Nature-Positive Solutions for Shifting Agrifood Systems to More Resilient and Sustainable Pathways
WP1 and WP3 in Vietnam: 2023 Report

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WP1

A. Project Background

Nature+ initiative aims to transform production systems in selected countries (including Vietnam) to meet the increasing demands of growing population by adopting agroecological principles, emphasizing biodiversity and improving soil and water management through integrated Nature Positive Solutions (NPS). WP1 and WP3 seek to assess the total soil microbial diversity according to the cropping land system/land use. In Vietnam, WP1 of Nature+ worked in Sapa District (Lao Cai Province) with two target villages: Thanh Binh and Muong Hoa. Muong Hoa's main livelihood activity is ecotourism while Thanh Binh focuses on agriculture. The study is the impact of crops, including rice, maize and home gardens with different soil fertility and characteristics on microbial diversity. The objectives of WP1 in Vietnam in 2023 were:

1. Define cropping land systems/land uses in 2 target villages, Muong Hoa and Thanh Binh, Sapa District, Lao Cai Province.
2. Work with NOMAFSI for soil physical and chemical assessment.
3. Initiate a preliminary investigation on the microbial diversity in soil and root of rice, maize plants and vegetables from home gardens using amplicon sequencing (Illumina technology).

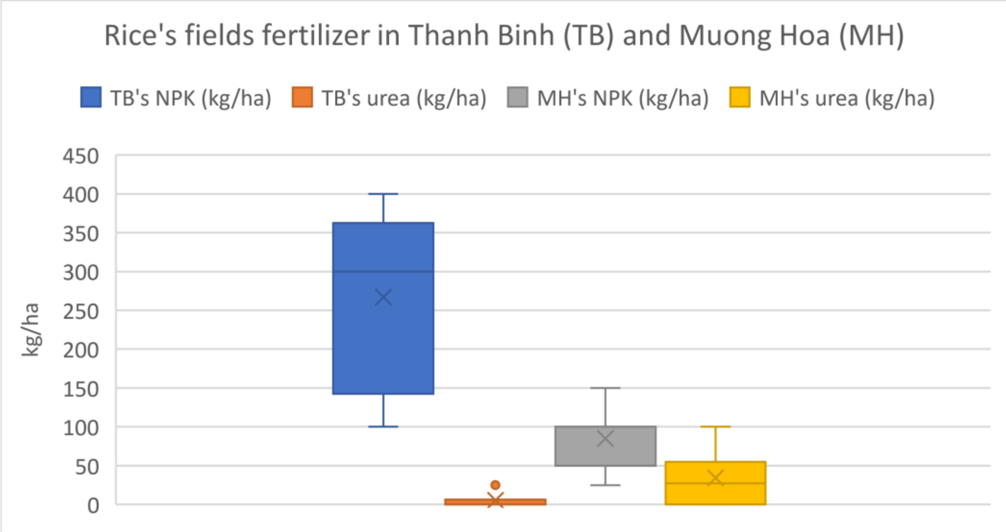
B. Work package activities and progress

1. Sample information and sampling process

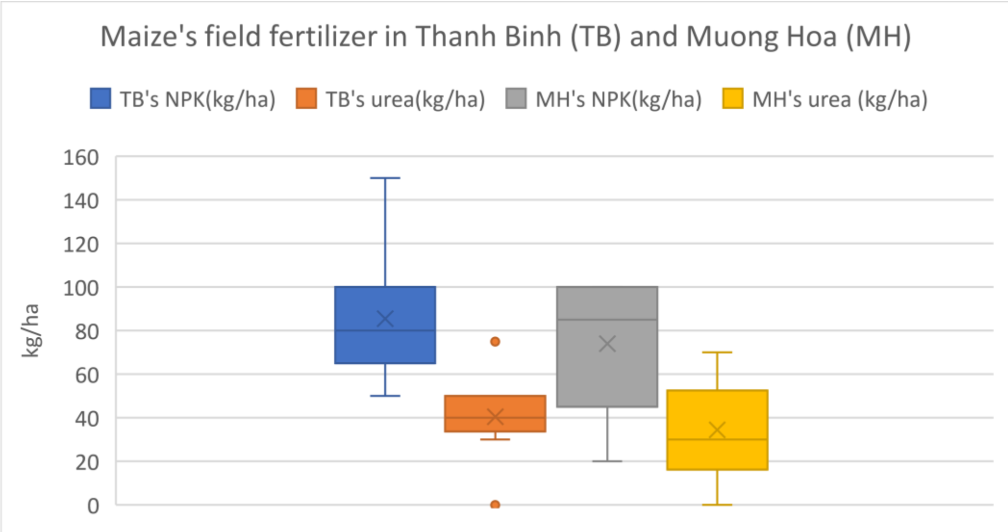
60 soil samples and 60 root samples were collected from rice, maize and home garden fields in 2 villages, Muong Hoa and Thanh Binh, Sapa, Lao Cai. The sampling protocol, farm locations, field area and amount of fertilizer were recorded in Appendix.

