Final narrative report
ACTEA Small Grant Facility
LEGINCROP Project

DO LEGUME-BASED INTERCROPS CONCURENTLY HALT SOIL EROSION, BOOST SOIL HEALTH AND STRENGTHEN (NATURAL) PEST CONTROL SERVICES IN CASSAVA CROPPING SYSTEMS IN NORTHERN VIETNAM

Contract in the Framework of ACTAE regional project
CANSEA component

Didier Lesueur – October 16th, 2018

Contents

Summary

In the Yen Bai Province, some farmers intercropped maize and cassava with legume species, mainly cowpea as it is easy to sale on local markets. However, most of the farmers were mainly doing cassava or maize mono cropping instead of intercropping crops with cowpea. After 2 years of LEGINCROP project, the number of farmers doing intercropping has more than doubled and the surface of land dedicated to intercropping cassava with cowpea was three times higher than in 2017 when we started the project. Absence of commercial rhizobial inoculants nodulating cowpea in Northern Vietnam (and may be in the whole Vietnam) shows the needs to isolate and screen under control conditions effective rhizobia strains nodulating cowpea for identifying the most effective strains. Twenty-one strains were isolated and tested under greenhouse conditions and about we selected eight strains as being very effective. Inoculated farm-trials were set up in the Mao Dong commune in Van Yen District. By practicing the inoculation of cowpea with native selected rhizobial strains, the nodulation, the yield, and the biomass were significantly higher than for the non-inoculated cowpea. The best inoculum was the one containing two native strains isolated from nodules collected in Mao Dong farms in 2017. LEGINCROP has demonstrated and quantified how soil fauna (macrofauna and microfauna) is significantly higher and more diverse in intercropping system than in the mono cropping fields. LEGINCROP has also assessed the capacity of both intercropped cowpea and cassava to form root symbiosis with native AMF concerning the landscape. Our results showed that if the mycorrhization of cowpea is much higher than the mycorrhization of cassava, there is no significant difference according to the landscape. Both crops are largely mycorrhized and there is no need to think about a possible utilization of commercial mycorrhizal inoculants to sustain the yields. Overall, by inoculating intercropped legumes with effective native rhizobia isolated in the Yen Bai province, nodulation and yield is significantly higher compare to un-inoculated legumes. It is possible to make available effective rhizobial inoculants for cowpea on local. Meanwhile, by taking more benefits of the legumes, farmers can significantly reduce the applications of

mineral fertilizers and save money and move on with more friendly environmental agricultural practices.

Acknowledgement

- The microbial analyses were performed on the Common Microbial Biotechnology Platform (CMBP) in Hanoi shared by the Agricultural Genetic Institute, CIAT and CIRAD.
- This work was part of the LEGINCROP project, funded by ACTAE through the French Agency of Development.

Partnership

- Centre de Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD): UMR Eco&Sols – Dr Didier Lesueur
- Northern Mountainous Agriculture and Forestry Sciences Institute (NOMAFSI)
- International Center for Tropical Agriculture in Asia (CIAT-Asia)
- The People's Committee of Yen Bai province and that of Van Yen and Van Chan districts.

The funded initiative

<u>Title</u> : Do legume-based intercrops concurently halt soil erosion, boost soil health and strengthen (natural) pest control services in cassava cropping systems in Northern Vietnam

Introduction

Intercropping systems with legumes and crops offer several advantages for farmers whose use them. It contributes to mitigate soil erosion by a better soil cover and through symbiotic nitrogen fixation, the legumes fix atmospheric N and a part of this nitrogen is released into the soil and benefits to the intercropped crop. In addition of nitrogen, intercropped legumes can significantly contribute to enhance soil carbon content through leaves and root systems remaining in the field after harvest. It shows the triple advantage of intercropping crops with legumes and the positive impacts it has on soil health/ soil fertility. This proposal is aiming to evaluate conservation agriculture practice based on intercropping legumes with maize (Van Chan district) or cassava (Van Yen district) and to see how the current system can be optimized by relevant and simple technologies easy to use by farmers in Northern Vietnam. Meanwhile, investigations will be carried out to assess if such agricultural systems also strengthen positively natural pest control services in order for end users to optimize and sustain both legumes and crop yields.

Location

Vietnam, Yen Bai province, Van Yen district, Mau Dong commune; Van Chan district, Son Thinh and Cat Thinh communes.

Backgroud of the intervention:

The Northern mountain region of Vietnam occupies around 103,000 km^2, about one third of the country's area, and contains about 12 million people, 15 percent of the national population, living in more than 2,000 communes. In this region, over 80 percent of cultural area is slope land. Due to an increase in population density and economic necessity, scope of extension of agriculture in lowlands is very limited so mountain dwellers have been using sloping lands to secure their livelihoods. Long-term cultivating on high slope areas leads to soil erosion and degradation. Moreover, the current wide use of mono-cropping systems on steep slopes resulted in low soil fertility and the imbalance of soil nutrient.

The decline in soil fertility as well as difficulties in crop production in uplands can be mitigated by agro-ecology practices, which can bring resilience and broad-based productivity to rural communities and provide important ecosystem services across the landscape. Various studies indicated that intercropped systems show plentiful advantages across the globe: better use of land, better utilization of labor, food security, increasing soil nutrient and moisture, natural pest control, and income benefits for smallholders. Intercropping might be the appropriate practice for slope land in managing soil fertility as well as enhancing crop productions, but a nearly-exclusive emphasis on yield enhancements through inter-cropping so far has prevented much-needed work. In Van Yen and Van Chan districts, cassava/maize productions and soil fertility are key priorities for their sustainable agriculture. However, not all farmers follow this intercropping option because it is time consuming to them and some don't believe in its good impacts. Thus, the local committees are very supportive for scaling up intercropping practice through farmer associations in order to mitigating soil degradation and improving soil health. The LEGINCROP's objective is to promote a balanced cropping system combining high BNF activity, less applications of mineral fertilizers and sustainable crop yields.

References and previous works

The cultivation of intercrops is one of the commonly suggested approaches for increasing carbon inputs (Mazzoncini, M. *et al.*, 2011) and reducing drought stress for the following crop when used as mulch cover in water limited systems. Intercropping systems have higher yield stability (Dapaah et al., 2003), reduced disease severity (Zinsou et al., 2004), and benefits weed control (Amanullah et al., 2007), especially when combined with nutrient addition (Olasantan et al., 1994) This is clarified that legumes can fix atmospheric nitrogen through Biological dinitrogen (N_2) fixation also called BNF in symbiosis with soil bacteria called rhizobia. According to Herridge, D. F. *et al.* (2008), symbiotically fixed N_2 in legumes ranged from 100 to 380 kg of N ha^{-1} $year^{-1}$, but exceptionally large amounts of more than 500 kg of N ha^{-1} $year^{-1}$ are also reported. Such an additional N source can explain higher biomass yields of mixtures compared to monocultures of non-legumes (Kirwan et al., 2009; Nyfeler et al., 2011). The positive interactions between N_2-fixing legumes and non-N_2 fixing plant species often contributed to a significantly larger extent to mixing effects in biomass yield than the interactions between other functional groups (Kirwan, L. *et al.*, 2009; Nyfeler, D. *et al.*, 2009; Spehn, E. *et al.*, 2002; Temperton, V. M. *et al.*, 2007). Contributions from BNF by the legumes cannot be expected to meet the N needs of the cassava crop, but may benefit the cassava crop. Makinde et al. (2007) observed 10–23% increase in cassava yield due to soybean residue incorporation, but only after two years of cassava–soybean intercropping. Intercropping can also show its advantages in resistance against herbivores or pathogens (Barbosa, P. *et al.*, 2009), hence, intercrops can effectively reduce pest populations and provide cost-free pest control services to farmers (Bugg, R. L. and C. Waddington, 1994). Neighboring plants frequently facilitate plant performance by reducing pest infestation (Bach, C. E., 1980; Risch, S., 1980) through mechanisms such as visual protection by tall associated plants, masking chemical cues used by herbivores, modified microclimate or even changes in soil community structure and corresponding plant performance. Intercropping with grain legumes (common beans, cowpea, groundnut, pigeon pea or soybean) generally increases productivity (land equivalency ratios of 1.2–1.9), with cassava yields either unaffected or decreased and legume yields least affected for species with short maturity periods (Mason et al., 1986; Mutsaers et al., 1993).

Historical presence in the area of the proposed project, and potential knowledge of local stakeholders in agriculture

The Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and the Northern Mountainous Agriculture and Forestry Science Institute (NOMAFSI) have a long- history of collaboration through the Platform in Partnership entitled CANSEA (Conservation Agriculture Network in South East Asia). NOMAFSI has people on the ground working closely with farmer associations Yen Bai Province.

The International Center in Tropical Agriculture (CIAT) has been working with both NOMAFSI and CIRAD through collaborative projects for many years and the leader of the project is one CIRAD's scientist posted at CIAT-Asia in Hanoi since March 2016. CIAT has a MOU with the Province of Yen Bai and leads the Climate Smart Agriculture activities undertaken at Ma Village by the Climate Change Agriculture and Food Security project.

Targeted beneficiaries of the intervention

- Local authorities and smallholders (direct benefits in terms of agronomy, social and economic benefits).
- Other scientists (knowledge achievement for further studies).
- Private sector (potential collaboration in the near future regarding commercial inoculant products and bio-fertilizers).

Main objectives of the funded initiative

This proposal is aiming to evaluate CA based cropping systems effects on maize yields (Van Chan district) and cassava yields (Van Yen district) and total economic benefits as well as on the soil quality with respect to legume intercropped, with specific objectives:

a. Field assessment of the natural nodulation by native rhizobia of intercropped legumes and assessment of the rate mycorrhizal root infection.

b. Soil mapping of both areas to make a link between BNF, soil fertility and crop yields.

c. Selection under greenhouse conditions of effective strains of rhizobia nodulating cowpea to identify possible inoculants for further inoculation field trials.

d. Field inoculation of intercropped legumes with selected strains of rhizobia to identify very effective inoculants to scale up through farmer associations.

e. Assess the impact of intercropping with legumes on soil fauna (diversity and richness).

Methodology

NOMAFSI has a long-history collaboration with farmer associations in Ban Loong village, located in Son Thinh Commune. This village represents about 3100 ha and the population living there is about 8500 residents. The size of the land cultivated by farmers from this village about 46.5 ha. The intercrop systems mainly used by these farmers are maize + peanut. The commune's people committee is supportive for scaling up such technology through farmer associations because sustain maize yields and soil fertility are key priorities for the agriculture in the district.

CIAT-Asia run projects in Van Yen, especially on promoting grass barriers on mono-cropped cassava fields, since the early 2000's. CIAT cassava scientists have introduced high yield cassava varieties and forage grass varieties to the region. Muoi village in Mau Dong commune participated in this promoted conservation agriculture since the beginning of the program. The village has a total land area of over 200 ha and 95 households. Land capacity per capita is rather average of 5,000 square meters per household. Muoi village is among the most productive cassava areas of Van Yen district, and therefore cassava is the number one priority crop in the village. Besides grass barriers, intercropped cassava with cowpea is promoted to improve soil nutrient stocks. However, not all farmers follow this intercropping option because it is time consuming to them and some do not believe in its good impacts. The government of Van Yen has been trying to out scale this practice yearly with an effort of mitigating soil degradation and improving soil health.

