
191 to decant, then the supernatant was filtered through a 0.2 µm Millipore filter (Merck, Darmstadt,
192 Germany) and stored in a sterile tube until analysis. A 50 g soil subsample was oven dried at 105 °C
193 for 48 hours to determine the dry weight of the extracted soil. Nitrate-N concentration was
194 determined using the colorimetric cadmium reduction and the Griess-Ilosvay reaction (Henriksen and
195 Selmer 1970) and the ammonium-N concentration using the indophenol blue method (Anderson and
196 Ingram 1989). Total inorganic N in the 0-80 cm soil layer ($\text{kg N}\cdot\text{ha}^{-1}$) was calculated from the nitrate-
197 and ammonium-N contents using soil bulk density measured in undisturbed soil cores of known
198 volume taken in all the soil layers.

199

200 *2.3.5. Soil nematodes*

201 In Year 1 and 3, nematodes were extracted at the flowering stage from 200 g fresh soil samples by
202 elutriation (Seinhorst, 1962) and were counted with a stereomicroscope. Nematodes were fixed in a
203 4% formaldehyde solution. Then, 200 individuals per sample were randomly selected on mass slides
204 and identified to genus or family level with a compound microscope. Taxa were assigned to trophic
205 groups as described by Yeates et al. (1993): bacterial feeders, fungal feeders, omnivores, carnivores
206 and plant feeders.

207

208 *2.3.5. Macrofauna sampling*

209 Sampling was performed in the four rotations using Tropical Soil Biology and Fertility (TSBF)
210 methodology (Anderson and Ingram, 1993). Samplings were done in the plots cropped with rice at
211 flowering. Two monoliths (25 x 25 cm) per plot were sampled. Three soil layers were considered in
212 each monolith: 0-10 cm, 10-20 cm, and 20-30 cm. All the organisms in the soil macrofauna found in
213 each soil layer were hand sorted. White grubs were separated and kept alive in separate flasks for
214 further identification. The other invertebrates were preserved in a flask with alcohol at 70 °C and
215 then separated, counted, and identified.

216 White grub attacks on rice were also recorded by counting the number of attacked rice plants in the
217 same quadrat as that used for yield.

218

219 *2.3.6. Data analyses*

220 As no effect of rotation could be expected in rice plots in Year 1, statistical analyses were done using
221 the data from years 2, 3 and 4.

222 *Agronomic variables*

223 All soil and rice variables were subjected to analysis of variance (ANOVA) for linear mixed effects
224 models. Rice grain yield and N content were tested with rotation and block as fixed effects, and
225 season, season × block and season × rotation interactions as random effects. Rice and weed biomass,
226 rice biomass N content, and soil inorganic N content were tested with rotation, block, date, and
227 rotation × date interactions as fixed effects, and season, season × block and season × rotation
228 interactions as random effects. In order to test the effect of rotation, some random effects were
229 selected for each variable analyzed using the Akaike and Schwarz information criteria (AIC and BIC).
230 Rotation means were then compared using Tukey's honestly significant difference test (Tukey's HSD).
231 When normality and variance assumptions were not respected, data were log-transformed (weed
232 biomass), or fixed effects (rotation, date, and rotation x date) were tested using the Kruskal-Wallis
233 test, and significant differences between means were compared using Dunn's test (N content in rice

234 biomass, in rice grains, and in the biomass of residues). Statistical analyses were done with R
235 software (R-4.0.0) using the packages lme4 (Bates et al., 2015), agricolae (Mendiburu, 2020) and
236 rstatix (Kassambara, 2018) for tests of linear mixed effects model fits and for post-hoc tests.

237

238 *Nematofauna and macrofauna*

239 Differences in the abundance of nematode trophic groups between rotations were assessed with a
240 one-way ANOVA coupled with post-hoc Tukey's HSD test in Year 1 (under rotation crops and rice)
241 and in Year 3 (under rice).

242 Attack rate and abundance of white grubs, and the abundance and diversity indices of macrofauna
243 were analysed with a mixed model with rotation and block as fixed effects, with season, rotation ×
244 season and block × season as random effects. To test the rotation effect, several random effects
245 were selected for each variable analysed using the Akaike and Schwarz information criteria (AIC and
246 BIC).

247 Raw data were used for species richness and the Shannon diversity index while transformed data
248 were used for other variables: the square root of arcsine for the attack rate, and the square root of
249 abundance data for white grubs and other macrofauna.

250

251 *2.3.7. Links between ecosystem functions, and assessment of ecosystem functions and profitability*

252 We computed different indicators to assess how the different rotations and rice monocropping
253 affected ecosystem functions. Six ecosystem functions were assessed: crop production, control of
254 weeds and plant-feeding nematodes, soil fertility, soil biodiversity and soil abundance. Crop
255 production was assessed using rice yield; weed biomass and abundance of plant-feed nematodes
256 were used to assess weed and nematode control, soil fertility using soil inorganic N content at rice
257 sowing, and soil biodiversity and abundance using macrofauna species richness and abundance,
258 respectively. For soil fertility, the amount of soil inorganic N at sowing in the RG and RSC rotations
259 was estimated from N in the residues and a relationship between soil N at sowing and N residues

260 defined with RR and RVC measurements. We consider the N returned by crop biomass to the soil is a
261 good proxy of N soil content (low mineralisation of residues during the dry and cold season before
262 the following cropping season and no other external nutrients applied). Links between these
263 ecosystem functions were assessed using a Pearson's correlations matrix.