In Muong Hoa village commune, the management of the vegetables in the home gardens is almost 100% organic (utilizing manure produced by cows and chickens). At the planting time, manure is mixed with NPK at a low rate. Applications of herbicides and/or pesticides are very rare. In Thanh Binh commune, Lech Dao village, manure is always mixed and applied with NPK and there are one or two applications for herbicides and/or pesticides per growing season according to the crops.

Globally, farmers in Thanh Binh used higher amounts of NPK fertilizer than in Muong Hoa for rice growth. Urea fertilizer was rarely used with rice. In comparison to the maize field, the figures for NPK and urea fertilizers were nearly similar in average amounts for both villages. Higher doses of NPK were applied for rice than for maize and urea was more commonly used in maize but not in rice (Figure 1a, 1b)



(a)



(b)

Figure 1. The amount of fertilizer used in recorded farms of rice and maize in Thanh Binh (a), and Muong Hoa (b) villages.

2. Soil texture and characteristics analysis

Soil texture and characteristics of 60 soil samples were analyzed by NOMAFSI. Analysis of soil texture showed a significant difference in sand and clay average percentage between Muong Hoa and Thanh Binh's soil (Table 1b). Besides, the amount of available Potassium(mg/100g) and Cation Exchange Capacity (meg/100g) in Rice soil (combining two villages) were significantly lower than maize and home garden soil (Table 1a).

Table 1a. Soil physicochemical characteristics comparing different crop types

Category	Soil Texture				Soil Characteristic						
	Sand (%)	Slit (%)	Limon (%)	Clay(%)	pH (H2O)	OM (%)	Total Nitrogen (%)	Phospho (mg/100g)	Potassium (mg/100g)	OC (%)	CEC (meg/100g)
Rice	16.35 a	25.73 b	31.29 a	26.63 a	5.3 a	3.24 a	0.52 a	0.25 b	5.76 b	2.21 a	1.77 b
Maize	17.71 a	32.44 ab	24.432 ab	25.41 a	5.1 b	3.08 ab	0.23 a	0.52 a	23.60 a	2.04 a	2.42 a
Home Garden	18.61 a	35.83 a	21.31 b	24.18 a	5.3 a	2.65 b	0.20 a	0.50 a	29.14 a	2.21 a	2.22 a

Note: For each variable, values followed by different letters are significantly different at p<0.05 (pairwise comparisons of the means using Tukey HSD tests).
Abbreviation: OM: Organic Matter, OC: Organic Carbon, CEC: Cation Exchange Capacity

Table 1b. Soil physicochemical characteristics comparing two villages

Category	Soil Texture				Soil Characteristic						
	Sand (%)	Slit (%)	Limon (%)	Clay(%)	pH (H2O)	OM (%)	Total Nitrogen (%)	Phospho (mg/100g)	Potassium (mg/100g)	OC (%)	CEC (meg/100g)
Muong Hoa	15.51 b	32.38 a	26.41 a	25.70 b	5.3 a	3.12 a	0.21 a	0.46 a	17.75 a	2.33 a	2.19 a
Thanh Binh	19.61 a	30.29 a	24.94 a	25.11 a	5.3 a	2.86 a	0.42 a	0.40 a	21.20 a	1.98 a	2.08 a

Note: For each variable, values followed by different letters are significantly different at p<0.05 (pairwise comparisons of the means using Tukey HSD tests).
Abbreviation: OM: Organic Matter, OC: Organic Carbon, CEC: Cation Exchange Capacity

