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Abstract

Plant diversification is one of the main ways to ecologically intensify agroecosystems to improve their sustainability and resilience. Rotations and/or a mixture of crops can mitigate pest and weed infestation, reduce diseases, and improve soil fertility and crop productivity. However, rainfed rice yields in the Malagasy highlands remain low despite the frequent use of cropping systems including crop rotations and mixtures. In this study, we compared three rainfed rice based short rotations with rainfed rice monocropping to quantify the benefits of plant diversification on different ecosystem functions such as weed and nematode control, soil fertility, soil macrofauna abundance and diversity, and rice yield over four cropping seasons. The three rotations were based on rice in rotation with one legume, groundnut (RG), a cereal-legume mixture, sorghum and cowpea (RSC), or a mixture of legumes, velvet bean and crotalaria (RVC). Rice growth, N content and yield, soil N content, weed biomass, nematofauna and macrofauna were assessed and a profitability analysis was performed at rotation scale. The legume mixture had a significant and positive effect on rice growth, N content and yield, soil N content, and weed and nematode control due to high biomass production in the cropping cycle including legume mixture, by limiting weed growth and leaving a large quantity of N-rich residues to enrich the soil for the following rice crop. The nematicide properties of the legume mixture may reduce the infestation of plant-feeding nematodes. The RG and RSC rotations produced intermediate results. While rice yields were higher in these rotations than when rainfed rice was grown alone, weed biomass remained high due to minimal competition with weeds during the crop rotation cycle especially with groundnut. For RSC, nematode control was limited as both sorghum and cowpea are host plants for nematodes. Despite a year with no crop income with the RVC rotation, profitability was higher mainly due to the increased rice yield and reduced field management costs. The choice of species is thus crucial to optimize ecosystem functions adapted to farmers' context and objectives.

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

Introduction

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

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

Including legumes in cropping systems, in rotation or/and in intercropping, is common in family farms in Africa (Waggoner, 1996), particularly for (i) their ability to fix atmospheric nitrogen (N), (ii) their potential to enrich soil mineral fertility via their N-rich residues, (iii) their facilitating effect on non-legume crops leading to a better exploration of soil resources, and (iv) their ability to suppress weeds. These benefits generally increase yields and reduce the use of external inputs (Chikowo et al., 2007; Namatsheve et al., 2020; Snapp et al., 2019). Consequently, they also play a major role in enhancing ecosystem functions. However, plant species have to be carefully chosen based on their functional traits related to the expected services (Blesh, 2017; Tribouillois et al., 2015) and to optimize their complementarity with non-legume species in space and over time to explore soil resources, and to compete for light (Bedoussac et al., 2015; Garcia et al., 2020; Vandermeer et al., 1998). Rice is the main staple food crop grown in Madagascar and is mainly cultivated in lowland areas. However, given population growth and the current need to import rice, increasing crop productivity is one of the main objectives of agricultural development, especially since average yields of irrigated rice (around 3 $t.ha^{-1}$, Naudin *et al.*, 2019) are well below the potential (yield gap of around 2.1 $t.ha^{-1}$; FAO, 2004). Rainfed upland rice, and more generally rainfed crops, are widely grown to satisfy the food needs of the growing population and also because lowlands are already saturated. In mid-western Vakinankaratra, one of the most productive areas of the country, rainfed rice accounts for around 15% of the area cultivated by family farms (Razafimahatratra et al., 2017). The constraints smallholder farmers have to face include numerous pests, weeds and diseases, poor soil fertility, and poor quality manure, while they lack access to external inputs (fertilizers, pesticides), and depend to a great extent on manual labour (Raboin et al., 2014; Raminoarison et al., 2020). Currently, farmers grow legumes, tubers, and other cereals in pure or crop mixtures in rotation with rainfed rice both to compensate for their limited access to exogenous inputs and to diversify their sources of income. Yet, given the many constraints encountered in this region, average rice yields are extremely low (1.6 $t.ha^{-1}$, Razafimahatratra *et al.*, 2017).