264 To gain insights into the potential economic sustainability of the different rotations we tested, we
265 estimated their relative costs and gains and calculated the gross margin at the scale of the rotation.
266 Gains were calculated based on averaged yields observed for rice, groundnut and cowpea and
267 averaged market prices. Weeding costs were estimated proportionally to the total labour required
268 for this task and the amount of weeded biomass measured under each rotation averaged over the
269 whole experiment (rice + crops rotation). We did not consider costs related to sowing or tillage, as
270 these costs were the same for all the plots in our controlled experimental context. Conversely, costs
271 related to organic manure were taken into account (fertiliser was only applied on rice), and
272 calculated based on market prices. The gross margin was used to assess profitability.

273 First, a correlation matrix was calculated between the ecosystem functions to reveal any possibly
274 links between them. The ecosystem functions and profitability indicators were then transformed to
275 obtain a score ranging from 0 to 1 using the following equations:

276 (1) $(V_T - V_{\min}) / (V_{\max} - V_{\min})$ when the lowest values observed corresponded to the lowest
277 performances

278 (2) $(V_T - V_{\max}) / (V_{\min} - V_{\max})$ when the highest values observed corresponded to the lowest
279 performances (i.e. for weed biomass and plant-feeder nematodes).

280 where V_T is the value observed in the rotation under consideration (RG, RSC, or RVC) or RR
281 monocropping, V_{\min} and V_{\max} are the minimum and maximum values observed in the four rotations.
282 For each criterion assessed, the highest performance is indicated by the score 1 and the lowest by
283 the score 0.

284 All experimental data are available online on CIRAD dataverse (Ripoche et al., 2021).

285 3. Results

286 3.1. Rice growth, yield, yield components, and N content in rice biomass and grain

287 Date, rotation, and the interaction date \times rotation had a significant effect ($p < 0.001$) on rice growth.

288 Considering the different measurement dates, the rotation effect was significant ($p < 0.001$)

289 throughout the rice crop cycle except at the first weeding (W1, Figure 2). From the second weeding

290 to harvest, rice biomass in the RVC (rice - velvet bean + crotalaria) rotation was 80-100% higher than

291 in RR (rainfed rice as monocrop), and 40% higher in RG (rice-groundnut) and RSC (rice - sorghum +

292 cowpea) than in RR. At the second weeding, rice biomass in the RVC rotation was twice higher than

293 in RR (2.54 vs 1.25 t.ha⁻¹) while the biomass in RG and RSN was intermediate (about 1.7 t.ha⁻¹ Figure

294 2). At flowering, rice biomass was significantly higher in the RVC and RSC rotations than in RR while

295 RG was intermediate. Finally, rice biomass was similar in RVC, RSC and RG but was significantly higher

296 than in RR at harvest (Figure 2).

297 The rotation effect on grain yield was highly significant ($p < 0.001$, Figure 2). Compared to the yield

298 observed in Year 1, it decreased only in the RR rotation (on average for Year 2 to 4 2.29 vs. 3.51 t.ha⁻¹

299 in Year 1). Yields in the RVC rotation were 80% higher than in RR ($p < 0.001$, 4.31 vs. 2.29 t.ha⁻¹) and

300 about 30% higher than in RG and RSC ($p < 0.05$, 3.19 and 3.25 t.ha⁻¹ respectively). These differences

301 were due to a significantly higher number of filled grains per panicle ($p < 0.01$) in all the rotations

302 compared to in RR (63.3 vs. 51.4, data not shown), and a significantly higher number of panicles per

303 hole ($p < 0.01$) in RVC than in the other rotations and RR (13.1 vs. 10.3, data not shown).

304 We observed similar trends in rice biomass N content to those we observed in rice biomass with a

305 highly significant effect of date and rotation, and their interaction ($p < 0.001$). At each date, we

306 observed the same differences between rotations as for rice biomass except at harvest, when rice N

307 content was twice higher in RVC and RG than in RR, while no significant difference was observed for

308 RSC (Figure 2). Grain N content differed less between rotations than grain yield. Grain N content was

309 similar in the RVC, RSC and RG rotations, and significantly higher than in RR (+ 40 to 70%).

310

311 3.2. Weed biomass

312 Rotation had a significant effect on weed biomass measured at the second weeding and on total
313 weed biomass (Table 4). Weed biomass at the first weeding was around 0.15 t.ha⁻¹ whatever the year
314 of experiment and the rotation considered. At the second weeding, weed biomass was three times
315 higher in RR and RG than in RVC while in RSC it was intermediate (Table 4). Total weed biomass was
316 more than twice as high in RR than in the RVC rotation ($p < 0.05$, 0.49 vs. 0.18 t.ha⁻¹ respectively)
317 while in RG and RSC, total weed biomass was intermediate (Table 4). In comparison to Year 1, weed
318 biomass only decreased in the RVC rotation (0.18 vs. 0.36 t.ha⁻¹ for total weed biomass).

319

320 3.3. N content in legume residues and soil

321 N content in groundnut and cowpea residues was significantly lower than in the crotalaria/velvet
322 bean mixture ($p < 0.001$; 23.3 and 21.5 kg N.ha⁻¹ vs. 113.7 N.ha⁻¹ respectively, data not shown) but
323 similar to the N content measured in rice straw (12.4 kg N.ha⁻¹).

324 A significant effect of the date ($p < 0.001$) and the date x rotation interaction ($p < 0.001$) were
325 observed on soil inorganic N content. Soil inorganic N content was significantly higher in RVC than in
326 the RR rotation on the two first measurement dates, i.e., at sowing and at the first weeding (+ 35 kg
327 N.ha⁻¹ and + 51 kg N.ha⁻¹ respectively, Table 5). At flowering and harvest, RR and RVC rotations
328 showed a similar value, around 15 kg N.ha⁻¹.