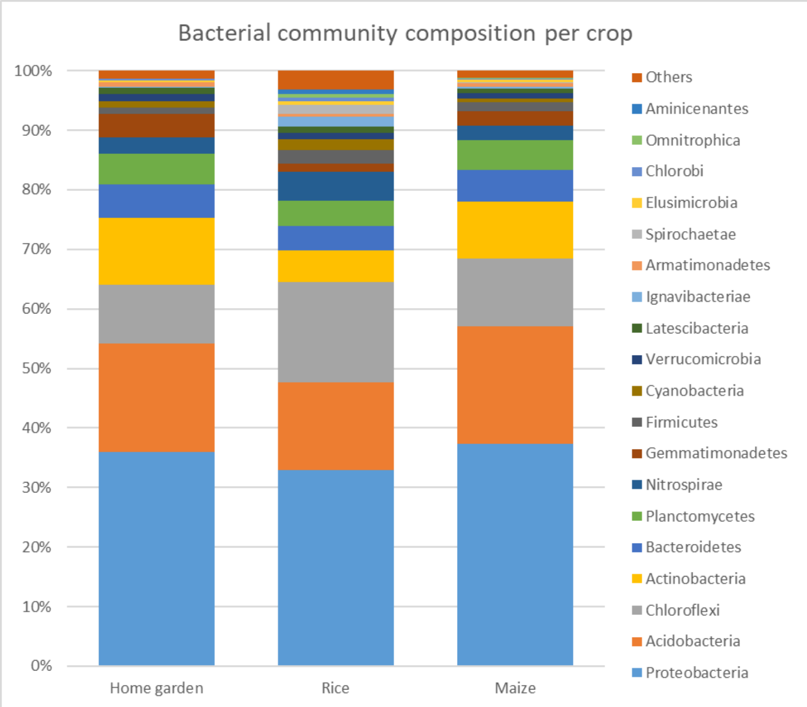
3. Microbial Diversity Analysis

The effect of 3 crops (rice, maize, and home garden) on the composition, structure, richness and diversity of the soil bacterial and fungal communities and of root arbuscular mycorrhiza fungi (AMF) community was assessed in the 2 villages. A total of 120 samples were analyzed by amplicon sequencing using the Illumina technology. Soil and root total DNA was extracted using the MP FastDNA Spinkit. The sequencing process was performed at Mingke Sequencing (www.mingkebio.com, Hangzhou City, China) using the primer pairs 515F - 907R for 16S (bacteria), ITS3F – ITS4R (fungi) and AMV4.5NF – AMDGR (mycorrhiza) amplicons, respectively.

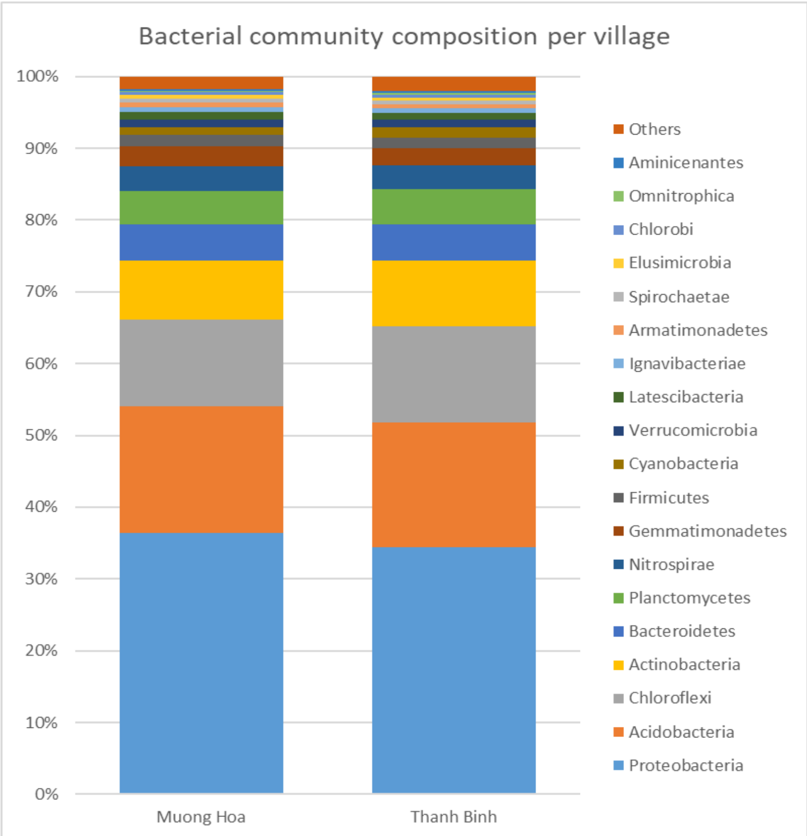
Initial analysis of metabarcoding

3.1 Bacterial community in soil

The bacterial community on the phyla level was dominated by Proteobacteria (35%), followed by Acidobacteria (17%), and Chloroflexi (12%) across all crops and villages. The composition per village and crop showed that the bacterial community was quite stable across the environmental conditions (Figures 2a and 2b)



(a)



(b)

Figure 2: Bacterial community composition per crop (a) and per village (b).