Main implemented activities

No.	Time	Activities	Products	Results
1	April 2017	- Collecting soil samples and GPS coordinates at Van Yen and Van Chan districts	- 87 soil samples - GPS coordinates of all farms and sampling points	- Soil characteristics - Soil maps
2	May 2017	- Assessing legume nodulation - Collecting plant samples	- No. of nodules - 500 legume samples and 500 maize/cassava samples	- Nodulation of cowpea at Van Yen and Van Chan (1st season) - Mycorrhizal infection rate of cowpea roots
3	October 2017	- Assessing legume nodulation in Son Thinh and Cat Thinh communes (2nd maize season) - Collecting nodules for isolation work at all the sites - Collecting cowpea roots for mycorrhizal infection assessment	- No. of nodules - 26 bottles of cowpea nodules being kept in -80^{o}C - 176 cowpea root samples	- Nodulation of cowpea at Son Thinh and Cat Thinh communes (2nd season) - 21 rhizobial strains were isolated and sequenced - Mycorrhizal infection rate of cowpea roots
4	January 2018	- Meeting with local government and farmers to evaluate what have done in 2017 and determine farms for 2018	- Getting feedback from local farmers and local authority	- Almost the farmers were very happy with the results and benefits from 2017 and registered for joining in 2018
5	March 2018	- New growing season at Mau Dong commune, Van Yen district	- Systematic inoculation of cowpea with native rhizobia isolated from both districts	- Lists of all inoculated and non-inoculated farms with area size, slope category and

				inoculated strains
6	May 2018	- Field sampling at Mau Dong, Van Yen at flowering stage	- No. of nodules - Fresh nodules and above-below biomasses for dry weight and root mycorrhizal infection assessment	- Nodulation - Shoot and root dry biomass - Shoot total N content - Mycorrhizal infection rate of cassava and cowpea roots - Nodule occupancy assessment
7	June-August 2018	- Conducting screening trial in the greenhouse at VNUA, Hanoi	- No. of nodules - Nodule, shoot and root biomasses for fresh and dry biomasses, shoot total N analyse - SPAD index	- Nodulation and nodule dry weight - Shoot and root fresh and dry biomass - Shoot total N content - SPAD value

Contribution to promote agroecology transition

LEGINCROP has contributed significantly to sustainable intensification cropping systems involving improved crop and legume seeds and conservation agriculture (CA) as potential avenues towards improved productivity. It brought the evaluation of CA based cropping systems effects on maize yields (Van Chan district) and cassava yields (Van Yen district) and total economic benefits as well as on the soil quality with respect to legume intercropped. After 2 years, the number of farmers doing intercropping has more than doubled and the surface of land dedicated to intercropping cassava with cowpea was three times higher than in 2017 when we started the project. Meanwhile, by taking more benefits of the legumes, farmers can significantly reduce the applications of mineral fertilizers and save money and move on with more friendly environmental agricultural practices.

Communication & dissemination activities

In the 1st year (2017), the project organized some field trips to all the sites in order to discuss with the local farmers and quickly assess the impacts of intercropping systems. In January of 2018, the big meeting was taken place with the participation of Van Yen district committee and about 100 local farmers. At that meeting, almost the smallholders were expressing their satisfaction about the success of cassava-cowpea intercropping which brought much of additional economic benefits to their cassava production. The local authority was also pleasant and supportive with the preliminary results from the project. The number of farmers willing to join the project in 2018 was at least three-fold increased. Some farmers said that they would still practice cassava-cowpea intercropping even without the support from the project.

Lessons learnt from the project

In the Yen Bai Province, some farmers intercropped maize and cassava with legume species, mainly cowpea as it is easy to sale on local markets. However, most of the farmers were mainly doing cassava or maize mono cropping instead of intercropping crops with cowpea.

- Absence of commercial rhizobial inoculants nodulating cowpea in Northern Vietnam (and may be in the whole Vietnam).
- Without inoculation, the field nodulation was most of the time low and very variable from one place to another one even within the same field.

Main outputs of the projects

By isolating rhizobia from nodules collected from all the sites in 2017, 21 rhizobial strains were isolated and sequenced. After screening and conducting inoculation trial in 2018, we found that the best inoculum was the one containing two indigenous strains isolated from nodules collected in Mau Dong farms. LEGINCROP has revealed that by inoculating intercropped legumes with effective native rhizobia strains, nodulation and yield are significantly higher compare to un-inoculated legumes. It is possible to make available effective rhizobial inoculants for cowpea on local.

LEGINCROP has demonstrated and quantified how soil fauna (macro-fauna and micro-fauna) is significantly higher and more diverse in intercropping system than in the mono cropping fields.

LEGINCROP also assessed the capacity of both intercropped cowpea and cassava to form root symbiosis with native AMF concerning the landscape. Our results showed that if the mycorrhization of cowpea is much higher than the mycorrhization of cassava, there is no significant difference according to the landscape. Both crops are largely mycorrhized and there is no need to think about a possible utilization of commercial mycorrhizal inoculants to sustain the yields.

AFD

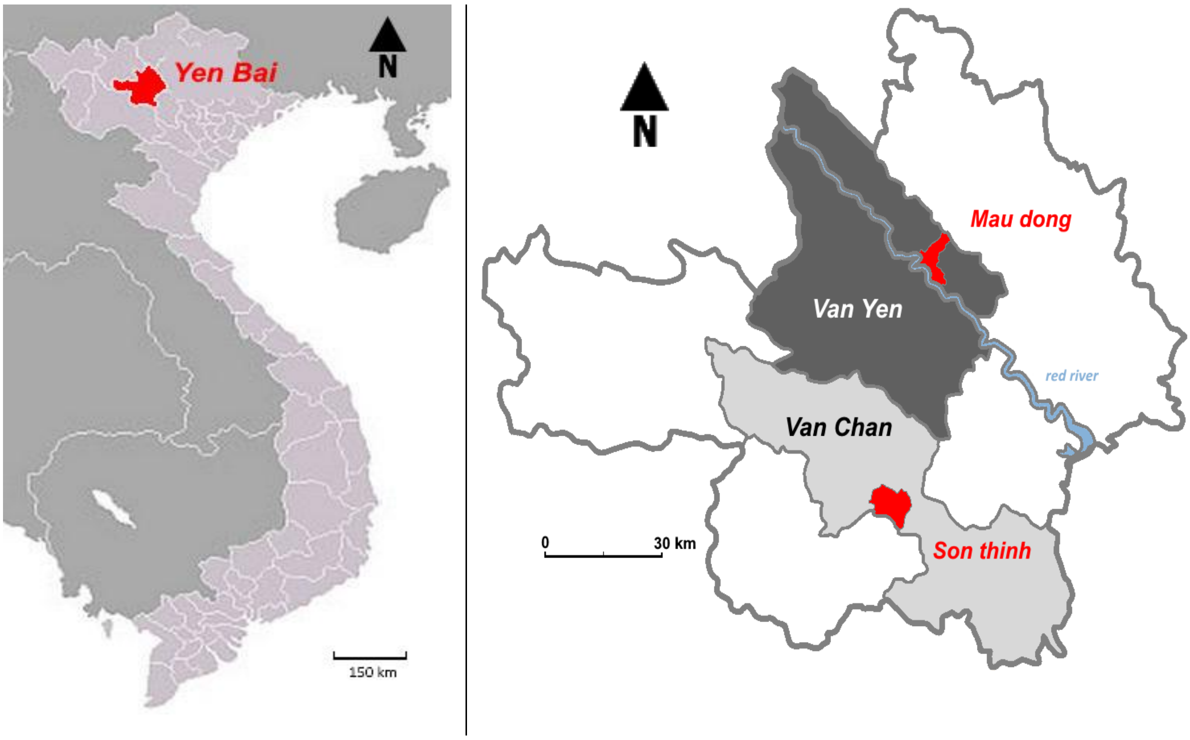
The present R&D grant has been financed by the French Agency for Development (AFD).
The ideas and the opinions presented in this document are the ones of its authors and do not represent necessarily those of the AFD.

APPENDICE: Full detailed technical report

Do legume-based intercrops concurrently halt soil erosion, boost soil health and strengthen (natural) pest control services in cassava cropping systems of Northern Vietnam? (LEGINCROP)

Project leader: Didier LESUEUR (CIRAD)

Full detailed report October 2018



Picture 1. Maps of Yen Bai province and the two research locations

1. Field experiment in 2017

1.1. Description of research sites

The on-farm trials were conducted in Van Yen and Van Chan district, Yen Bai province, Vietnam. There is a cassava intercropping system with cowpea at Mau Dong commune, Van Yen and a maize intercropping system with cowpea at Son Thinh and Cat Thinh commune, Van Chan district. At Mau Dong, there are 5 selected farms in 5 areas for comparing cropping systems on: steep slope (≥ 15%), moderate slope (> 5% and < 15%), and gentle slope (≤ 5%). The total area is 3.7 hectares. In Van Chan district, there are 7 farms (4 at Son Thinh and 3 at Cat Thinh, respectively) with different slope levels: steep slope (≥ 15%), moderate slope (5-15%), and gentle slope (≤ 5%). The total area is about 1.5 hectares.