329

330 3.4. Nematofauna

331 Rotation had a significant impact on the density of plant-feed ($p < 0.05$) in both Year 1 and 3 (Figure
332 3), and the patterns were similar in the two years. The abundance of plant-feeders was highest in RR
333 in both years while it was significantly lower in the RG and RVC rotations (respectively -79% and -69%
334 of the density observed in the RR rotation in Year 1, and -67% and -68% of the density observed in
335 the RR rotation in Year 3). On the other hand, the density of plant-feeders was intermediate in RSC in
336 both years and did not differ significantly from that in the other rotations. The density of omnivores

337 and carnivores' nematodes differed in the same way between rotations as the density of plant
338 feeders, albeit only significantly in Year 3, when the highest densities were found in RR ($1,068 \pm 322$
339 ind kg^{-1} soil), and were significantly lower in RG and RVC (respectively -80% and -72% of the density
340 observed in the RR rotation), while in RSC, intermediate values were observed (692 ± 573 ind kg^{-1}
341 soil). Finally, rotation had no significant effect on the density of bacterial- and fungal-feed nematodes
342 in Year 1 or 3 ($p > 0.05$, Figure 3).

343

344 3.5. Macrofauna

345 Due to marked variability, rotation had no significant effects on macrofauna biomass, density and
346 diversity (Table S1). Density ranged between 21 and 28 ind. m^{-2} and was mainly represented by social
347 insects such as ants and termites and Coleopteran larvae. No earthworms were collected during the
348 experiment. Detritivores were slightly higher in RVC, and herbivores slightly higher in RR but the
349 difference was not significant. In the same way, species richness and the Shannon index were lower
350 in RR than in the other rotations but not significantly so. In the same way, the rotation had neither
351 significant effect on the number of attacks by white grubs nor on the density of white grubs despite
352 higher density in RSV and RG compared to the other rotations. In general, populations of white grubs
353 were very small (Table S1). Three main species were found, among the most common in the zone:
354 *Enaria melanictera* (Melolonthidae), *Heteroconus paradoxus* (Dynastidae) and SpS1, identified as
355 *Hyposerica* sp (Sericidae, Lacroix, 1994).

356

357 3.5. Assessment of links between ecosystem functions and profitability

358 Crop production was positively and significantly correlated with weed and nematode control ($r = 0.66$
359 and 0.64 respectively, p -values < 0.01 , Table 6) as well as with soil fertility ($r = 0.54$ $p < 0.05$). Weed
360 control was also correlated with soil fertility ($r = 0.62$, p -value < 0.01). In contrast, no significant
361 correlation was found with soil macrofauna abundance or biodiversity.

362 Profitability was highest in RVC and lowest with RSC (Table 7). In RVC, profitability was mainly
363 explained by the low cost of weeding (lowest weed biomass, Table 4) and higher income from the
364 crops (highest rice grain yield, Figure 2) while the income from crops was the lowest in RSC (mean
365 rice yield and low cowpea yield) with a medium weeding cost. RR and RG profitability were similar in
366 both crop production years (rice in RR and rice and groundnut in RG) but were offset by the higher
367 cost of manure and of weeding in RR and RG, respectively.

368 In the assessment of the ecosystem functions and profitability analysis, RR monocropping obtained
369 the lowest scores (null score) for four criteria whereas profitability, soil biodiversity and soil
370 macrofauna abundance obtained medium to high scores (> 0.45 ; Figure 4). In contrast, RVC had the
371 highest scores for all the criteria except soil macrofauna abundance, for which the score was medium
372 (0.36). RG and RSC scores were intermediate. RG obtained medium to high scores (between 0.45 and
373 0.98) for crop production, profitability, soil biodiversity and nematode control, but low to null scores
374 (< 0.11) for soil macrofauna abundance, soil fertility and weed control. RSC obtained medium scores
375 for nematode control, crop production and weed control (between 0.32 and 0.48), low scores
376 (around 0.10) for soil fertility and soil macrofauna abundance, and null scores for soil diversity and
377 profitability.

378

379 **4. Discussion**

380 **4.1. Effect of rotation on soil fertility and rice N uptake**

381 Rotations with legumes had a positive impact on soil fertility and N content in rice biomass and grain,
382 as reported in other studies (Rodenburg et al., 2020; Saito et al., 2008). The observed differences
383 between rotations and monocropping may be due to differences in biomass production and N
384 returned by residues because the ability of legume to fix N was similar among the different crops
385 (around 70% of fixed N, Razafintsalama pers. comm.) and similar to those reported by Peoples et al.
386 (2009). The complementarity between velvet bean and crotalaria in terms of plant habit, expected to
387 produce more biomass and especially green manure, was effective given the amount of N returned

388 by residues in the RVC (rice - velvet bean + crotalaria) rotation, which was five to nine times higher
389 than in the other rotations or rice monocropping. Differences were less contrasted in rice N uptake,
390 particularly in rice grain N uptake, as all rotations showed similar N content but higher than observed
391 in RR (rainfed rice grown as monocrop). High N uptake could have a positive effect on protein
392 content and food quality even if relationships between N content and grain quality remain complex
393 (Gu et al., 2015). Different studies have shown the ability of velvet bean to compete with weeds and
394 supply N, and also a positive effect on water supply (Akanvou et al., 2001; Masikati et al., 2014).
395 However, significant inputs of N in legume residues with no inputs of other deficient nutrients, i.e., P
396 and Ca, in these soils (Raminoarison et al., 2020), limit N use efficiency. Even if plants can store more
397 nitrogen than required, thereby increasing plant N content (Figure 2b), N could be subject to leaching
398 when the amount of N fixed is high. Indeed, another study showed that including legumes in poor
399 environments can lead to soil acidification, which, in the long term, may hamper soil biological
400 activity and reduce soil phosphorus availability, another crucial nutrient for crop yield (Fujii et al.,
401 2018). To mitigate potential disservices, this point should thus be taken into consideration when
402 choosing the best crops for the rotation and in the management of fertilization depending on the
403 selected crops and on the biophysical constraints faced by farmers.