In terms of richness at the OTU level, rice showed the significant higher level of diversity, according to the chao and exp(H') indices, compared to maize and home garden. On the other hand, diversity indices demonstrated no notably distinction between the two villages (Table 2).

Table 2. Richness and diversity indices of the bacterial community at the OTU level

	Village		Crop		
	Muong Hoa	**Thanh Binh**	**Rice**	**Maize**	**Home Garden**
Chao	5220.8 a	4972.2 a	6095.60 a	4547.95 b	5541.60 b
Exp(H')	1070.06 a	1060.36 a	1370.10 a	867.49 b	1167.28 b
1/D	378.42 a	391.88 a	511.92 a	289.83 b	422.81 b

Chao: species richness index; Exp(H'):exponential Shannon index; 1/D: inverse Simpson index..Values followed by different letters are significantly different at $p<0.05$

Noticeably, sample MHRC6S (Muong Hoa, Rice, Farm 6) showed a significant difference in the diversity in terms of both composition and structure, compared to other samples from Muong Hoa's rice soil samples. For instance, it is lacking *Nitrospiraceae*, which plays a critical role in the nitrogen cycle by converting nitrite to nitrate (Figure 3). This may be related to the intensive application of pesticides in this particular farm (4 applications of pesticide while others farms used 0-2 times only).

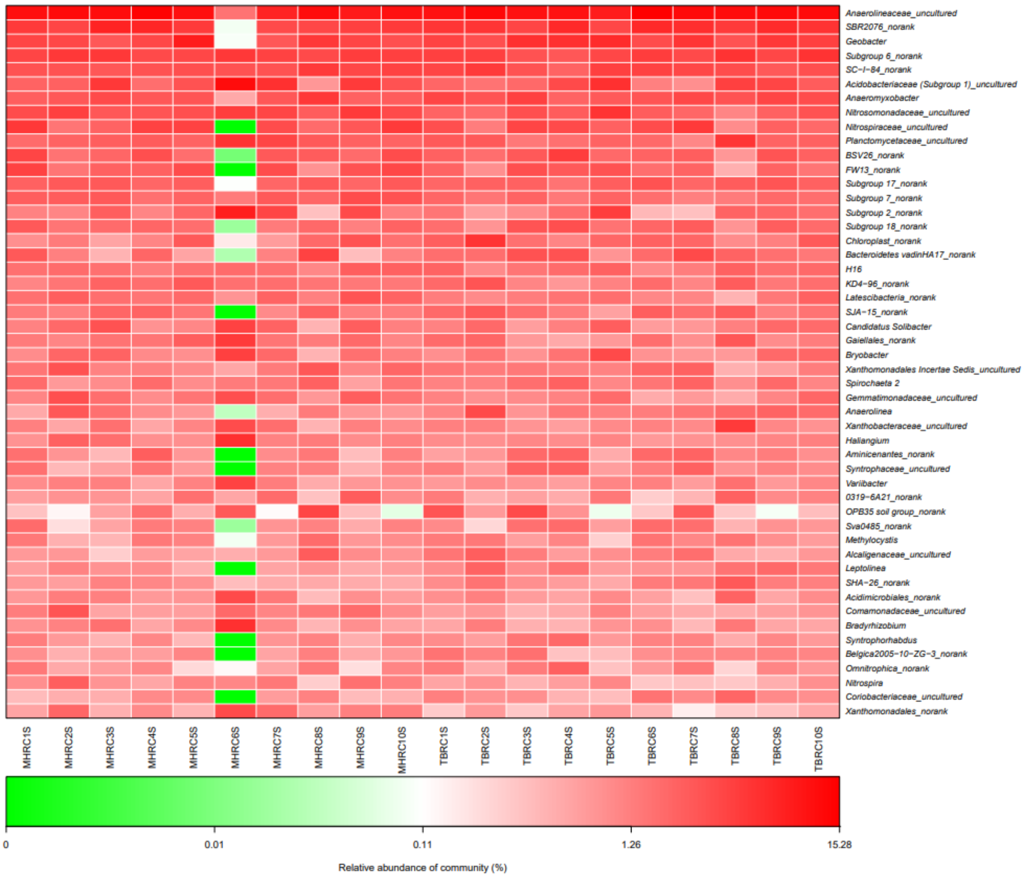
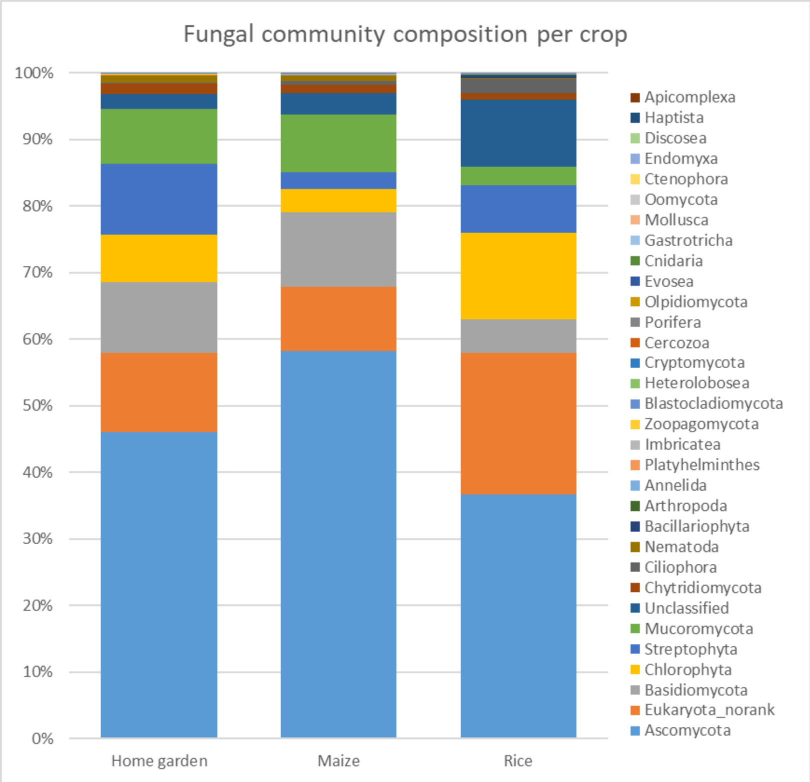