Picture 2. Fields at Mau Dong commune, Van Yen district

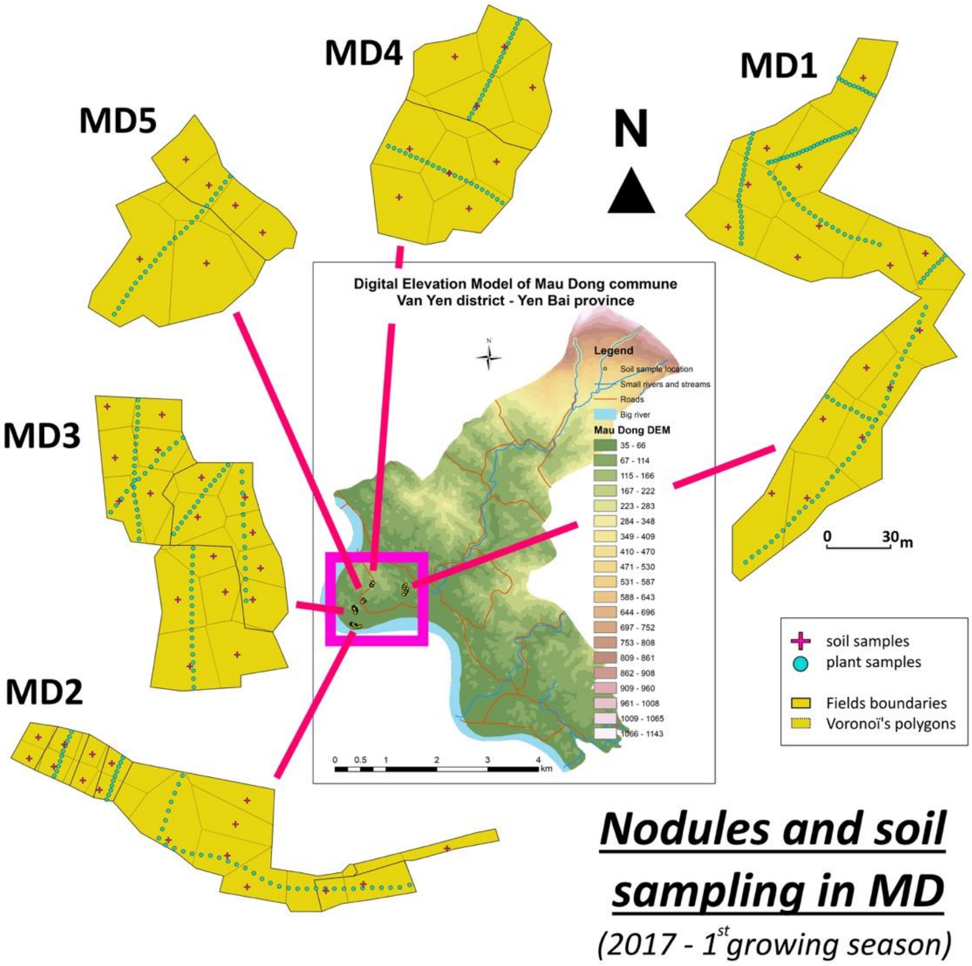


Figure 1. Soil and plant sampling at Mau Dong commune, Van Yen district

Picture 3. Fields at Son Thinh and Cat Thinh commune, Van Chan district

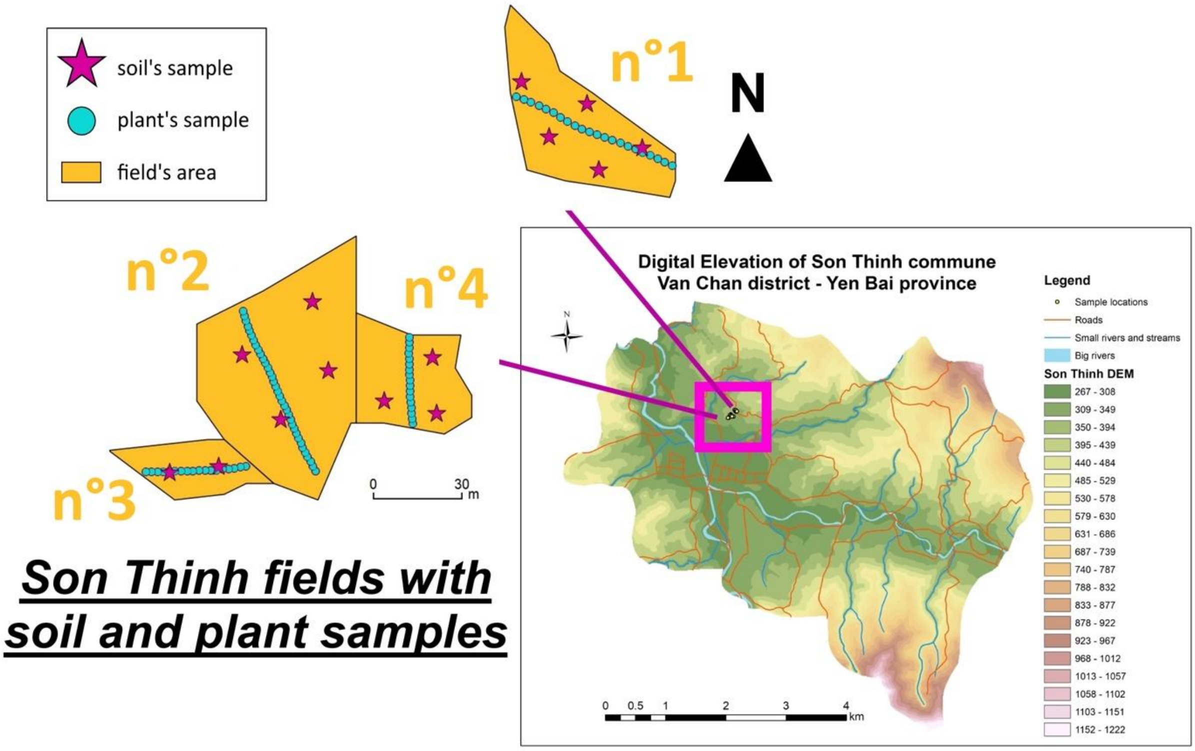


Figure 2. Soil and plant sampling at Son Thinh commune, Van Chan district

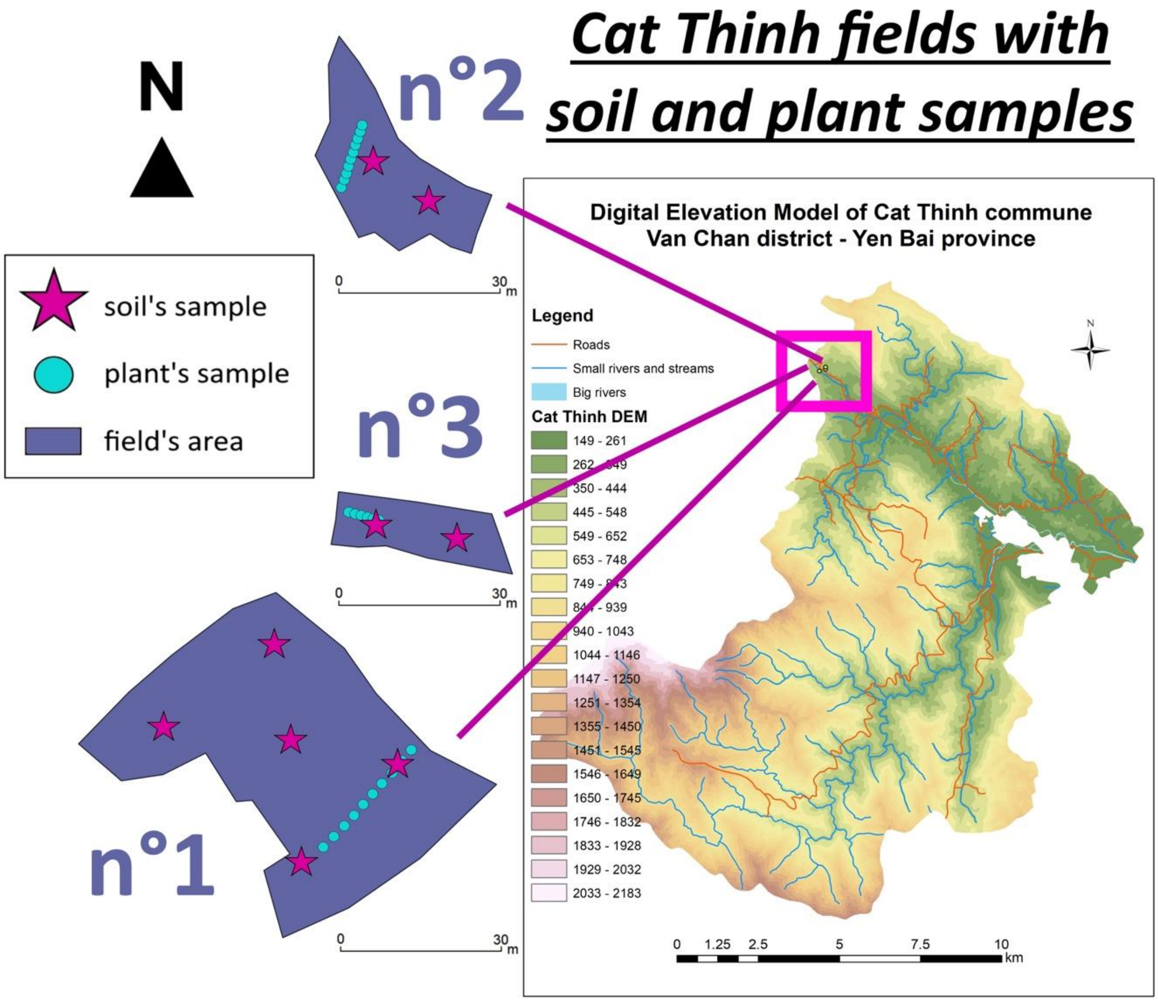


Figure 3. Soil and plant sampling at Cat Thinh commune, Van Chan district

Regarding to the protocols to collect the data and samples, we determined the lines based on the water flows and the number of sampling points in each line. After gathering GPS location and other information (date, name of farmer, number of sample, slope degree and characterization) at each point, we dig cowpea plants up by the spades, cut off the above-ground biomass, carefully removed the soil around the roots, then smoothly cleaned them in basins of water with 2-3 times. Hence, we counted the effective nodules (with reddish/pink color). After that, by using the small scissors, we cautiously detached nodules from cowpea roots and dip them in 70% ethanol solution, then lightly shake in 10 seconds before picking up the nodules by the forcep and putting them into labelled Fill McCartney bottle occupied with about 10ml of glycerol. The remain root parts were kept in labelled paper bags. At each sampling point, we also simultaneously dig one maize plant nearby sampled cowpea plant, then collect the clean root part by the method described above and keep it in the labelled paper bags.

1.2. Results obtained in 2017

a) Field information

Fields								Samples		
Sites	*No. of farms*	*Total area (ha)*	*Altitude (m)*	*Crop*	*Fertilization*	*NPK (5:10:3) (kg ha^{-1})*	*Soil*	*Nodulation (with 0)*	*Nodulation (without 0)*	*% of 0*
ST	4	0.75	[330; 350]	M	Moderate	[260; 500]	14	61	54	11.5
MD	5	3.54	[43; 131]	C	Very high	[1160; 1430]	64	336	174	48.2
CT	3	0.65	[305; 370]	M	Very high	[1000; 1500]	9	26	25	3.8