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

Material and methods

2.1. Study site

This study was carried out at Ivory station, located in mid-western Vakinankaratra region (19°33’18.90’’ lat. S, 46°24'53.83’’ long. E, 930 m a.s.l.) over four cropping seasons 2015/2016, 2016/2017, 2017/2018 and 2018/2019, hereafter referred to as Year 1, Year 2, Year 3, and Year 4. Cropping season corresponds to the rainy season, which lasts from November to the end of March/beginning of April in the study region. An automatic weather station (CIMEL, Electronique, Paris France) located near the experimental field recorded daily weather data. Average temperature during the four cropping seasons was 24.7 ± 0.6 °C and annual rainfall was 1225 ± 84 mm, with monthly variations between seasons (Figure 1).

At the start of the experiment in 2015, soil samples were collected at six randomly selected points in the experimental field in the 0-10, 10-20, 20-40 cm soil layers to determine selected physical-chemical soil properties (Table 1). The soil type at the experimental site was a sandy-clay-loamy Ferralsol (FAO classification) with 32-18-50% clay-silt-sand composition in the 0-40 cm soil layer. Soil pH (H_2O) was measured using a glass electrode (Kalra, 1995). Available phosphorus (P) was determined using the Olsen method (Olsen et al., 1954), cation exchange capacity (CEC) using the cobaltihexamine chloride method (Fallavier et al., 1985) and total carbon (C) and N by dry combustion using a Carbon Hydrogen Nitrogen (CHN) microanalyzer (ThermoFisher Flash 2000, USA).

2.2. Experimental design and crop management

The field experiment was set up in November 2015 in a field cropped in the previous year with maize and cassava. Three two-year rotations including legumes alone or in a crop mixture, namely (i) rice after groundnut (RG), (ii) rice after sorghum-cowpea (*Vigna unguiculata)* intercropping (RSC) and (iii) rice after velvet bean (*Mucuna pruriens)*-crotalaria (*Crotalaria spectabilis)* intercropping (RVC) were compared with rainfed rice monocropping (RR) in a factorial randomized block design with four replications. Rice was considered as the main crop. In each year of the experiment, each crop or crop mixture in the rotation was grown in an individual 45.9 m^2 plot. The cultivars used for the experiment were the rice cultivar Nerica 4, the groundnut cultivar Marabe, the sorghum cultivar IS 2787, the cowpea cultivar Farimaso (Malagasy cultivar) and the velvet bean cultivar utilis. The three rotations were selected for different purposes based on expert knowledge: RG was selected to provide a cash crop and green manure made of groundnut residues, RSC to provide sorghum (grain and vegetative biomass) as forage for livestock, and grain for food and green manure with cowpea, and RVC for its potential to produce large quantities of green manure thanks to the combination of an erect and a climbing plant, and to control plant-feeder nematodes.

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

2.3. Sampling and analyses

2.3.1. Rice biomass and N content

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

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

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

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

2.3.2. Weed biomass

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

2.3.3. N content in legume residues

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

2.3.4. Soil inorganic N content

N measurements were made in the rice crop in the RR and RVC rotations, which were the two most contrasted rotations in terms of biomass production. In the RG and RSC rotations, N provided by groundnut, sorghum and cowpea was assessed based on the amount of residues left on the field and their N content. Soil sampling was carried out in Years 2 and 3 on four different occasions (at sowing, first weeding, flowering and harvest); in year 4, soil samples were only collected at sowing and flowering. Considering that the effect of rotation on the rice crop would not be noticeable in the year the experiment was established, no soil samples were collected in Year 1. Soil was sampled in the 0-10, 10-20, 20-40, 40-60 and 60-80 cm soil layers in three locations in the plot (the same locations as for rice, weed and pest measurements, except at sowing). Samples from the three locations were pooled and stored in a freezer at -4 °C. Soil inorganic N (ammonium and nitrate) was extracted with 2 N KCl solutions by shaking the suspension (30 g soil per 100 ml of solution) for 1 h. Samples were left to decant, then the supernatant was filtered through a 0.2 µm Millipore filter (Merck, Darmstadt, Germany) and stored in a sterile tube until analysis. A 50 g soil subsample was oven dried at 105 °C for 48 hours to determine the dry weight of the extracted soil. Nitrate-N concentration was determined using the colorimetric cadmium reduction and the Griess-Ilosvay reaction (Henriksen and Selmer 1970) and the ammonium-N concentration using the indophenol blue method (Anderson and Ingram 1989). Total inorganic N in the 0-80 cm soil layer (kg $N.ha^{-1}$) was calculated from the nitrate- and ammonium-N contents using soil bulk density measured in undisturbed soil cores of known volume taken in all the soil layers.