404

405 **4.2. Effect of rotation on pest control**

406 As also reported in other studies (Sester et al., 2014; Tamburini et al., 2020), we observed a positive
407 effect of rotation on pest control, but the intensity of this positive effect varied with the species
408 chosen.

409 In our study, the soil cover provided by the crop before rice and soil fertility seemed to be the main
410 factors responsible for the different degrees of weed control provided by the different rotations.
411 Indeed, groundnut was the crop with the lowest soil cover and biomass production, resulting in
412 higher weed infestation in the rice (Table 4) and groundnut crop cycle (data not shown), whereas the
413 opposite trend was observed in the RVC rotation (i.e., high soil cover and biomass production,

414 leading to low weed infestation during the mixed crop and rice cycle). As noted by Akanvou et al.
415 (2001), the ability of velvet bean and crotalaria to grow faster may limit weed growth, thanks to
416 increased competition for light and soil nutrients with weeds, hence depleting the weed seed bank
417 and leading to lower infestation in the following year (Mhlanga et al., 2015). The combination of
418 better nutrition due to higher soil N availability at rice sowing and lower infestation during their crop
419 cycle may also have benefitted rice growth and development in the RVC rotation (Becker and
420 Johnson, 1999), exacerbating competition between rice and weeds in favour of rice. In addition, we
421 can assume that the velvet bean-crotalaria mixture may have had allelopathic effect on weed growth
422 and/or germination. Actually, these effects were observed in different situations and on various
423 species (Adler and Chase, 2007; Farooq et al., 2011; Galon et al., 2021). Therefore, these three
424 factors combined (better soil cover, soil N-enrichment beneficial to the rice crop and allelopathic
425 effect) may have explained the high and significant difference between RVC and the other rotations
426 considering weed control.

427 The positive impact of rotation on plant-feeders nematodes could be mainly linked to two factors: (i)
428 the host/non-host status of the crops grown in the rotation (Inomoto and Asmus, 2010), and (ii) the
429 potential allelopathic effect of legume species on plant-feeders nematodes (Farooq et al., 2011;
430 Wang et al., 2002). Indeed, rice monocropping, and to a lesser extent RSC, had the highest
431 abundances of plant-feeders nematode likely due to the host status of the cereals (rice and sorghum)
432 and of cowpea to these pests (Bridge et al., 2005; Sikora et al., 2005). Nevertheless, the density of
433 plant-feeders was lower in the three rotations than in rice monocropping, as reported in other
434 studies (Alvey et al., 2001; Bagayoko et al., 2000). Indeed, legumes may excrete metabolites that
435 reduce the presence of plant-feeders nematodes in soil (Rao, 1990).

436 Our results concerning infestations by white grubs did not allow us to draw conclusions, perhaps due
437 to the conventional management practices applied in our study that led to low levels of infestation as
438 already shown in similar biophysical conditions (Rakotomanga et al., 2016; Ratnadass et al., 2017).

439

440 **4.3. Consequences for yield and profitability**

441 As reported in Tamburini et al. (2020), significant positive effects of plant diversification were mainly
442 observed on weed control and on the control of plant-feeding nematodes, rice growth and yield, and
443 soil fertility. Based on our results, the observed positive effects on rice yield were mainly due to
444 better pest control and soil fertility (Table 6). Lower levels of weed infestation and lower densities of
445 plant-feeders nematodes provided better growth conditions for rice (less competition for nutrients
446 and fewer pathogens), and, as explained above, this was emphasised by higher soil fertility. The
447 significant correlations between yield and these variables suggest that all three factors positively
448 affected rice productivity, albeit less for soil fertility ($r = 0.54$) than the others ($r \geq 0.64$).

449 These effects were also beneficial in terms of profitability, the highest profitability was observed in
450 the case of rotation with no cash crops as in RVC, due to the combination of (i) higher rice yields, (ii)
451 lower weeding costs, and (iii) lower manure costs because the rotation crops were not fertilised. RG
452 also scored well on profitability due to (i) a higher market price than for rice and (ii) a high average
453 rice yield, which led to a higher profit. Despite the assumptions we made to assess profitability based
454 on our experimental data, we think these results are relevant. We possibly underestimated the profit
455 to be obtained with a RG (rice/groundnut) rotation, as in our experiment, groundnut was sown at a
456 lower plant density which should reduce the cost of labour, and for RSC we were unable to attribute
457 a value to the fact that sorghum straw and grains can be used for livestock. Cost-benefits analysis is
458 now needed in real farms to be able to recommend the crop rotations best adapted to farmers'
459 conditions and objectives, but also related to market demand (Kleijn et al., 2019).