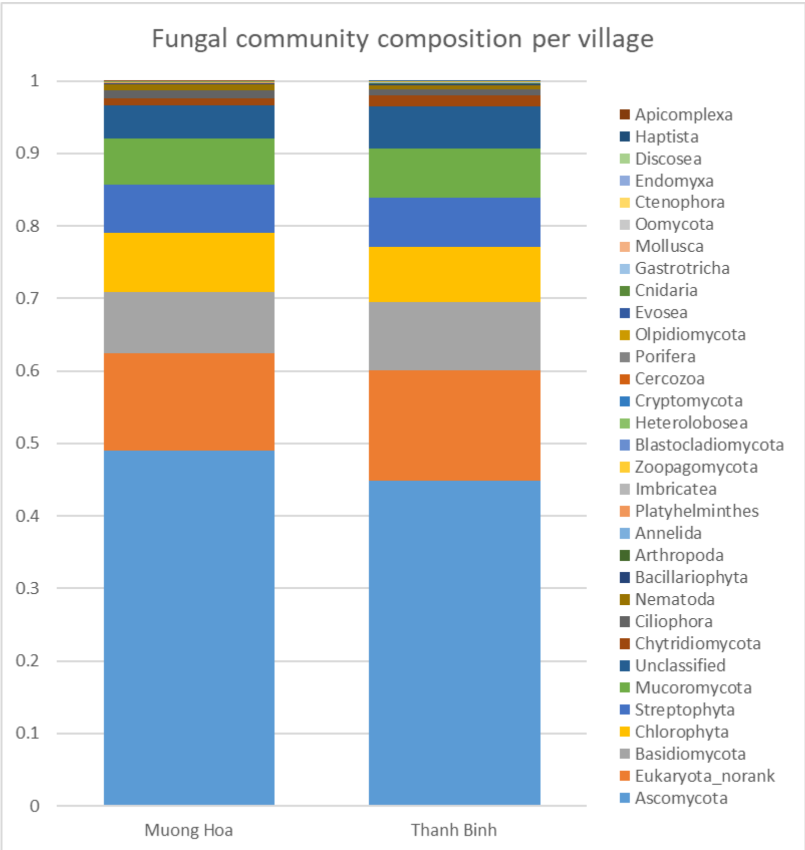
Figure 3. Heatmap analysis in genus level of soil samples from rice fields in Muong Hoa and Thanh Binh villages.

3.2 Fungal community in soil

Ascomycota (47%) is the dominant fungal phylum in all samples. (Figures 3a and 3b)



(a)



(b)

Figure 4. Fungal community composition per crop (a) and per village (b).