2.3.5. Soil nematodes

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

2.3.5. Macrofauna sampling

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

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

2.3.6. Data analyses

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

Agronomic variables

All soil and rice variables were subjected to analysis of variance (ANOVA) for linear mixed effects models. Rice grain yield and N content were tested with rotation and block as fixed effects, and season, season × block and season × rotation interactions as random effects. Rice and weed biomass, rice biomass N content, and soil inorganic N content were tested with rotation, block, date, and rotation × date interactions as fixed effects, and season, season × block and season × rotation interactions as random effects. In order to test the effect of rotation, some random effects were selected for each variable analyzed using the Akaike and Schwarz information criteria (AIC and BIC). Rotation means were then compared using Tukey's honestly significant difference test (Tukey's HSD). When normality and variance assumptions were not respected, data were log-transformed (weed biomass), or fixed effects (rotation, date, and rotation x date) were tested using the Kruskal-Wallis test, and significant differences between means were compared using Dunn's test (N content in rice biomass, in rice grains, and in the biomass of residues). Statistical analyses were done with R software (R-4.0.0) using the packages lme4 (Bates et al., 2015), agricolae (Mendiburu, 2020) and rstatix (Kassambara, 2018) for tests of linear mixed effects model fits and for post-hoc tests.

Nematofauna and macrofauna

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

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

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

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

We computed different indicators to assess how the different rotations and rice monocropping affected ecosystem functions. Six ecosystem functions were assessed: crop production, control of weeds and plant-feeding nematodes, soil fertility, soil biodiversity and soil abundance. Crop production was assessed using rice yield; weed biomass and abundance of plant-feed nematodes were used to assess weed and nematode control, soil fertility using soil inorganic N content at rice sowing, and soil biodiversity and abundance using macrofauna species richness and abundance, respectively. For soil fertility, the amount of soil inorganic N at sowing in the RG and RSC rotations was estimated from N in the residues and a relationship between soil N at sowing and N residues defined with RR and RVC measurements. We consider the N returned by crop biomass to the soil is a good proxy of N soil content (low mineralisation of residues during the dry and cold season before the following cropping season and no other external nutrients applied). Links between these ecosystem functions were assessed using a Pearson's correlations matrix.

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

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

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

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

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

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

3. Results

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

Date, rotation, and the interaction date × rotation had a significant effect ($p < 0.001$) on rice growth. Considering the different measurement dates, the rotation effect was significant ($p < 0.001$) throughout the rice crop cycle except at the first weeding (W1, Figure 2). From the second weeding to harvest, rice biomass in the RVC (rice - velvet bean + crotalaria) rotation was 80-100% higher than in RR (rainfed rice as monocrop), and 40% higher in RG (rice-groundnut) and RSC (rice - sorghum + cowpea) than in RR. At the second weeding, rice biomass in the RVC rotation was twice higher than 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 2). At flowering, rice biomass was significantly higher in the RVC and RSC rotations than in RR while RG was intermediate. Finally, rice biomass was similar in RVC, RSC and RG but was significantly higher than in RR at harvest (Figure 2).

The rotation effect on grain yield was highly significant ($p < 0.001$, Figure 2). Compared to the yield observed in Year 1, it decreased only in the RR rotation (on average for Year 2 to 4 2.29 vs. 3.51 $t.ha^{-1}$ 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 about 30% higher than in RG and RSC ($p < 0.05$, 3.19 and 3.25 $t.ha^{-1}$ respectively). These differences were due to a significantly higher number of filled grains per panicle ($p < 0.01$) in all the rotations compared to in RR (63.3 vs. 51.4, data not shown), and a significantly higher number of panicles per hole ($p < 0.01$) in RVC than in the other rotations and RR (13.1 vs. 10.3, data not shown).