460

461 **4.4 Diversifying low input rice cropping systems in space and over time**

462 We showed that even in short-term rotations, ecosystem functions, yield, and profitability can be
463 enhanced by plant diversification. Nevertheless, different crops or crop mixtures should be included
464 in rotations with rice to avoid possible the drawbacks of short rotations (Bennett et al., 2012). As
465 explained by Gaba et al. (2015), different space and time arrangements are possible to optimise

466 ecosystem functions but require experimentation and field trials to identify the best management
467 plan depending on the constraints and objectives of the farmers concerned. Here, we compared
468 three legume diversification schemes in bi-annual rice-based rotations with rice monocropping: (i) as
469 a pure crop (RG), (ii) intercropped with a cereal (RSC), (iii) as a short fallow (RVC). These three
470 schemes offered a gradient in terms of diversity and contrasted services but also in feasibility. Low
471 input based cropping systems are characterised by the need to find trade-offs between short-term
472 issues, in our case by production, profitability, and pest control, and long-term issues such as
473 sustainability and soil fertility (Shiferaw and Holden, 1998). The one legume-rice rotation, which
474 could be the most feasible for farmers thanks to groundnut production, represented a first step in
475 enhancing ecosystem function compared to rice monocropping and maintained a satisfactory level of
476 profitability (Figure 4). The cereal-legume intercrop in rotation with rice did not lead to significant
477 improvement mainly due to the negative impact of nematodes and relatively variable yields,
478 probably linked to the high variability of biomass production by the crop mixture over the course of
479 the experiment. These two examples call for more research to select the species the best adapted to
480 the farmers' objectives and the services they expect, and local constraints (Garcia et al., 2020; Tixier
481 et al., 2011). The short legume fallow resulted in the highest performances in terms of ecosystem
482 functions and profitability but its social acceptance requires additional studies as it provides no
483 income for half of the surface usually cropped. Therefore, despite increased profitability, it may be
484 difficult for farmers to adopt this practice if they receive no technical support, subsidies, or other
485 means of compensating as they mainly live under the poverty threshold (Razafimahatratra et al.,
486 2017). Modelling approaches may help to summarize knowledge to assess plant diversification
487 scenarios, to be able to guide farmers in their choice (Jourdain et al., 2001). Thus, multidisciplinary
488 research is needed to better support the introduction of ecological intensified practices in a systemic
489 way (Gaba et al., 2015; Tamburini et al., 2020).

490

491 **Conclusion**

492 In family farms in the Malagasy highlands, plant diversification is often a way to for smallholders to
493 compensate for their lack of access to external inputs, to be more resilient, and to increase their
494 income. In this study, we quantified the benefits of plant diversification in short rainfed rice-based
495 rotations, in terms of crop production, weed and nematode control, soil fertility, macrofauna
496 biodiversity and abundance, and profitability. We showed that rotations have varying levels of
497 positive impacts on these different ecosystem functions depending on the crops chosen for the
498 rotation or for crop mixtures. At the rotation scale, profitability was always better in rotations than in
499 rice monocropping due to the rapid and marked decrease in rice yield in rice monocropping. Better
500 results were observed in the rotation with non-cash crops, suggesting that different ways of support
501 should be introduced to help poor farmers include a period of fallow with no income. It is crucial to
502 improve our knowledge of crop species development according to the diversification practices
503 chosen; to combine experimentation in controlled and real conditions so as to be able to give
504 contextualised technical advice. Modelling approaches could be a great help in extending our
505 knowledge and in providing guidance to farmers to test different plant diversification options and
506 assess their performances with respect to farm sustainability.

507

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517

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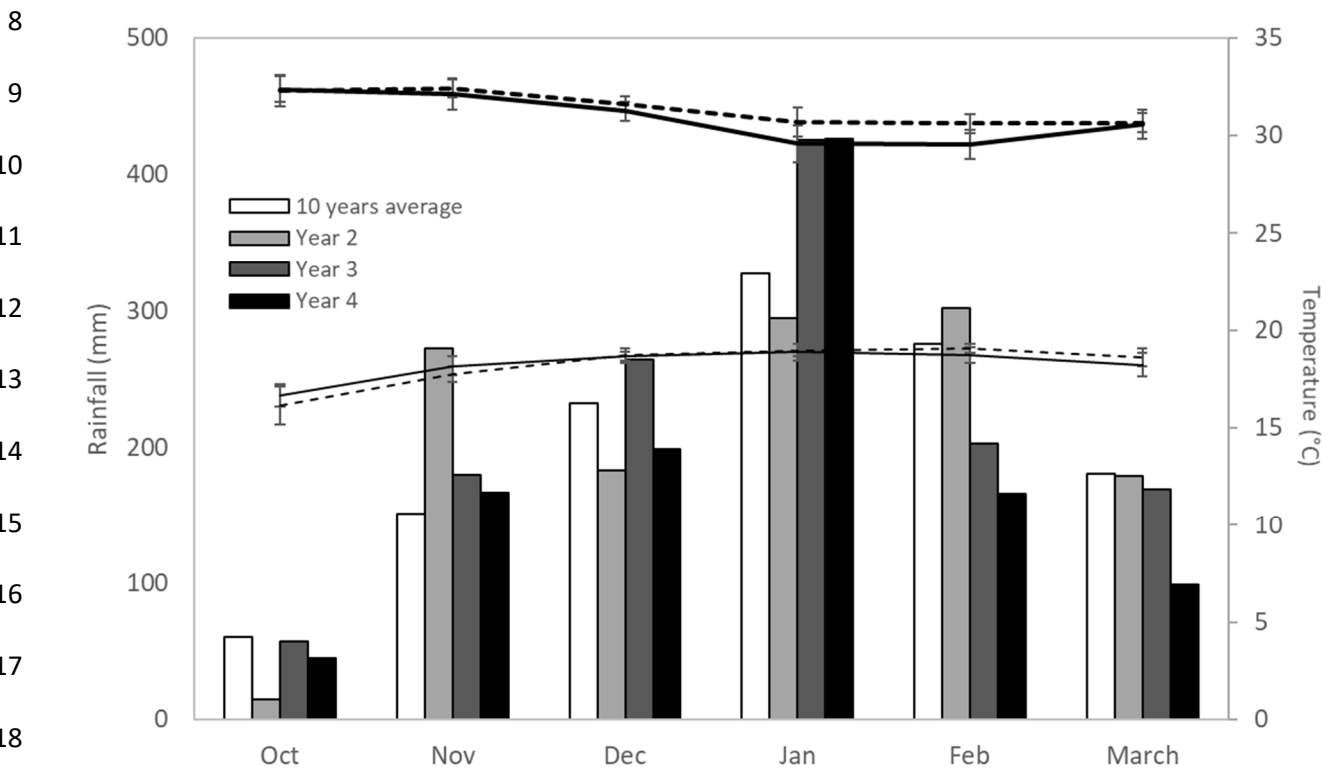
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1 Figure 1. Average monthly rainfall and average maximum and minimum temperatures recorded at
2 Ivory station over the four cropping seasons of the experiment and averaged over 2006-2015 (Rainfall
3 in mm, Temperature in °C ± standard error on the left and right axis, respectively).