** M: maize; C: cassava*

b) Cowpea nodulation

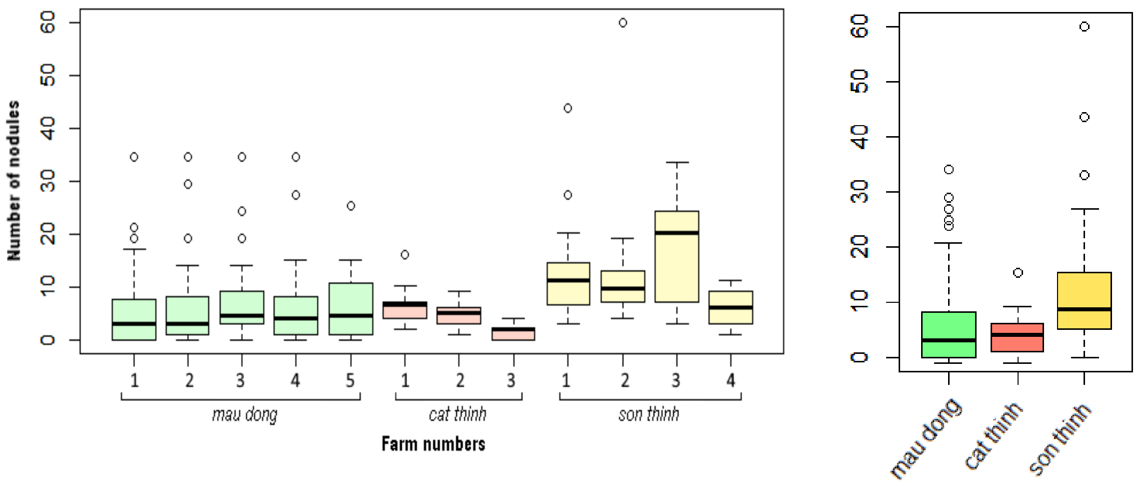


Figure 4. Distribution of the number of nodules at each field

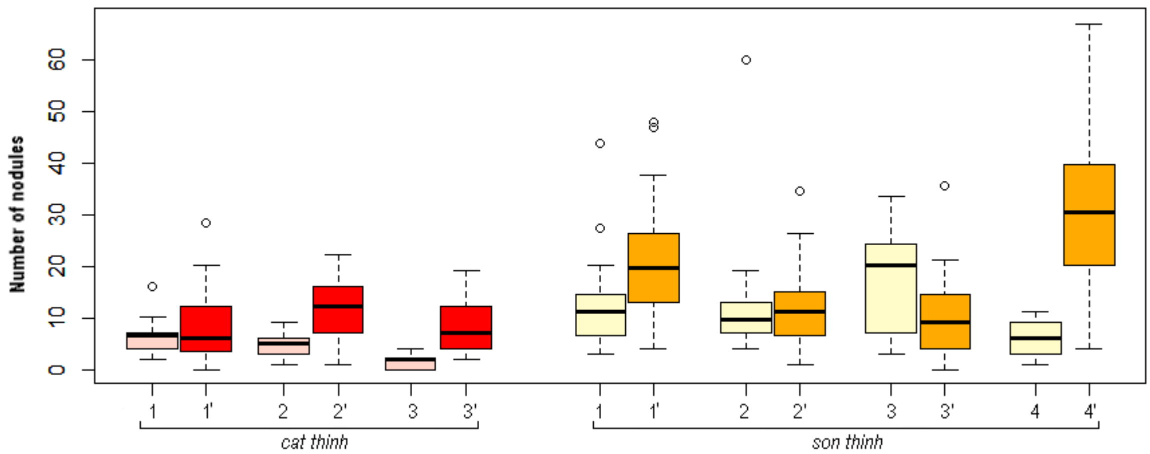


Figure 5. The number of nodules between two seasons at Son Thinh and Cat Thinh

The highly variable nodulation of cowpea at 3 locations is showed in Figure 4 and 5. According to the above figures, the number of nodules is highest in Son Thinh commune while that of Mau Dong showed the lowest value. Besides, when comparing the number of nodules at Son Thinh and Cat Thinh, the number of the

2nd season at both communes is higher than that from the 1st season. This is pertinent to the climatic conditions that was more favorable for crops at the 2nd season. Nonetheless, these results showed that the numbers of cowpea nodules are quite low at all fields. That is the reason why we did not do the ^{15}N analyses for estimating BNF from cowpea at these locations, as well as showing the need for conducting inoculation trials to enhance the ability of nitrogen fixation of cowpea at these sites.

c) *Soil quality comparison between Mau Dong and Son Thinh*

Table 1. Soil characteristics at Mau Dong and Son Thinh commune

		Slope	**Bulk density**	**Fine sand**	**Silt**	**Total N**	**pH**	**Ca**	**Mg**	**Na**
Wilcoxon test	W	343	80	56	379	274	370	301	385	349
	P value	0.000	0.004	0.000	0.000	0.038	0.000	0.004	0.000	0.000
		***	**	***	***	**	***	**	***	***
Son Thinh	min	15	1.21	12.9	26.8	0.12	4.33	2.61	2.43	0.22
	1qt	25	1.22	18.2	28.8	0.14	4.94	3.07	2.56	0.26
	med	27	1.28	20.9	29.6	0.15	5.00	4.00	2.77	0.32
	3qt	27	1.35	24.2	33.5	0.16	5.10	4.60	3.31	0.39
	max	29	1.45	28.4	34.7	0.22	5.47	5.73	3.97	0.50
Mau Dong	min	3	1.15	10.1	8.4	0.08	3.63	1.59	0.12	0.12
	1qt	5	1.28	27.2	13.9	0.11	3.83	2.04	0.28	0.15
	med	10	1.44	34.8	16.1	0.13	3.98	2.67	0.44	0.19
	3qt	17	1.53	43.4	19.1	0.15	4.15	3.41	0.80	0.23
	max	30	1.68	55.1	29.9	0.21	5.24	4.58	1.54	0.38

The total of 87 soil samples were collected at all the research sites and the analysing results are showed in Table 1. According to the results of soil characteristics, the pH at Son Thinh is significantly higher than at Mau Dong commune, while there are also greater contents of some mineral nutrients at Son Thinh commune. Therefore, Son Thinh seems to be the better site for plant to grow. That can also be the explanation for the greater number of nodules at that commune.

d) *PCA analysis for the data at Son Thinh commune*

There is only the result for Son Thinh commune because the variables for the others are less well represented. At this location, it seems to be no closely link between texture and bulk density, which is quite surprising. However, there is a very strong anti-correlation between clay and sand contents that is logical. The soils of steep slopes also contain the most sand and the least clay. Also logically, the pH and the calcium content are quite close. In addition, overall the different ion contents and the pH evolve is in the same direction, while the texture seems to be related to the presence of some ions (more calcium in clay soils, more sodium for sandy soils).

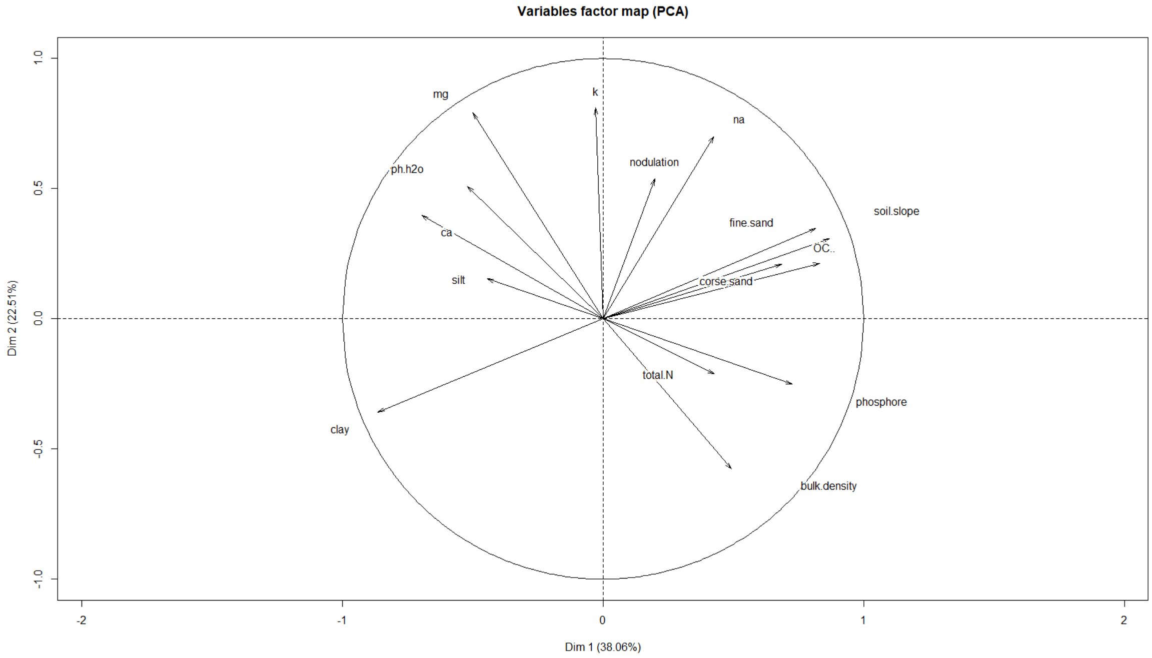


Figure 6. PCA analysis for the data at Son Thinh commune

Regarding to the nodulation, the soils with the highest potassium and calcium levels appear to be those where the number of nodules is highest. Besides, the nodulation seems to be quite dependent on the texture because the richer sand soils would be more favorable to the number of nodules. Conversely, soils that are poor in sands and rich in clays are unfavorable. However, since there is a strong link between sand and clay contents (which are among the variables best represented on the map), it is not possible to say whether this supposed difference in nodulation is rather related to the sand content, the clay content or both. Finally, the intensity of the slope is quite close to nodulation. It seems, however, that this connection is rather indirect.

e) Correlation between sodium content and number of nodules at Son Thinh

As shown in Fig. 7, there is a strong positive correlation between the sodium content and the number of nudules per plant at Son Thinh commune. The number of nodules is lowest in the plots with poorest sodium content and this is highest in the plots with richest amount of sodium.

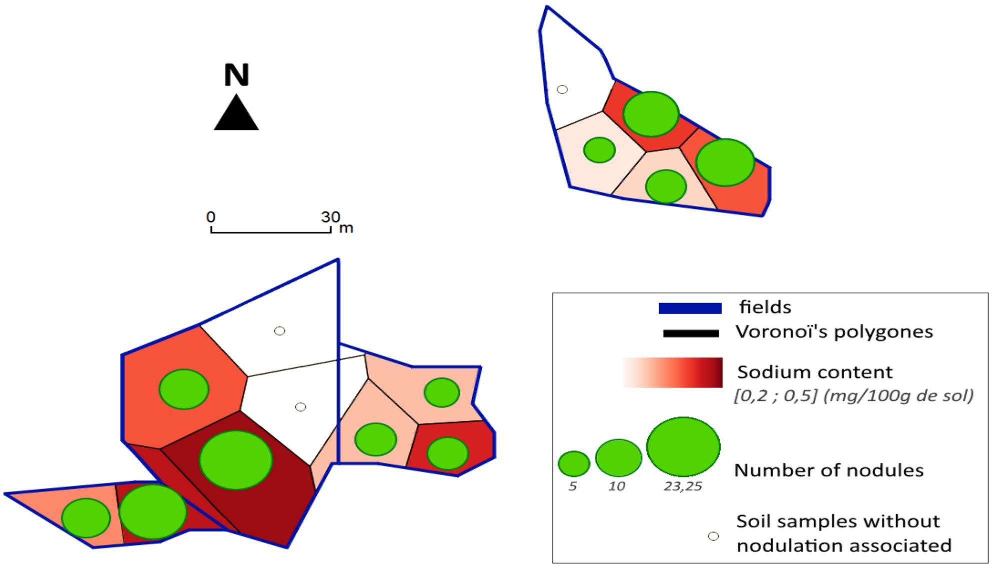


Figure 7. Sodium content and number of nodules at Son Thinh commune described in Voronoi's polygon

f) Mycorrhizal infection rate of cowpea roots at Son Thinh and Cat Thinh

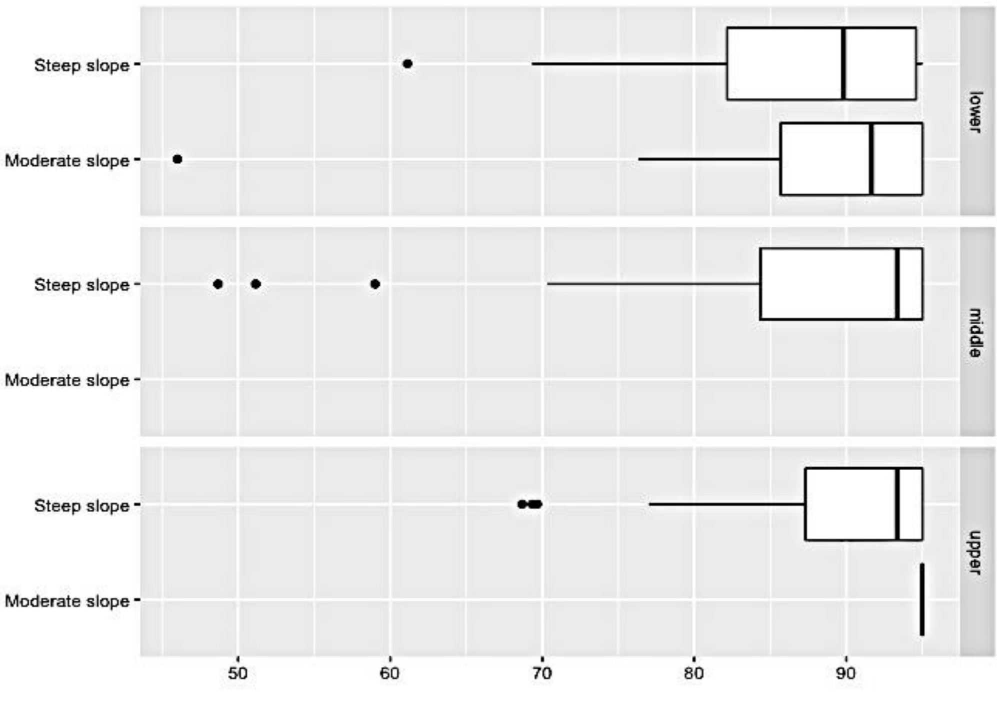


Figure 8. Mycorrhizal infection rate of cowpea roots across different slope degrees and slope categories at Son Thinh