We observed similar trends in rice biomass N content to those we observed in rice biomass with a highly significant effect of date and rotation, and their interaction ($p < 0.001$). At each date, we observed the same differences between rotations as for rice biomass except at harvest, when rice N content was twice higher in RVC and RG than in RR, while no significant difference was observed for RSC (Figure 2). Grain N content differed less between rotations than grain yield. Grain N content was similar in the RVC, RSC and RG rotations, and significantly higher than in RR (+ 40 to 70%).

3.2. Weed biomass

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

3.3. N content in legume residues and soil

N content in groundnut and cowpea residues was significantly lower than in the crotalaria/velvet 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 similar to the N content measured in rice straw (12.4 kg $N.ha^{-1}$).

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

3.4. Nematofauna

Rotation had a significant impact on the density of plant-feed ($p < 0.05$) in both Year 1 and 3 (Figure 3), and the patterns were similar in the two years. The abundance of plant-feeders was highest in RR in both years while it was significantly lower in the RG and RVC rotations (respectively -79% and -69% of the density observed in the RR rotation in Year 1, and -67% and -68% of the density observed in the RR rotation in Year 3). On the other hand, the density of plant-feeders was intermediate in RSC in both years and did not differ significantly from that in the other rotations. The density of omnivores and carnivores' nematodes differed in the same way between rotations as the density of plant feeders, albeit only significantly in Year 3, when the highest densities were found in RR (1,068 ± 322 ind kg^{-1} soil), and were significantly lower in RG and RVC (respectively -80% and -72% of the density observed in the RR rotation), while in RSC, intermediate values were observed (692 ± 573 ind kg^{-1} soil). Finally, rotation had no significant effect on the density of bacterial- and fungal-feed nematodes in Year 1 or 3 ($p > 0.05$, Figure 3).

3.5. Macrofauna

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

3.5. Assessment of links between ecosystem functions and profitability

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

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

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

4. Discussion

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

Rotations with legumes had a positive impact on soil fertility and N content in rice biomass and grain, as reported in other studies (Rodenburg et al., 2020; Saito et al., 2008). The observed differences between rotations and monocropping may be due to differences in biomass production and N returned by residues because the ability of legume to fix N was similar among the different crops (around 70% of fixed N, Razafintsalama pers. comm.) and similar to those reported by Peoples et al. (2009). The complementarity between velvet bean and crotalaria in terms of plant habit, expected to produce more biomass and especially green manure, was effective given the amount of N returned by residues in the RVC (rice - velvet bean + crotalaria) rotation, which was five to nine times higher than in the other rotations or rice monocropping. Differences were less contrasted in rice N uptake, particularly in rice grain N uptake, as all rotations showed similar N content but higher than observed in RR (rainfed rice grown as monocrop). High N uptake could have a positive effect on protein content and food quality even if relationships between N content and grain quality remain complex (Gu et al., 2015). Different studies have shown the ability of velvet bean to compete with weeds and supply N, and also a positive effect on water supply (Akanvou et al., 2001; Masikati et al., 2014). However, significant inputs of N in legume residues with no inputs of other deficient nutrients, i.e., P and Ca, in these soils (Raminoarison et al., 2020), limit N use efficiency. Even if plants can store more nitrogen than required, thereby increasing plant N content (Figure 2b), N could be subject to leaching when the amount of N fixed is high. Indeed, another study showed that including legumes in poor environments can lead to soil acidification, which, in the long term, may hamper soil biological activity and reduce soil phosphorus availability, another crucial nutrient for crop yield (Fujii et al., 2018). To mitigate potential disservices, this point should thus be taken into consideration when choosing the best crops for the rotation and in the management of fertilization depending on the selected crops and on the biophysical constraints faced by farmers.