Home garden showed the lowest OTU richness by Chao and exponential Shannon indices, compared to rice and maize. A lower Chao index for a metagenomic sample suggests that the observed diversity (in terms of OTUs) is lower than the estimated total diversity, taking into account potential unobserved or rare OTUs. Comparing the 2 villages in terms of the fungal community of maize soil samples, Muong Hoa is also lower in the Chao index when compared to Thanh Binh but without statistical significance. (Table 3)

Table 3. Richness and diversity indices of the fungal community at the OTU level

	Village		Crop		
	Muong Hoa	**Thanh Binh**	**Rice**	**Maize**	**Home Garden**
Chao	1793.43 a	1910.67 a	1982.80 a	1930.75 a	1642.60 b
Exp(H')	180.26 a	199.21 a	220.18 a	201.95 a	147.08 b
1/D	53.55 a	55.06 a	54.95 ab	65.40 a	42.57 b

Chao: species richness index; Exp(H'):exponential Shannon index; 1/D: inverse Simpson index. Values followed by different letters are significantly different at $p<0.05$

3.3 AMF community in roots

At the class level, Glomeromycetes were more abundant in samples from maize (32%) and home garden roots (23%) than in rice root samples (2%) in both villages. Glomeromycetes are known to facilitate the uptake of nutrients, especially phosphorus, by their host plants. The higher abundance in maize and home garden roots may be linked to the nutrient demands of these plants and the efficient nutrient uptake facilitated by Glomeromycetes (Figure 5). Looking at the genus level, Scutellospora was the most abundant in rice but a very low abundance was recorded in home garden and maize. Several genera such as: *Funneliformis*, *Gigaspora*, etc. were not identified from data of rice. Meanwhile, between two villages, there was not a significant alteration regarding to the AMF community (Figure 6)