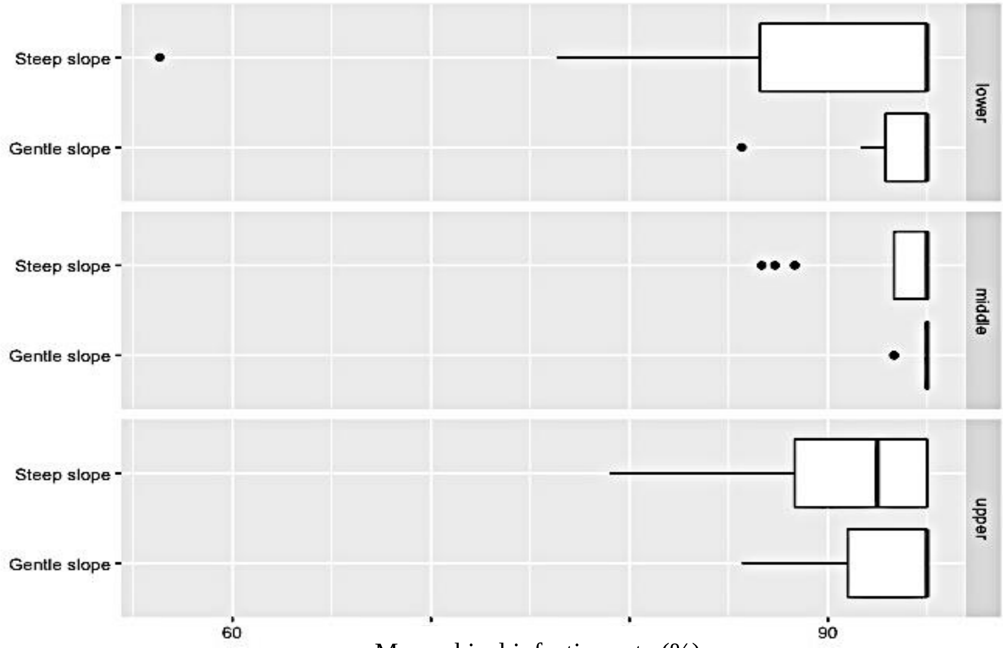


Figure 9. Mycorrhizal infection rate of cowpea roots across different slope degrees and slope categories at Cat Thinh

As shown in Figure 8 and 9, mycorrhizal infection rates of cowpea roots in both locations were very high (≥ 90%) across different slope degrees and slope categories. This result showed the significant advantage of intercropping cowpea with other crops regarding to nutrient solubilization.

2. Screening trial of native rhizobial strains isolated from cowpea nodules at different sites

According to the results in 2017, the numbers of cowpea nodules at all the sites are quite low, that showed the need to enhance the ability of nitrogen fixation of cowpea by inoculating with rhizobial inoculants. Moreover, currently in Vietnam, there is no commercial rhizobial inoculant for cowpea, so this is necessary to isolate the native rhizobia strains from the nodules and conduct screening trial in the greenhouse and inoculation experiment in the fields.

2.1. Experiment design

The pot trial was carried out in a greenhouse (VNUA, Vietnam) to assess rhizobial strains forming effective symbiotic association with cowpea. There was a total of 5 treatments; 3 rhizobial strains isolated from cowpea nodules at Mau Dong (Van Yen district) and Cat Thinh commune (Van Chan district), a negative control (no inoculation, and no N application), and a positive control (no inoculation, with N applied as KNO_3 (480 mg N pot^{-1}) (Table 1). The trial was arranged in a completely randomized design with five replications.

Table 2. Description of the treatments in pot trial in 2018

No.	Treatment	No. of replication	Strains	N application
1	Control	5	No inoculation	0
2	N (+)	5	No inoculation	480 mg N pot^{-1} (KNO_3)
3	CMBP037	5	Individual strain	0
4	CMBP054	5	Individual strain	0
5	CMBP065	5	Individual strain	0

2.2. Inoculant preparation and seed inoculation

Rhizobia inoculant cultures were prepared by picking single purified colonies of the individual strains from plates cultures on YEMA (Yeast Extract Mannitol Agar – 0.5 g l^{-1} KH_2PO_4, 0.2 g l^{-1} $MgSO_4$, 0.1 g l^{-1} NaCl, 1 g l^{-1} Yeast Extract, 10 g l^{-1} Mannitol, 15 g l^{-1} Agar). Each colony was transferred into 50 ml of fresh YEM broth in 200 ml Erlenmeyer flasks and incubated at 28^{o}C at 200 rpm for 2 days for *Rhizobium* species and 4 days for *Bradyrhizobium* species. Before applying to the pots, direct cell count for each inoculum was done using spread plate method (SOP-MI10 LH-V01) to ensure that at least 10^6 rhizobia cells ml^{-1} was reached before inoculation.

The cowpea plants (local variety, green entrails) were grown in plastic pots with 5 drainage holes in the bottom (12 cm diameter and 16 cm length) sterilized with 70% Ethanol. 1.3 kg of sterilized sand was filled and 150 ml of distilled water was applied to each pot in preparation for sowing.

Before planting, cowpea seeds were surface sterilized by soaking in 3.3% NaOCl solution for 5 minutes and rinsed thoroughly several times with sterile distilled water. Surface sterilized seeds were immersed in water for 1 h to initiate germination, and afterwards placed in Petri dishes with moistened sterile cotton wool for germination (in a growth chamber at 28°C in the dark for 24 h) for the radicle to emerge and then three pre-germinated seeds were sown per pot. For each pot, 3 ml of the inoculant was added at the base of each seedling (1 ml per seedling), at 4 days after sowing (DAS). Plants were thinned to 2 healthy plants per pot at 7 DAS.

Nutrients were applied as Broughton and Dilworth (1971) nutrient solutions at every Monday, Wednesday and Friday, and each pot was applied with 140 ml of distilled water every other day.

2.3. Sampling and data assessment of pot trial

Plants were harvested at the flowering stage to assess all the parameters. Before harvesting, the Soil Plant Analysis Development (SPAD) index of the youngest fully developed cowpea leaves were determined using a Minolta SPAD-502 chlorophyll meter (Minolta corporation, Ltd., Osaka, Japan). The above biomass was collected by cutting at 1 cm above soil surface using a clean, sharp knife, weighed for fresh weight and oven dried at 60^{o}C for 2 days. Shoot dry weight (SDW) was weighed and analyzed for total N content (%). The root parts were washed gently and weighed for root fresh biomass. Nodules were separated from the roots, counted the number of nodules and dried for measuring nodule dry weight (NDW) as well as root dry weight (RDW).

2.4. Results and discussion

a) *Nodulation and nodule dry weight of cowpea*

Table 3 shows the effect of different rhizobial strains on nodulation and nodule dry weight of cowpea. Significant higher numbers of nodule per plant (59.2 and 44.9 nodules per plant, respectively) were observed in CMBP054 and CMBP065 strains comparing to the control, while there was no significant different between CMBP037 and the control (non-inoculated treatment). Regarding to nodule dry weight, it was highest in CMBP065, but there was no significant difference between CMBP037, CMBP054 and the control.

Table 3. Nodulation and nodule dry weight of cowpea plants

Treatment	Nodule per plant as compared to negative control	Nodule dry weight as compared to negative control (g plant^{-1})
CMBP037	6.2 n.s.	0.05 n.s.
CMBP054	44.9 *	0.07 n.s.
CMBP065	59.2 *	0.15 *

Values are expressed as mean. n.s. = not significant. * = significantly different at *P<0.05*. Means separated by Tukey's test.

b) Biomass yield and SPAD index of cowpea

Table 4. Biomass yield of cowpea plants inoculated with different rhizobial strains

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	SPAD value
Control	3.04^{d}	0.48^{d}	0.83^{d}	0.12^{c}	17.54^{b}
N+	17.92^{a}	4.65^{a}	6.83^{a}	0.76^{a}	33.33^{a}
CMBP037	5.33^{cd}	0.87^{cd}	3.07^{b}	0.27^{b}	30.03^{a}
CMBP054	7.86^{bc}	1.37^{bc}	1.66^{c}	0.23^{b}	35.72^{a}
CMBP065	9.69^{b}	1.92^{b}	2.51^{bc}	0.33^{b}	38.35^{a}

Means followed by different letters are significantly different at $P<0.05$. Means separated by Tukey's test.

As shown in Table 4, there were significant differences in fresh and dry biomass of cowpea between different treatments. Positive control had the highest values of shoot fresh and dry weight, as well as root fresh and dry weight, while the negative control had smallest values. Shoot fresh and dry weight in CMBP065 was significant higher than CMBP037, but there is no significant difference in biomass fresh or dry weight between CMBP065 and CMBP054. The lowest SPAD value was observed in the negative control, but it was not significant different from CMBP037, CMBP054, CMBP065 and mineral N treatment.

3. Field inoculation trial

The field inoculation trial was conducted at Mau Dong commune, Van Yen district, Yen Bai province to assess the symbiotic effectiveness of native rhizobial strains inoculated with cowpea in field conditions. Twenty-four farmers' fields were selected to set up the inoculation experiment in 2018. There are three treatments: 1) Non-inoculated (Non_I); 2) Inoculation with the mixture of strains CMBP037+054; 3) Inoculation with strain CMBP065. Inoculant preparation is described by the above method. Before applying to the pots, direct cell count for each inoculum was done using spread plate method (SOP-MI10 LH-V01) and cell concentration of each strain was at least 10^8 CFU ml^{-1} of inoculant. Cowpea seeds were surface sterilized in NaOCl (0.5M; 2 min) and rinsed several times in distilled water to eliminate any rhizobia from the surface. Afterwards, inoculation was done at a rate of 50ml of inoculant kg^{-1} of seed resulting in 10^4 to 10^5 CFU rhizobia per seed. All inoculation was done just before sowing under the shade to maintain the viability of bacterial cells. Seeds were allowed to air dry for a few minutes and were then planted. Seeds were immediately covered with soil after sowing.