4.2. Effect of rotation on pest control

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

In our study, the soil cover provided by the crop before rice and soil fertility seemed to be the main factors responsible for the different degrees of weed control provided by the different rotations. Indeed, groundnut was the crop with the lowest soil cover and biomass production, resulting in higher weed infestation in the rice (Table 4) and groundnut crop cycle (data not shown), whereas the opposite trend was observed in the RVC rotation (i.e., high soil cover and biomass production, leading to low weed infestation during the mixed crop and rice cycle). As noted by Akanvou et al. (2001), the ability of velvet bean and crotalaria to grow faster may limit weed growth, thanks to increased competition for light and soil nutrients with weeds, hence depleting the weed seed bank and leading to lower infestation in the following year (Mhlanga et al., 2015). The combination of better nutrition due to higher soil N availability at rice sowing and lower infestation during their crop cycle may also have benefitted rice growth and development in the RVC rotation (Becker and Johnson, 1999), exacerbating competition between rice and weeds in favour of rice. In addition, we can assume that the velvet bean-crotalaria mixture may have had allelopathic effect on weed growth and/or germination. Actually, these effects were observed in different situations and on various species (Adler and Chase, 2007; Farooq et al., 2011; Galon et al., 2021). Therefore, these three factors combined (better soil cover, soil N-enrichment beneficial to the rice crop and allelopathic effect) may have explained the high and significant difference between RVC and the other rotations considering weed control.

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

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

4.3. Consequences for yield and profitability

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

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

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

We showed that even in short-term rotations, ecosystem functions, yield, and profitability can be enhanced by plant diversification. Nevertheless, different crops or crop mixtures should be included in rotations with rice to avoid possible the drawbacks of short rotations (Bennett et al., 2012). As explained by Gaba et al. (2015), different space and time arrangements are possible to optimise ecosystem functions but require experimentation and field trials to identify the best management plan depending on the constraints and objectives of the farmers concerned. Here, we compared three legume diversification schemes in bi-annual rice-based rotations with rice monocropping: (i) as a pure crop (RG), (ii) intercropped with a cereal (RSC), (iii) as a short fallow (RVC). These three schemes offered a gradient in terms of diversity and contrasted services but also in feasibility. Low input based cropping systems are characterised by the need to find trade-offs between short-term issues, in our case by production, profitability, and pest control, and long-term issues such as sustainability and soil fertility (Shiferaw and Holden, 1998). The one legume-rice rotation, which could be the most feasible for farmers thanks to groundnut production, represented a first step in enhancing ecosystem function compared to rice monocropping and maintained a satisfactory level of profitability (Figure 4). The cereal-legume intercrop in rotation with rice did not lead to significant improvement mainly due to the negative impact of nematodes and relatively variable yields, probably linked to the high variability of biomass production by the crop mixture over the course of the experiment. These two examples call for more research to select the species the best adapted to the famers' objectives and the services they expect, and local constraints (Garcia et al., 2020; Tixier et al., 2011). The short legume fallow resulted in the highest performances in terms of ecosystem functions and profitability but its social acceptance requires additional studies as it provides no income for half of the surface usually cropped. Therefore, despite increased profitability, it may be difficult for farmers to adopt this practice if they receive no technical support, subsidies, or other means of compensating as they mainly live under the poverty threshold (Razafimahatratra et al., 2017). Modelling approaches may help to summarize knowledge to assess plant diversification scenarios, to be able to guide farmers in their choice (Jourdain et al., 2001). Thus, multidisciplinary research is needed to better support the introduction of ecological intensified practices in a systemic way (Gaba et al., 2015; Tamburini et al., 2020).