4 The bold solid line corresponds to the maximum temperature averaged over 2006-2015 and bold
5 dashed line to the average maximum temperature over the four years of the experiment. The regular
6 solid line corresponds to the minimum temperature over 2006-2015, and the regular dashed line
7 corresponds to the minimum temperature averaged over the four year of the experiment.



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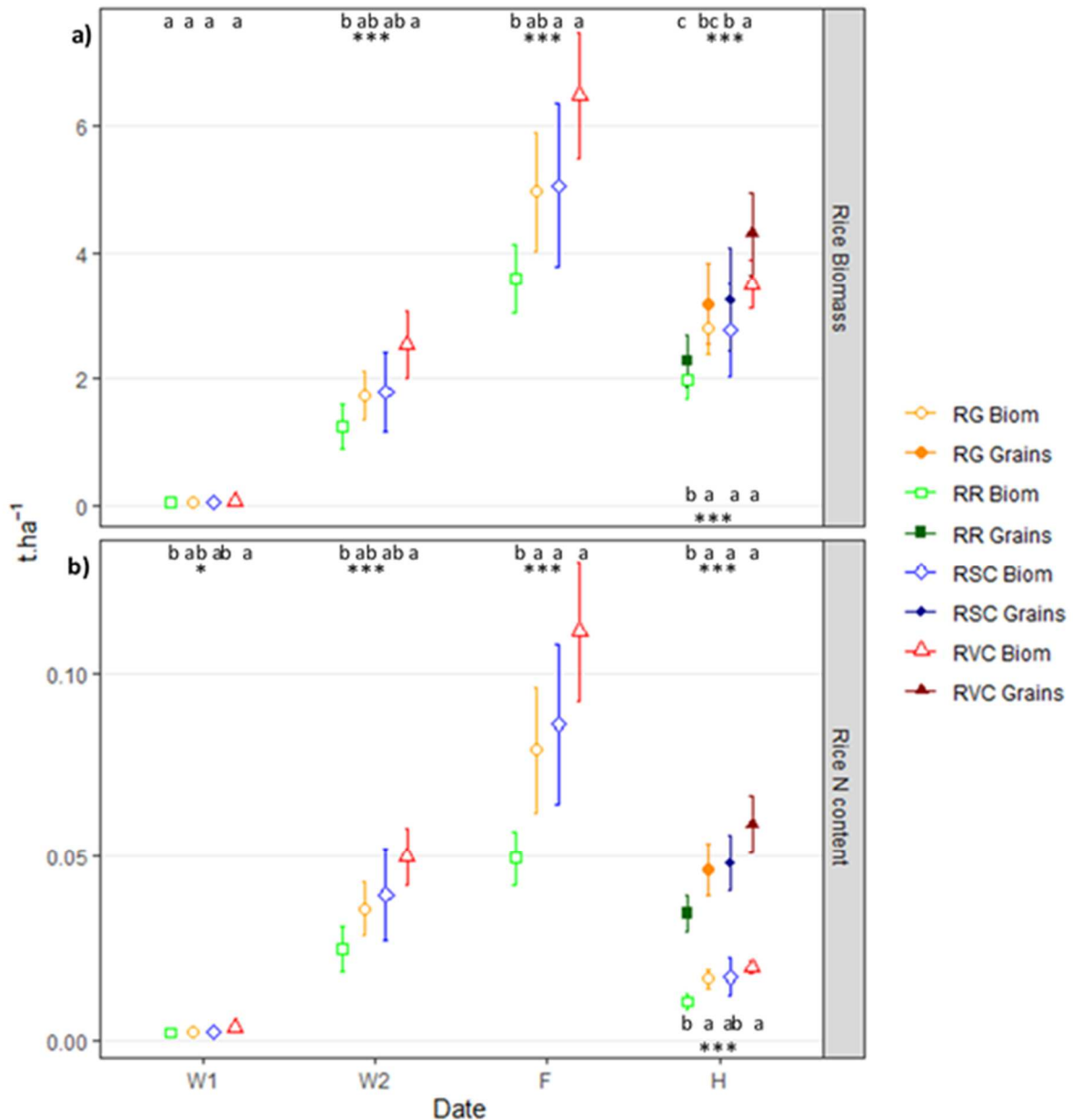
27 Figure 2. Average rice biomass (vegetative and grain) (a), and its N content (b) observed during rice
 28 crop cycle in the four different rotations.