3.1. Maps of Mau Dong commune and farm locations

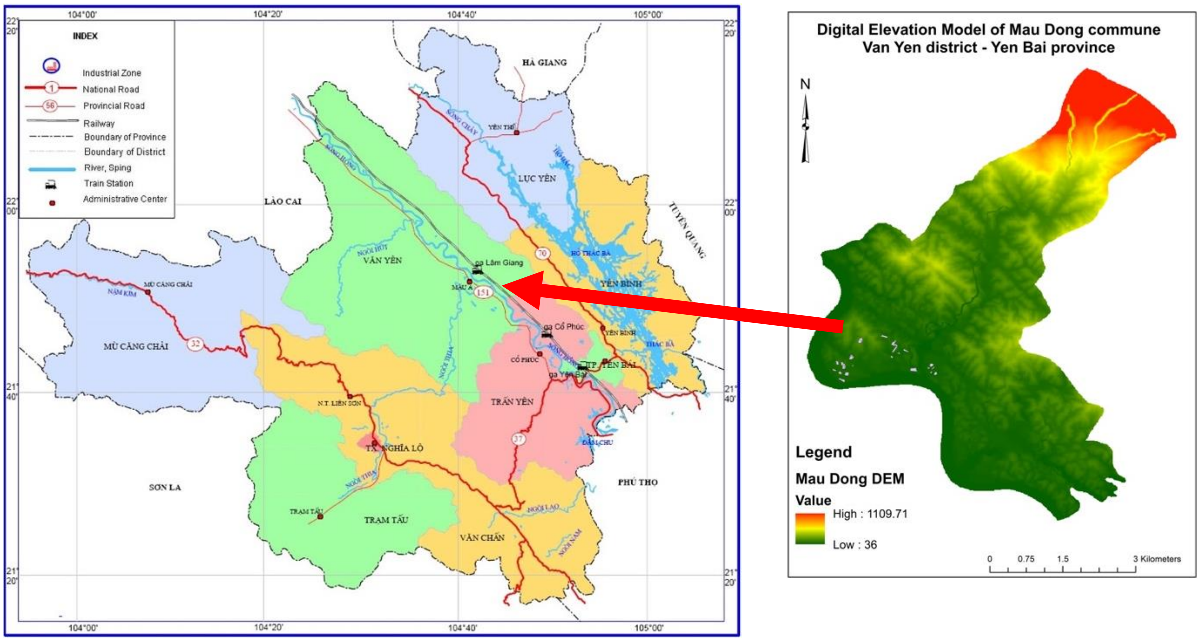


Figure 10. Maps of Yen Bai province and Mau Dong commune

3.2. Results

a) Soil analysed results

Table 5. Soil characteristics of experimental sites

Treatment		Sand	Silt	Clay	pH H_2O	OC (%)	Total N (%)	Available P (mg P_2O_5/100g of soil)	Available K (mg K_2O/100 g of soil)
CMBP065	Max	55.36	16.26	40.06	4.43	1.95	0.16	24.77	14.10
	Min	46.10	4.74	33.82	4.10	1.36	0.14	1.33	4.82
	Mean	51.90	11.08	37.02	4.26	1.68	0.15	7.83	7.18
CMBP037+ 054	Max	65.12	53.28	33.76	6.72	2.23	0.14	51.94	15.91
	Min	25.10	12.22	12.98	4.40	0.92	0.08	11.30	6.39
	Mean	48.10	29.20	22.70	5.03	1.50	0.11	22.87	9.88
Non_I	Max	55.64	44.06	50.76	5.82	2.03	0.18	28.53	16.75
	Min	34.12	6.88	16.06	3.90	0.64	0.04	3.19	4.22
	Mean	47.43	19.96	32.61	4.40	1.46	0.12	9.97	8.24

b) Cowpea nodulation, biomass yield, shoot total N and yield

Table 6. Number of nodules, shoot dry weight, root dry weight and shoot total N of cowpea plants inoculated with different rhizobial strains

Treatment	Nodulation (number of nodules per plant)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Shoot total N (%)	Yield (kg ha^{-1})
Non_I	11.3^{b}	17.62^{b}	1.76^{a}	2.88^{b}	384.26^{a}
CMBP037+054	19.2^{a}	23.14^{a}	1.81^{a}	3.02^{a}	407.56^{a}
CMBP065	12.3^{ab}	16.21^{b}	1.57^{a}	2.85^{b}	424.66^{a}

Means followed by different letters are significantly different at *P<0.05*. Means separated by Tukey's test.

As shown in Table 6, rhizobial inoculation significantly affected nodulation in cowpea. The number of nodules per plant from CMBP037+054 inoculant was highest comparing to the others. Similarly, CMBP037+054 had the highest shoot dry weight per plant and shoot total N content. Whereas, root dry weight and cowpea yield was not significant different between all treatments. The yield of cowpea observed from strain CMBP065 and CMBP037+054 seems to be higher than the non-inoculated treatment but the difference was not significant.

c) Mycorrhizal infection rate of cowpea and cassava

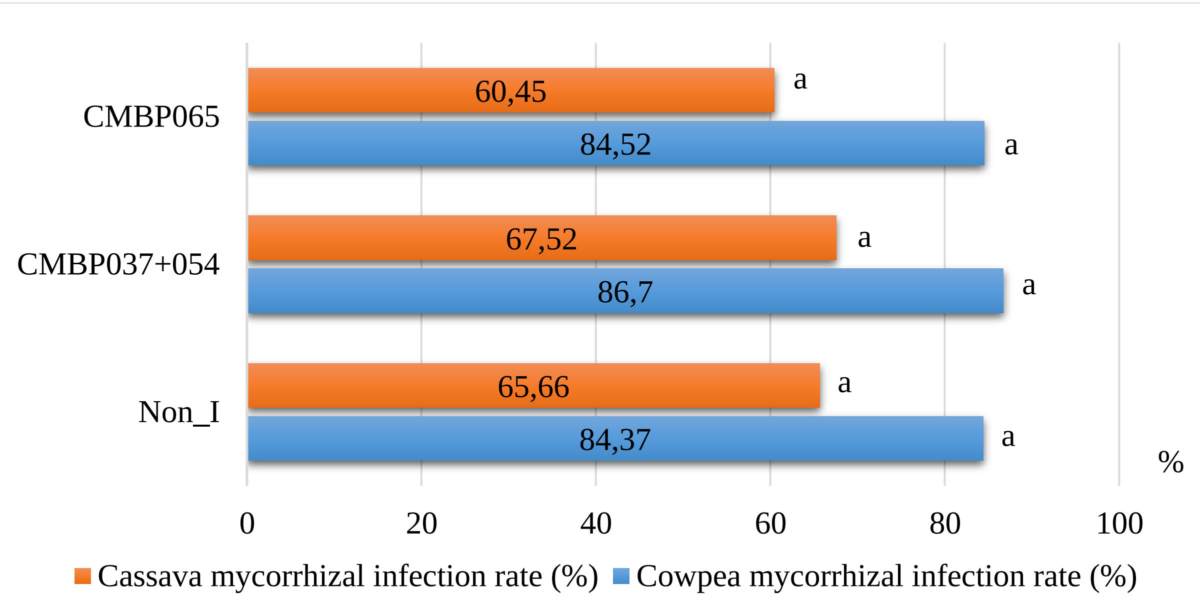


Figure 11. Mycorrhizal infection rate of cowpea (*Vigna unguiculate* var. Cylindrica) and cassava (*Manihot esculenta*) roots inoculated with different rhizobial strains. For each crop, means followed by different letters are significantly different at *P<0.05*. Means separated by Tukey's test.

Inoculation did not significantly affect mycorrhizal infection rate of both cowpea and cassava. In cowpea, the root mycorrhizal infection was quite high (more than 80%) in all treatments, while it seems to be lower in cassava roots (60.45-67.52%).

d) Nodule analysis

With the total of 24 farms, PCR-RFLP analysis was done on a total of 173 nodules: 64 nodules from farms located on gentle slope; 34 nodules from farms on moderate slope; and 75 nodules from steep slope farms (Figure 13). There were 4 different IGS profiles as shown in Figure 12 in which: group IGS 1 is the profile of strain CMBP065 (*Bradyrhizobium elkanii*); IGS 3 is strain CMBP054 (*Bradyrhizobium elkanii*); IGS 4 is strain CMBP037 (*Rhizobium freirei*; and IGS 2 group was a new profile found in the nodules.

Table 7. Nodule occupancy of different treatments

Profile	CMBP037+054	CMBP065	Non_I	Total
IGS 1	26	19	39	84
IGS 2	17	25	39	81
IGS 3	0	0	3	3
Others	2	0	3	5
Total	45	44	84	173

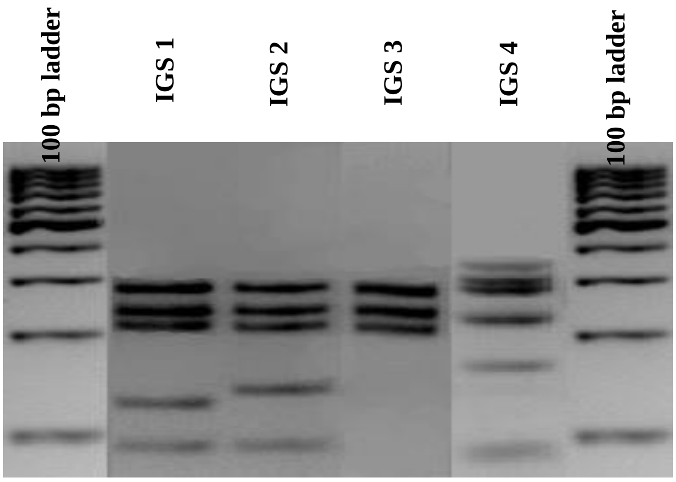


Figure 12. Intergenic spacer region (IGS) profiles obtained from isolated rhizobial strains

IGS 1 occupied the highest percentage of the nodules from the treatment combination CMBP037+054, while IGS 2 reported the highest percentage in treatment CMBP065 (Figure 13). In control (non-inoculated) treatments, there was equal percentage of nodules (46.43%) with both IGS 1 and IGS 2 profiles. Only a few nodules (8 nodules) reported other profiles referred to as "Others" (Figure 13).

This study also looked at the impact of different landscapes on nodule occupancy as shown in Figure 14. The percentage of IGS 1 profile is highest on gentle slope and lowest on steep slope farms. On the contrary, profile IGS 2 is dominant on steep slope, lowest on gentle slope, and equal to profile IGS 1 on moderate slope farms. Among different landscapes, the CMBP065 profile (IGS 1) is higher on gentle slope and moderate slope as compared to steep slope. The IGS 3 profile (strain CMBP037+054) is only found in few nodules from the control plots on the gentle slope.