Conclusion

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

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

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

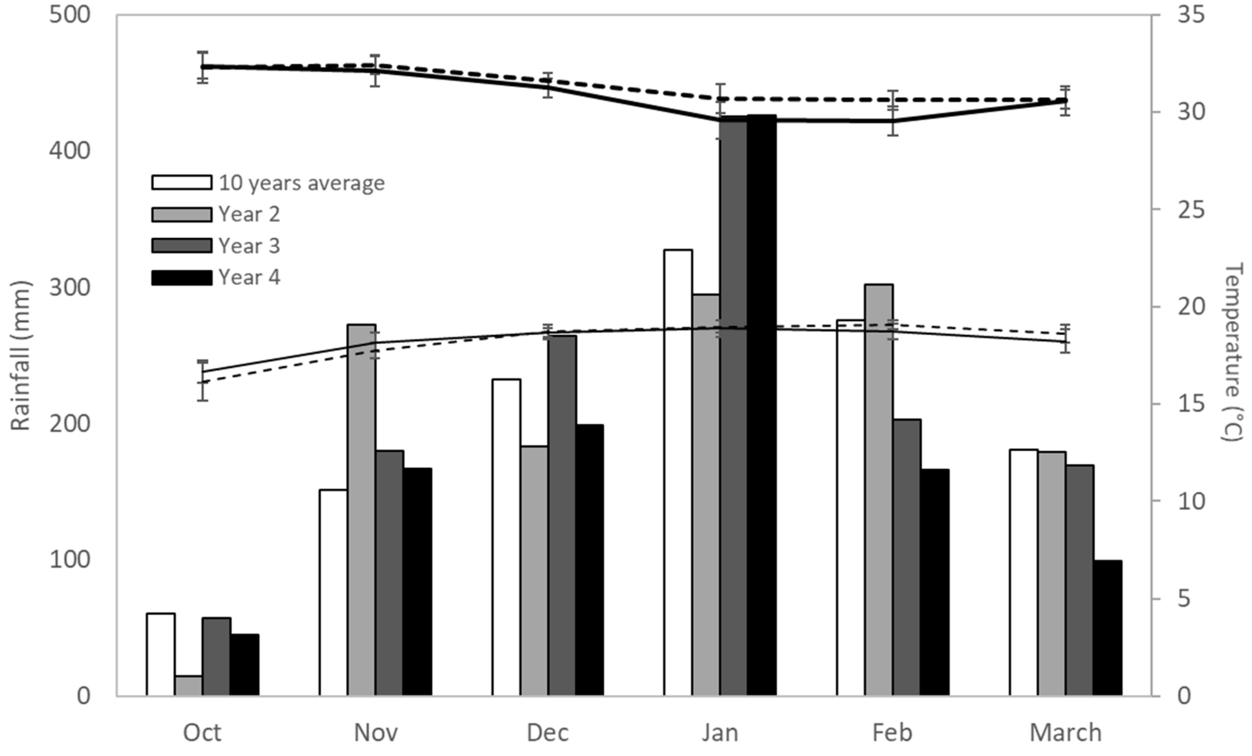

Figure 2. Average rice biomass (vegetative and grain) (a), and its N content (b) observed during rice crop cycle in the four different rotations.