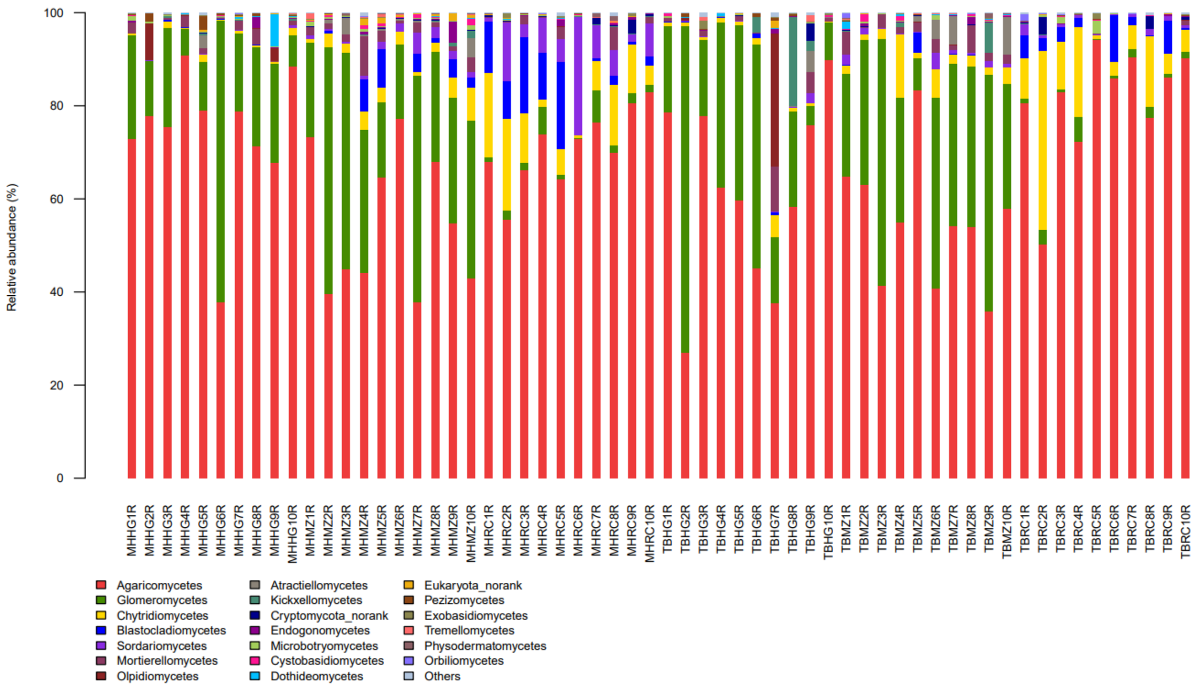
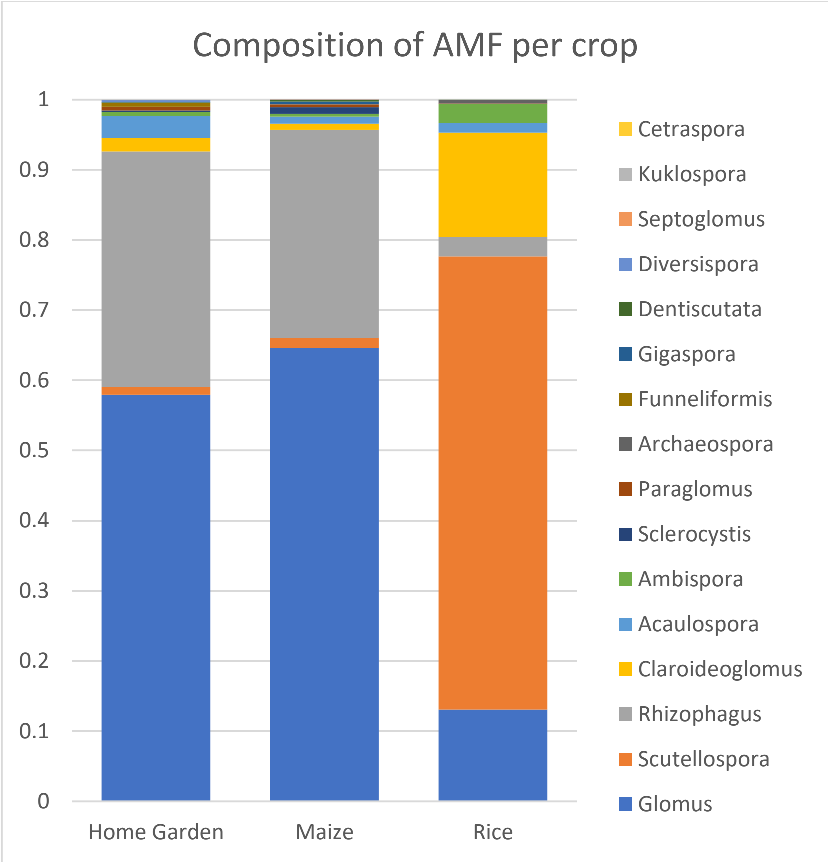
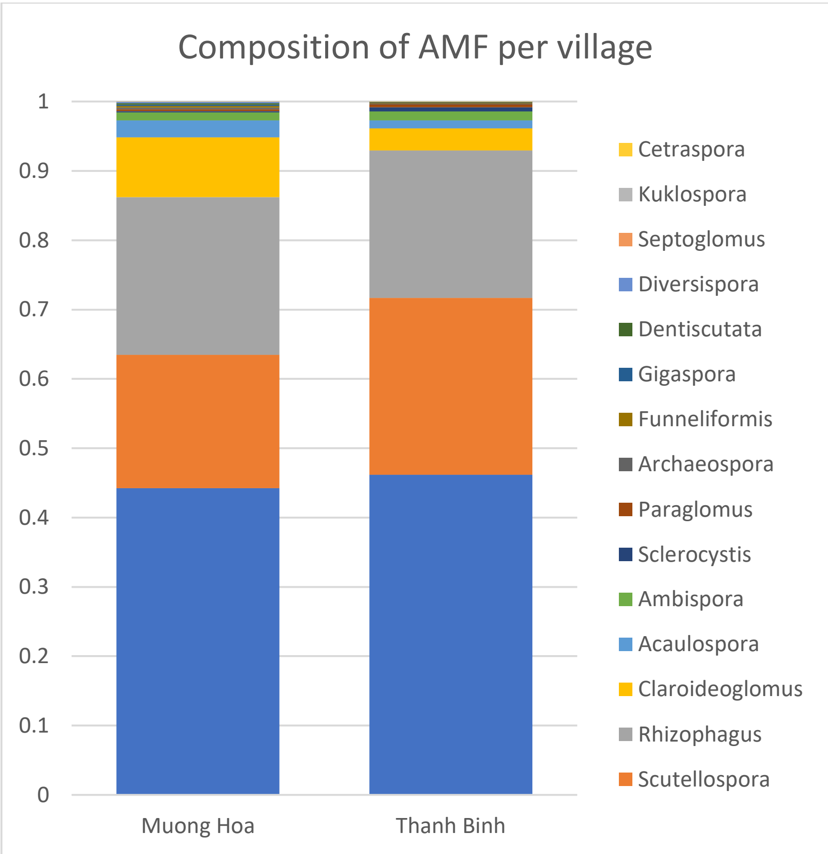


Figure 5. Fungal community composition at the class level of every root sample from the AMF-targeted amplicon sequencing data.