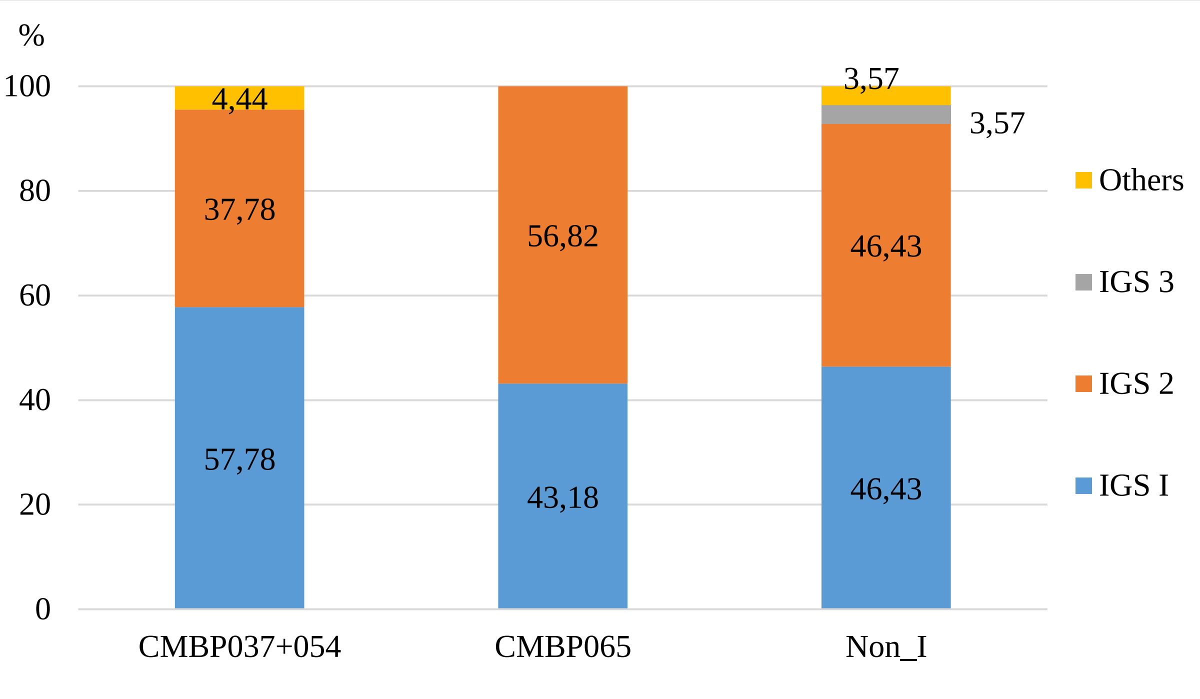


Figure 13. Nodule occupancy of different rhizobial strains

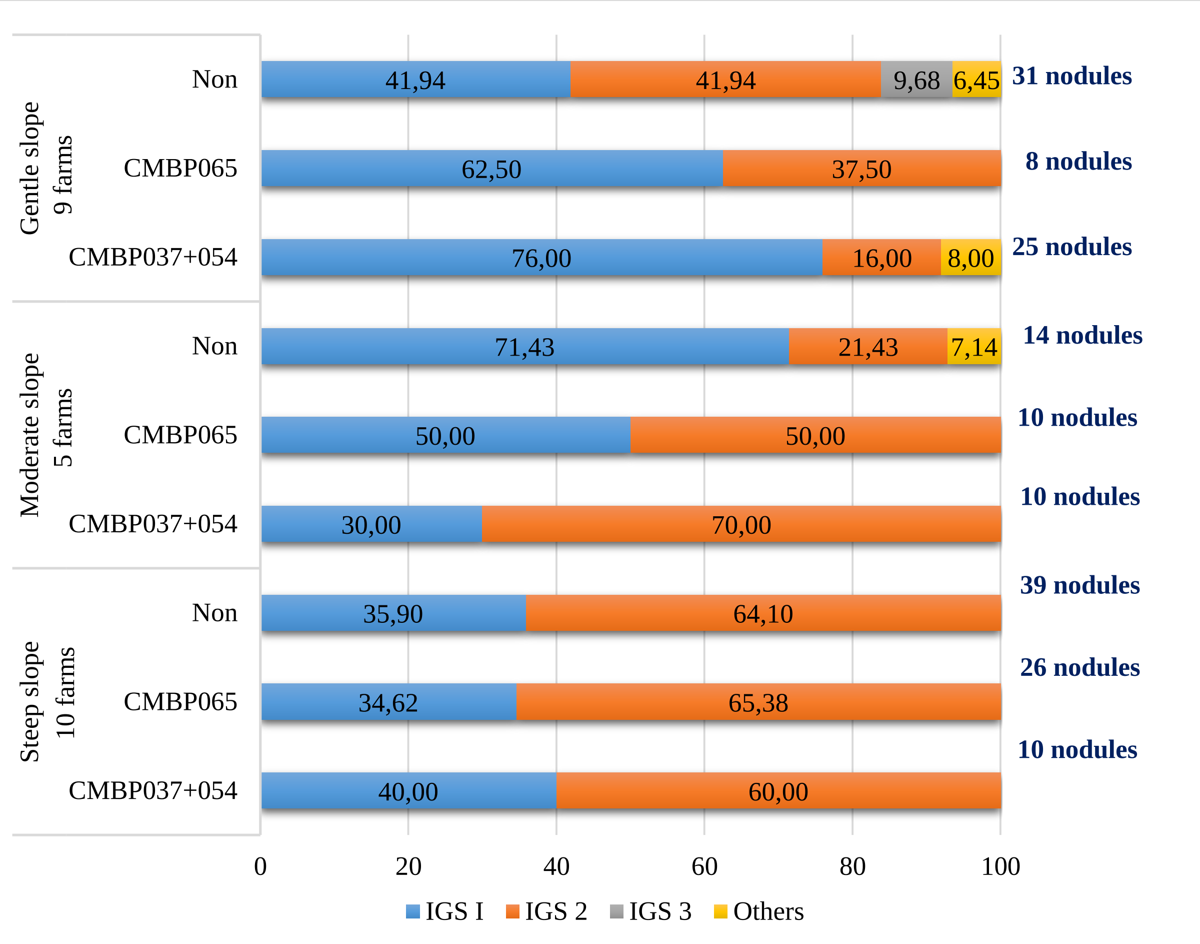


Figure 14. Nodule occupancy of different rhizobial strains classified by slope category

4. Diversity of soil fauna and microbial communities in cassava-cowpea intercropping system

The investigation was conducted at Mau Dong commune, Van Yen district, Yen Bai province in 2018. There were the total of 18 farms: 9 intercropped farms of cassava-cowpea; 9 cassava monocrop farms.

4.1. Materials and methods

a. Macrofauna extraction

One monolith of 30*30 cm and with 30 cm depth was extracted per plot using the TSBF (Tropical Soil Biology Fertility Institute of CIAT) method (Moreira et al., 2008). Macrofauna was hand-sorted and put into a 50 mL falcon tube filled with 70% ethanol. From this monolith, soil sample were taken and put into ziplocked bag. Samples are kept at room temperature and take back to the laboratory to be analysed and identified with a determination key.

b. Microfauna extraction

To extract the soil microfauna, the sucrose gradient concertation method from Velsasco-Castrillòn & al. (2018) was used. 100 g of soil per plot were used for the microfauna extraction. First, a sieve 400 µm and rinsed with distilled water. Suspended soil was poured into a 38 µm sieve. The retained soil was washed into a 50 mL Falcon tube and filled up with distilled water and mix by inversion and centrifuge during 5 minutes at 500 RCF. The sediment and water through the sieve were discarded. The supernatant is passed through the 38 µm sieve and the sediment are collected. Then the tube is filled up with 1.3 M sucrose solution, the pellet is resuspended and centrifuged at 500 RCF for 1 minutes. The aqueous layer is decanted through the 38 µm sieve and the retained particles are washed with distilled water and put in a 50 mL Falcon Tube which is kept at -20°C. The identification was done under an optical microscope with a determination key.

c. Bacterial and fungal communities

DNA extraction was done using 0.5 g of soil using MP116004-500 *FastDNA Spin Kit* for soils (MP Biomedical, Santa Anna, CA). The sequencing was done by MR DNA (www.mrdnalab.com, Shallowater, TX, USA) using the *Illumina TruSeq DNA library preparation protocol* method.

For the data processing, the protocol efficiency has been evaluated with a rarefaction curve. The sequencing depth per sample was standardized by retaining randomly a number of sequences, where the number retained is the minimum of read count for each sample (respectively 65 583 and 40 890 for the bacterial and fungal communities).

d. Soil parameters

500 g of soil per plot were sampled and the analysis were performed by SFRI (Soils and Fertilizers Research Institute) in Hanoi to analysed different soils parameters: organic matter, pH (in H_2O), N_{total}(%), available phosphorus (P_2O_5) and the soil texture from 2 mm to 0.002 mm. The soil texture gives information on the content on coarsed sand (% of particles comprised between 2 mm and 0.2 mm), fine sand (from 0.2 to 0.02 mm), silt (0.02 and 0.002%) and clay (% of soil particles inferior as 0.002mm).

e. Data processing

Calculation of index where done to assess the species richness and abundance with the Shannon index, Simpson index, evenness and species number and were calculated for the macrofauna, microfauna and bacterial and fungal communities.

Statistics have been made using the version 3.2.2 of R software. ANOVA test was proceeded to see if they were significative differences between intercropping and monocropping with the different index. All the tests were done with a 95% confidence interval and the homoscedasticity, residues centre, normality and independence hypothesis were verified and confirmed. PERMANOVA for the metagenomic analysis.

4.2. Results

a. Macrofauna

Index	Intercropping	Monocropping
Shannon Index	1,13623268	0,58244113
Simpson Index	0,23658825	0,26087826
Pielou Index	0,67214067	0,52977816
S (number of species)	5,33333333	2,66666667

Means of the index for both system: intercropping and monocropping

198 animals were handsorted, earthworm was the most abundant species (23.7%) in the monocropping and weevil (28.5%) in the intercropping field. In total, 14 species were found in intercropping field and 12 species in monocropping field. The mean of number of species found is twice higher in intercropping system (5.33) than in monocropping system (2.6). The indexes were calculated. ANOVA showed a significative difference of the species richness (P= 0.002264) and the Shannon Index (P= 0.0335) in both systems. Intercropping has a positive impact on specific richness and richness of the soil macrofauna. However, there is no significative differences between the number of animals per plot and the cultivation practice (P=0.06215), Pielou Index (P= 0.4183) and Simpson Index (P=0.8631). Intercropping doesn't impact the abundance and the evenness but increase the species richness.

b. Microfauna

In total, 159 individus were identified with ascari (46.7%) and ciliates (26.7%). More animals were extracted in intercropping system than in monocropping system (P=0.04859). However, there is no significative differences for the Shannon Index (P= 0.5594) and Simspon Index (P = 0.457) for both monocropping and intercropping systems. Intercropping did not have any impact on the diversity and abundance of species but there was an increase in the number of animals.