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

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

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

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

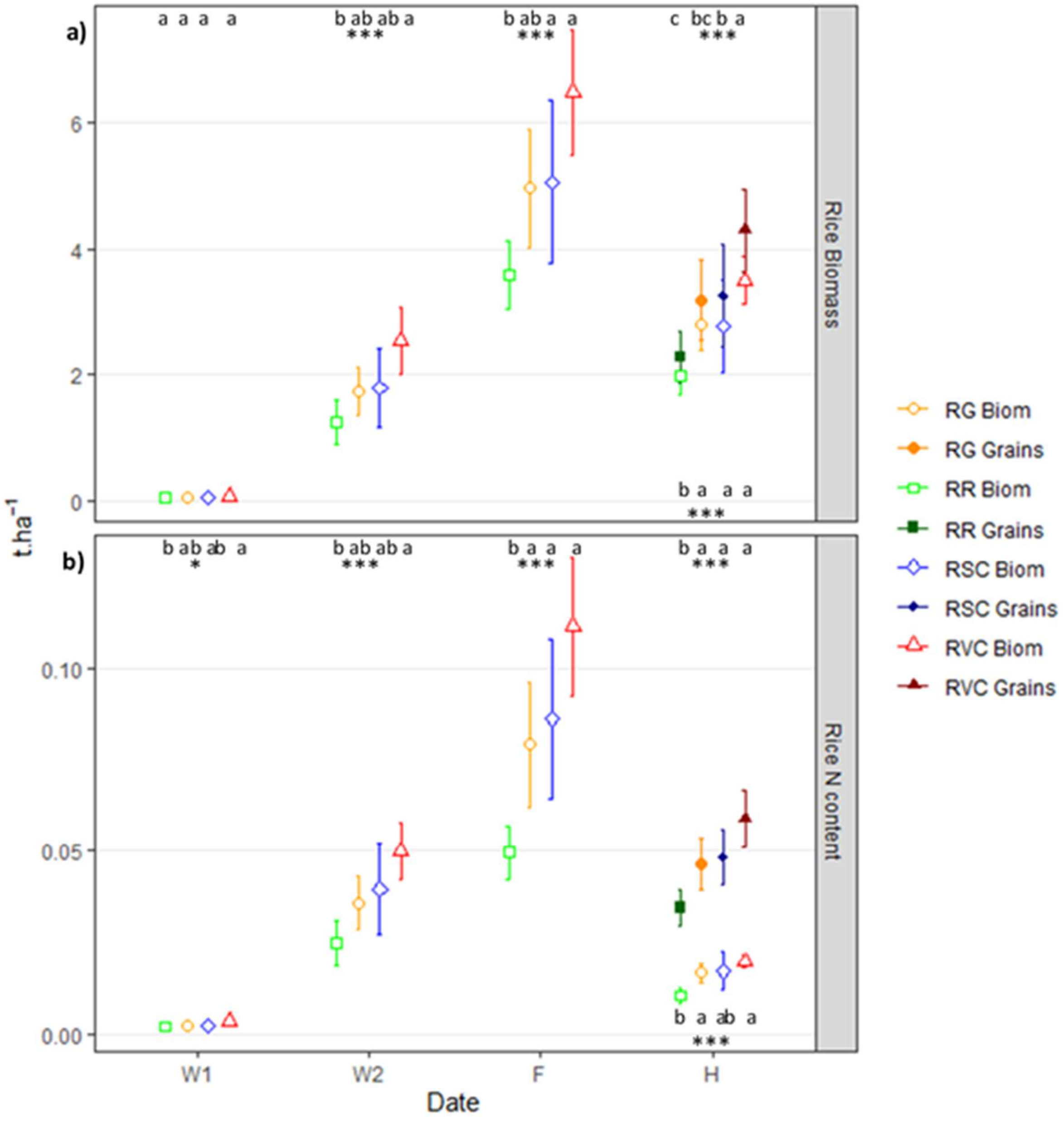

Figure 3. Abundance of trophic groups of nematodes in the four different rotations in Year 1 of the experiment (a) under rice (RR) or rotation crops (groundnut for RG, sorghum-cowpea mixture for RSC, and velvet bean-crotalaria mixture for RVC) and in Year 3 of the experiment (b) under rice in RR and the three rotations (in ind.kg^{-1} soil).

Error bars are 95% confidence intervals. Different letters indicate significant difference ($p < 0.05$) between rotations for each trophic group.