29 Means are calculated over Year 2 to 4 of the experiment, error bars are 95% confidence intervals.

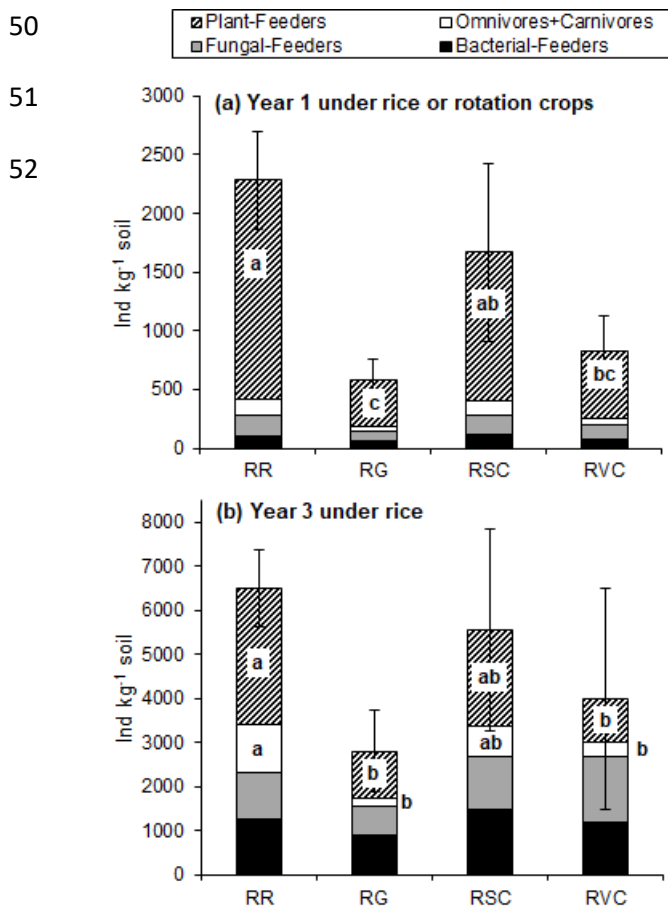
30 * and *** indicate significant rotation effect at $p < 0.05$ and $p < 0.001$ and different letters indicate
 31 significant differences between rotations. At harvest (H), the letters at the top indicate significant
 32 differences in grain yield. The letters at the bottom indicate significant differences in vegetative
 33 biomass.

34 RR = Rainfed rice monocropping, RG = Rice-Groundnut rotation, RSC = Rice-Sorghum + Cowpea
 35 rotation, RVC = Rice-Velvet bean + Crotalaria rotation.

36 W1 = first weeding, W2 = second weeding, F = rice flowering, H = rice harvest



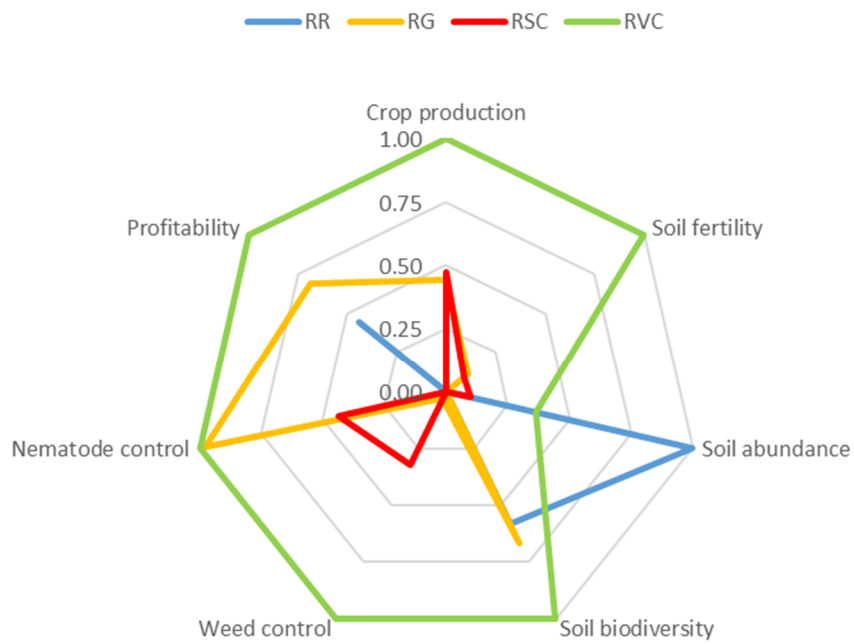
44 Figure 3. Abundance of trophic groups of nematodes in the four different rotations in Year 1 of the
 45 experiment (a) under rice (RR) or rotation crops (groundnut for RG, sorghum-cowpea mixture for RSC,
 46 and velvet bean-crotalaria mixture for RVC) and in Year 3 of the experiment (b) under rice in RR and
 47 the three rotations (in ind.kg⁻¹ soil).
 48 Error bars are 95% confidence intervals. Different letters indicate significant difference (p < 0.05)
 49 between rotations for each trophic group.



53 Figure 4. Ecosystem functions and profitability assessment for the three rotations compared to rice
54 monocropping averaged over the experiment.

55 RR = Rainfed rice monocropping, RG = Rice-Groundnut rotation, RSC = Rice-Sorghum + Cowpea
56 rotation, RVC = Rice-Velvet bean + Crotalaria rotation.

57



1 Table 1. Selected soil physical and chemical properties (mean and standard error, n = 4) of the
 2 experimental field at the Ivory station located in the mid-western region, Madagascar.