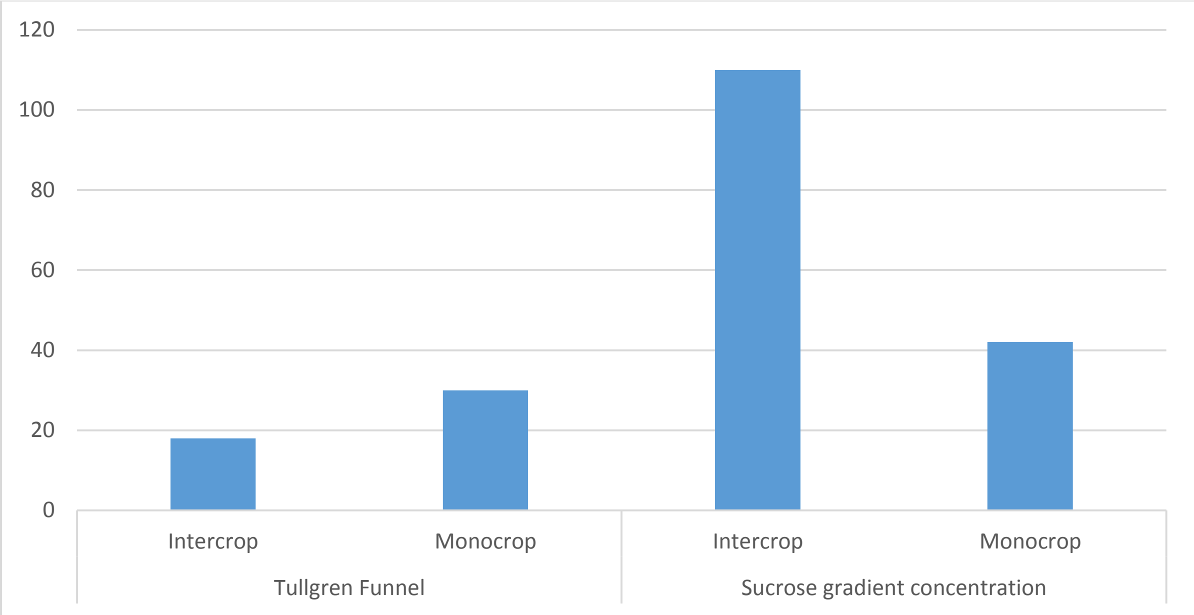


Figure 15. Comparison of the 2 methods to extract microfauna

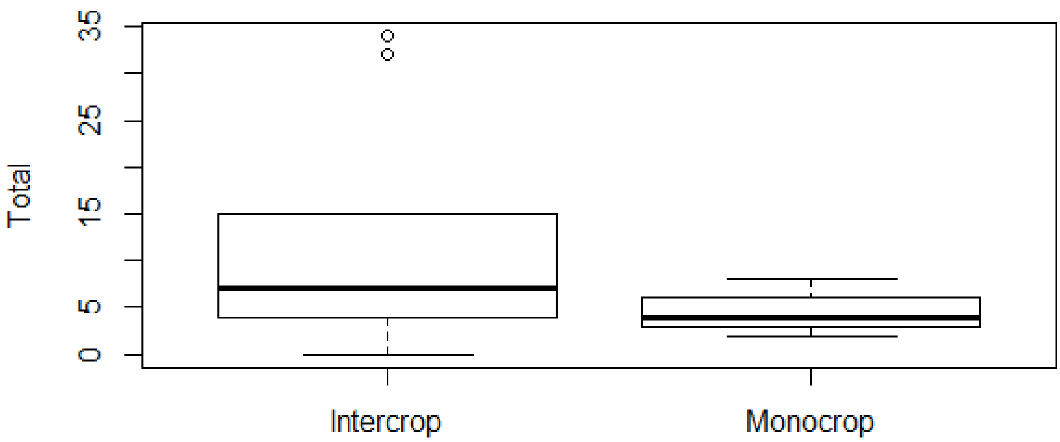


Figure 16. Microfauna population in intercropping and monocropping systems

c. Bacterial communities

Bacterial communities are dominate by Acidobacteria (27.5%), Proteobacteria (29.6%) and Chloroflexis (11%) (figure?). The most present classes in intercropping and monocropping are Acidobacterias, respectively 15% and 18% and α-proteobacteria, 15% and 12% (Figure 17). In intercropping, the average species richness is 764 with a maximum of 928 and in monocropping field the mean is 688 species with a maximum of 835. ANOVA shows that the specific richness (P=0.02768) and the diversity (P=0.03262) are higher in intercropping system. Moreover, there is a better repartition of abundance in intercropping system (P=0.009961). The PERMANOVA showed a significant difference between intercropping and monocropping (P=0.016) and it is confirmed by the NMDS.

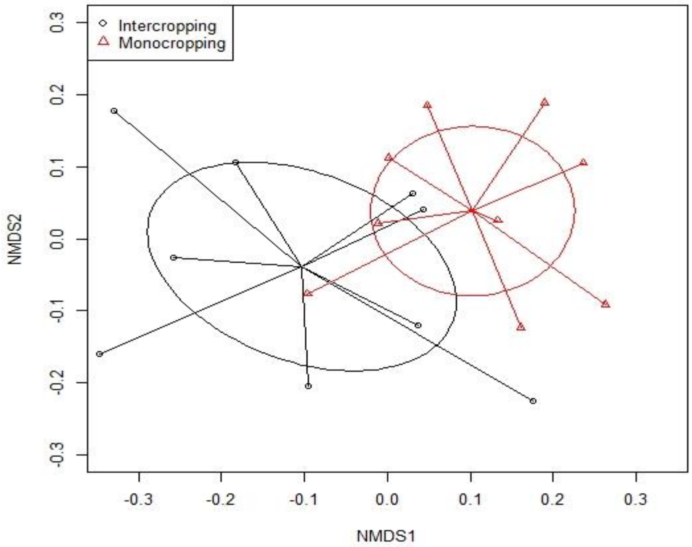


Figure 17. Bacterial communities in intercropping and monocropping systems

d. Fungal communities

Fungal communities are dominated by Ascomycetes (54.3%) and Basidiomycetes (27.3%). Most abundant species are the same in intercropping and monocropping system Sordariamycetes (25%), Agaricomycetes (20%) et Eurotiomycetes (13%) (Figure 18). In intercropping, the mean 710 species with a maximum of 804 and in monocropping a mean of 655 species and a maximum of 723. No significative differences between intercropping and monocropping for the indexes. No impact of intercropping on abundance (P= 0.832) and the distribution of abundance (P=0.4398), specific richness (P=0.1354) for fungal communities. PERMANOVA showed no significative differences between both systems (P=0.589). The NDMS confirms that distribution of groups is the same between the 2 systems. Intercropping doesn't affect the fungal communities.

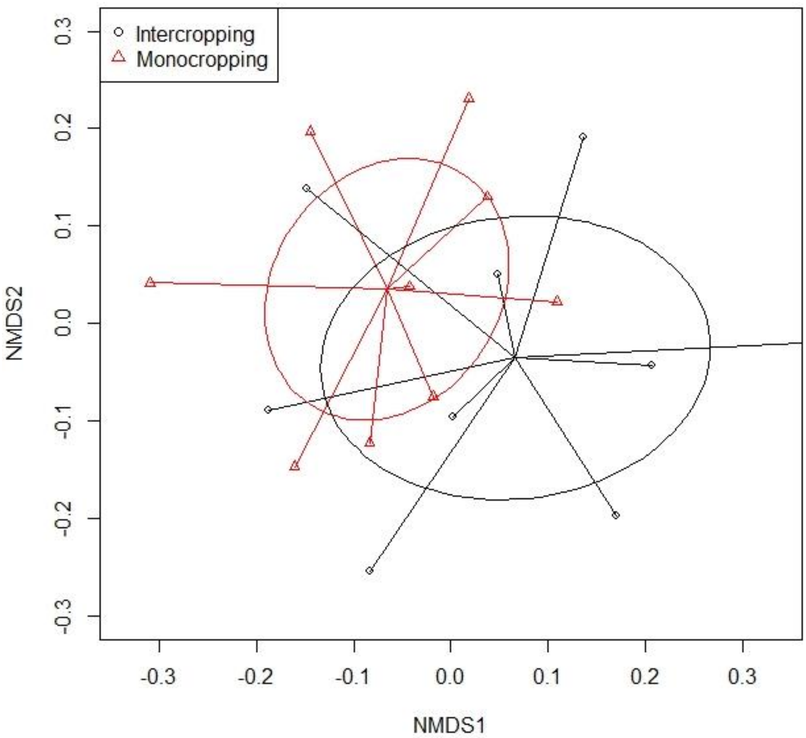


Figure 18. Fungal communities in intercropping and monocropping systems

e. Soil characteristics

Soil in all field were sandy soil and acidic (4.01 to 5.24) The mean of the different soil parameters is close for the monocropping and intercropping fields (table 1). For example, for the mean of organic matter (%) is the same for both system: 2.1%. The distribution of the data is similar for intercropping and monocropping system and ANOVA shows no effect of intercropping on soil parameters: pH (P=0.6171), organic matter (P=0.9516), nitrogen (P=0.913). Some values of the available P are very high (39.5%, 28.7%) in comparison to the mean (6.7%). These data were not included in the statistical analysis and intercropping has no impact on the avaible phosphorus (P=0.1786). Intercropping has no impact on the soil parameters.

Table 8. Soil characteristics in intercropping and monocropping farms

System	Species	pH	OM	N	P	2 - 0.2 mm	0.2-0.02 mm	0.02-0.002 mm	< 0.002 mm
Intercropping	Cassava cowpea	4,428	2,104	0,108	6,701	18,459	33,781	13,860	33,900
Monocropping	Cassava	4,344	2,089	0,103	6,655	18,952	32,259	10,953	37,836

5. Discussion

The results from screening trial in greenhouse showed that strain CMBP037 had a lowest symbiotic effectiveness among all strains. CMBP065 and CMBP054 only had significant effects on nodulation and nodule dry weight. The highest nodule dry matter recorded by CMBP065 shows its potential in enhancing cowpea nitrogen fixation and yield.

In the field inoculation experiment, the inoculant of strains CMBP037+054 showed significant differences in nodulation, shoot dry weight and shoot total N but there was no significant difference in nodule occupancy assessment. The low effectiveness of inoculated strains can be attributed to several reasons. First, high abundance of native rhizobia may inhibit nodule formation of introduced strains, even the concentrations of inoculated strains were quite high ($>10^8$ CFU ml^{-1}). Secondly, there was the presence of effective native rhizobia, or high competition but ineffective indigenous strains. This is consistent with the results from various authors ((Cheminingâ, Theuri, & Muthomi, 2011; Mathu et al., 2012; Thies, Woomer, & Singleton, 1995).

The soil fauna analysis showed that the species number in intercropping and monocropping is significantly different. The cassava-cowpea intercropping system had more species. We can explain these differences with a better soil cover and a better soil biology. Accumulation of litter and the canopy created by the cowpea induced an importance richness and biomass of the soil fauna. Intercropping induced a change in microclimatic factors through a better soil cover which will protect soil fauna from water stress and high temperature (Sileshi & Mafongoya, 2005; Kautz et al., 2006). Moreover, the modification of the vegetation structure affected the composition of the soil community because a higher diversity of food for a large diversity of consumer (Li, 2014). The input of leaves from the legume can favour the growth of detritivores arthropods such as earthworms, myriapods and termites (Sileshi & al., 2008). Intercropping increase the herbivore arthropods diversity through a diversified food supply. In general, intercropping increased specific richness without affecting the abundance because intercropping allowed the direct control of pests and biological control (Fuente & al, 2014).

The metagenomics analysis showed that intercropping positively affected the bacterial communities, with a higher abundance and diversity. However, this system did not affect fungal communities. The soil fungal community was changed with the amount of NPK fertilizer, the effect is species-specific (Donnison & al., 2000). The study of Marschner & al. (2003) showed that the addition of organic amendment at long-term increases at a low rate increase bacterial biomass but had no effect on fungal biomass.

6. Conclusions

This result suggests that it would make sense to isolate and do screening more indigenous rhizobial strains in order to get effective strains. Further studies should also be conducted to better characterize indigenous populations of rhizobia for cowpea at this site. The good competition of IGS 1 profile (CMBP065) on gentle and moderate slope showed its potential in improving cowpea nitrogen fixation and yield by appropriate inoculation in Northern mountainous areas of Vietnam.

The species richness and abundance were increased in terms of soil fauna and bacterial community in intercropping farms. But there is no impact on the fungal communities and the soil characteristics. This is one of the first study assessing both bacterial and fungal communities in 2 different agricultural systems (monocropping and intercropping) in SE Asia.

List of people whom contributed to the project:

Mrs Hai Nguyen (NOMAFSI)
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Mr. Nguyen Tuan Anh (Bachelor student – VNUA)
Ms. Le Thuy Hang (Bachelor student – VNUA)

7. Appendix: some photos of the activities at research sites






	

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