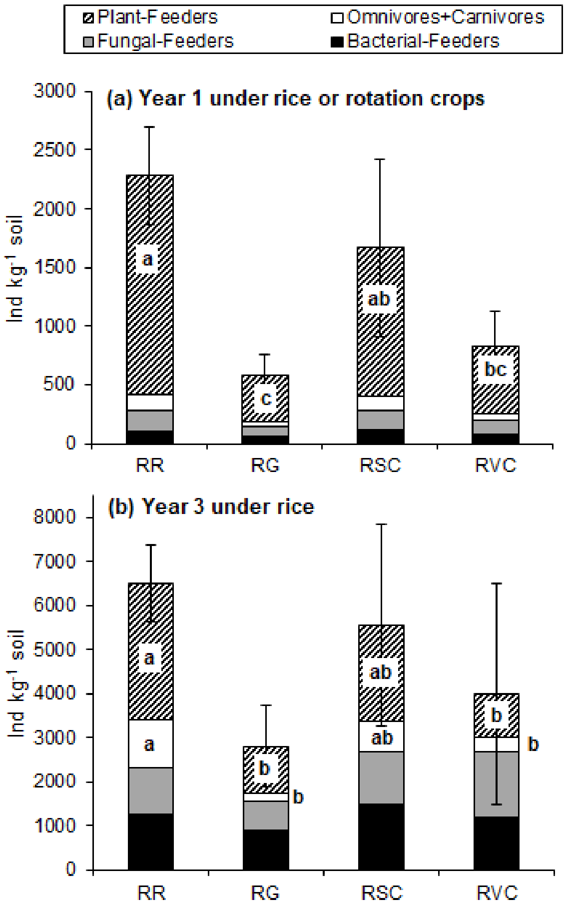

Figure 4. Ecosystem functions and profitability assessment for the three rotations compared to rice monocropping averaged over the experiment.

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

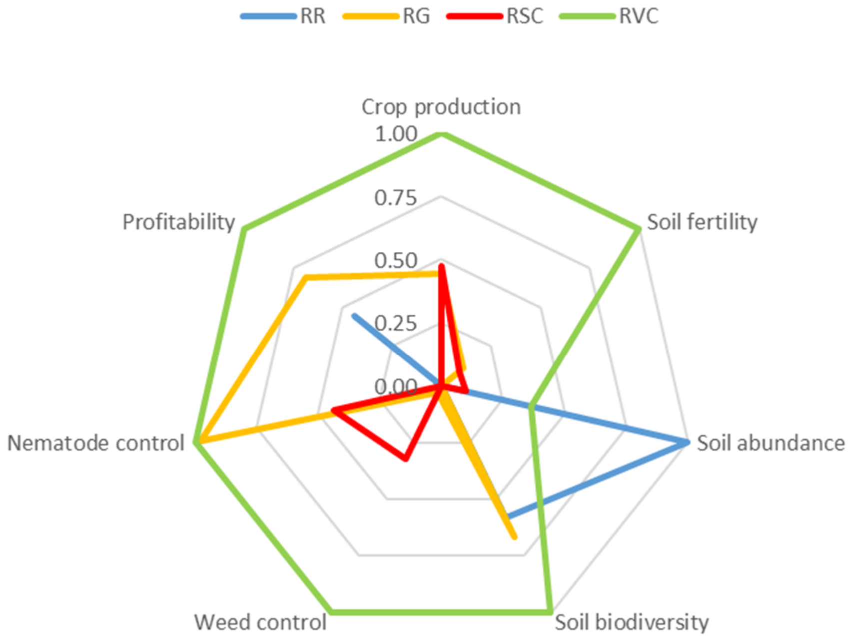

Table 1. Selected soil physical and chemical properties (mean and standard error, n = 4) of the experimental field at the Ivory station located in the mid-western region, Madagascar.

Soil layer (cm)	pH (H2O)	Olsen P	CEC	Total C	N	MO	Clay	Silt	Sand
		mg.kg^{-1}	cmol.kg^{-1}	%	‰	%	%	%	%
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

Table 2. Rice management practices over the four cropping seasons of the experiment. WBS = Week(s) before sowing, WAS = Weeks after sowing. Mixture harvest corresponds to the harvest 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

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

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

	Quantity of manure applied (t.ha^{-1}of DM)	% DM	OM %	N %	C %	P %	K %	Ca %	Mg %
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

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

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

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

	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 ***

Table 5. Soil inorganic N content (in kgN.ha^{-1}) during the rice crop cycle in the RR and RVC rotations. Mean ± confidence interval at 95% and p-value associated with the rotation effect. Means are calculated over Year 2 to 4 of the experiment.

S = sowing, W1 = first weeding, F = rice flowering, H = rice harvest. Letters indicate significant difference between rotations.

	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

Table 6. Pearson’s correlation coefficients (r) matrix between the different ecosystem functions. Significant correlations (* p > 0.05 and ** p < 0.01) are indicated in bold.

	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

Table 7. Income from crops, weeding and manure costs and gross margin calculated for rice monocropping and the three different rotations on a per hectare basis.

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