3

Soil layer (cm)	pH (H ₂ O)	Olsen P mg.kg ⁻¹	CEC cmol.kg ⁻¹	Total C %	N ‰	MO %	Clay %	Silt %	Sand %
0 - 10	4.9 ± 0.1	4.7 ± 0.9	3.1 ± 0.2	1.7 ± 0.2	1.3 ± 0.1	3.0 ± 0.3	33.1 ± 4.4	17.4 ± 1.1	49.6 ± 4.9
10 - 20	4.9 ± 0.2	3.5 ± 0.5	3.0 ± 0.2	1.5 ± 0.4	1.2 ± 0.1	2.6 ± 0.7	34.1 ± 7.7	18.0 ± 0.4	48.0 ± 7.9
20 - 40	5.0 ± 0.0	2.9 ± 0.6	3.0 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	2.2 ± 0.3	27.7 ± 3.5	19.7 ± 2.6	53.4 ± 1.1

4

5 Table 2. Rice management practices over the four cropping seasons of the experiment.

6 WBS = Week(s) before sowing, WAS = Weeks after sowing. Mixture harvest corresponds to the harvest

7 of velvet bean and crotalaria in the RVC rotation.

	Year 1	Year 2	Year 3	Year 4
Tillage	5 WBS	3 WBS	6 WBS	5 WBS
Sowing	2 & 3/12/15 = W0	23 & 24/11/16 = W0	24 & 25/11/17 = W0	27 & 28/11/2018 = W0
Weeding 1	4 WAS	3 WAS	3 WAS	4 WAS
Weeding 2	9 WAS	10 WAS	8 WAS	11 WAS
Rice harvest	16 WAS	17 WAS	17 WAS	17 WAS
Groundnut harvest	23 WAS	20 WAS	24WAS	20 WAS
Cowpea harvest	23 WAS	23 WAS	23 WAS	26 WAS
Mixture harvest	23 WAS	24 WAS	23 WAS	26 WAS

8

9

10 Table 3. Quantity and characteristics of the manure applied to rice over the four years of the
11 experiment at Ivory station.

12 Values are expressed in % of dry matter (DM). OM = organic matter.

13

	Quantity of								
	manure	%	OM	N	C	P	K	Ca	Mg
	applied	DM	%	%	%	%	%	%	%
	(t.ha ⁻¹ of DM)								
Year 1	12.4	0.74	20.8	0.69	8.45	0.17	1.03	0.50	0.27
Year 2	7.5	0.76	14.3	0.50	6.13	0.14	1.14	0.54	0.28
Year 3	5.0	0.48	21.7	0.83	9.88	0.22	1.47	0.70	0.38
Year 4	5.5	0.72	17.6	0.57	7.10	0.18	1.18	0.71	0.31

14

15 Table 4. Weed biomass (in t DM.ha⁻¹) at the first and second weeding and cumulated over the rice crop
16 cycle in the four rotations over Year 2 to 4 of the experiment.

17 Mean ± confidence interval at 95% and p-value related to the rotation effect. Means are calculated
18 over Year 2 to 4 of the experiment.

19 W1 = first weeding, W2 = second weeding. Letters indicate significant difference between rotations.

20

	W1	W2	Total
RR	0.17 ± 0.09	0.31 ± 0.09 (a)	0.49 ± 0.16 (a)
RG	0.16 ± 0.08	0.32 ± 0.13 (a)	0.48 ± 0.23 (ab)
RSV	0.13 ± 0.08	0.26 ± 0.11 (ab)	0.39 ± 0.18 (ab)
RVC	0.09 ± 0.05	0.10 ± 0.02 (b)	0.18 ± 0.05 (b)
Rotation effect	ns	< 0.01 **	< 0.001 ***

21

22 Table 5. Soil inorganic N content (in kgN.ha⁻¹) during the rice crop cycle in the RR and RVC rotations.
 23 Mean ± confidence interval at 95% and p-value associated with the rotation effect. Means are
 24 calculated over Year 2 to 4 of the experiment.
 25 S = sowing, W1 = first weeding, F = rice flowering, H = rice harvest. Letters indicate significant difference
 26 between rotations.

27

	S	W1	F	H
RR	51.2 ± 14.8 (b)	85.2 ± 14.8 (b)	13.6 ± 7.0	13.5 ± 4.2
RVC	86.6 ± 25.4 (a)	136.1 ± 23.7 (a)	18.3 ± 16.5	12.9 ± 4.8
Rotation effect	< 0.001***	< 0.001***	ns	ns

28

29

30 Table 6. Pearson's correlation coefficients (r) matrix between the different ecosystem functions.

31 Significant correlations (* p > 0.05 and ** p < 0.01) are indicated in bold.

32

	Weed control	Nematode control	Soil fertility	Soil diversity	Soil abundance
Crop production	0.66**	0.64**	0.54*	0.17	0.40
Weed control		-0.44	0.62**	0.03	0.39
Nematode control			-0.24	0.45	0.43
Soil fertility				-0.10	0.16
Soil diversity					0.35

33

34

35 Table 7. Income from crops, weeding and manure costs and gross margin calculated for rice
36 monocropping and the three different rotations on a per hectare basis.
37 Incomes and costs were calculated for two years, corresponding to the time of the rotation.

	RR	RG	RSC	RVC
Crops income (\$·ha ⁻¹)	932	850	725	878
Weeding cost (\$·ha ⁻¹)	41	47	34	23
Manure cost (\$·ha ⁻¹)	255	127	127	127
Gross margin (\$·ha ⁻¹)	636	676	564	728

38