(a)



(b)

Figure 6: Root AMF community composition at the level of genus per crop type (a) and per village(b)

4. Next steps

Based on the activities conducted so far, the objectives for WP1 in 2024 are as follows:

- Detailed analysis of metabarcoding data, assess the effect of fertilizer usage on microbial diversity and environmental sustainability.
- To complete this analysis by integrating soil physical and chemical data for each plot, to determine the main environmental drivers of the microbial community changes.

WP 3

A. Project Background

Nature+ initiative aims to transform production systems in selected countries (including Vietnam) to meet the increasing demands of growing populations by adopting agroecological principles, emphasizing biodiversity and improving soil and water management through integrated Nature Positive Solutions (NPS). WP1 and WP3 seek to assess the total soil microbial diversity according to the cropping system/land use. In Vietnam, WP 3 of Nature+ worked in Son La Province, targeting Longan fruit plantations. The study targets the impact of agricultural practices (monocropping and intercropping) and the level of chemicals used on the microbial diversity of Longan plantations. The objectives of WP3 in Vietnam in 2023 were:

1. Define cropping systems/land uses in Son La Province and process sampling
2. Work with NOMAFSI for soil texture and soil characteristics assessment
3. DNA extraction and amplicon sequencing - Illumina technology.

B. Work package activities and progress

1. Samples information and processing

In Son La, we are targeting the agroforestry systems with longan (fruit tree). The treatments are as follows:

- Longan monocropping with organic/low chemical input (inorganic fertilizer 0-50 kg/ha, no pesticides/herbicides)
- Longan monocropping with high chemical input (>100 kg/ha, and/or pesticides or herbicides)
- Longan intercropping with organic/low chemical input (inorganic fertilizer 0-50 kg/ha, no pesticide/herbicide)
- Longan intercropping with high chemical input (>100 kg/ha, and/or pesticide or herbicide)

The sampling process was carried out in October 2023 on 4 treatments as previously described, following the protocol in Appendix 1. A total of 40 soils and 40 root samples were collected.

The effect of agricultural practices and level of chemical on the composition, structure, richness and diversity of the soil bacterial and fungal communities and of root arbuscular mycorrhiza fungi (AMF) community was assessed. A total of 80 samples were analyzed by amplicon sequencing using the Illumina technology. Soil and root total DNA was extracted using the MP FastDNA Spinkit. The sequencing process was performed at Mingke Sequencing (www.mingkebio.com, Hangzhou City, China) using the primer pairs 515F - 907R for 16S (bacteria), ITS3F – ITS4R (fungi) and AMV4.5NF – AMDGR (mycorrhiza) amplicons, respectively.

2. Soil texture and characteristics analysis

Soil samples were sent for texture and characteristics analysis by NOMAFSI. The analysis of soil physicochemical characteristics showed that there was an interaction between agricultural practices and the level of chemicals that affect the average values of pH, cation exchange capacity (CEC) and the percentage of slit (Table 3)

Table 3: Soil's physicochemical characteristics analysis with interaction between agricultural practices and level of chemical

Plantations	Soil Texture				Soil Characteristics						
	Sand (%)	Slit (%)	Limon (%)	Clay (%)	pH(H2O)	OM (%)	Total Nitrogen (%)	Phospho (mg/100g)	Potassium (mg/100g)	OC (%)	CEC (meg/100g)
Monocrop*Low	34.72 (8.56) a	24.85 (3.48) b	35.04 (10.83) ab	5.4 (4.46) a	5.4 (0.38) a	3.20 (0.78) a	0.18 (0.03) a	23.99 (11.42) a	11.27 (8.58) a	2.38 (0.53) a	2.76 (0.35) ab
Intercrop*High	25.69 (7.86) ab	33.25 (5.99) a	36.24 (7.48) ab	4.54 (2.5) a	5.12 (0.12) ab	3.32 (0.54) a	0.21 (0.04) a	15.86 (4.37) a	15.14 (9.06) a	2.36 (0.49) a	3.14 (0.4) a
Intercrop*Low	29.77 (11.09) ab	34.08 (7.05) a	31.64 (9.4) b	4.51 (3.16) a	4.82 (0.24) b	3.13 (0.32) a	0.20 (0.03) a	16.46 (7.82) a	8.06 (5.21) a	2.61 (0.28) a	2.76 (0.5) ab
Monocrop*High	19.83 (6.79) b	33.92 (6.20) a	43.56 (7.49) a	2.71 (1.38) a	5.03 (0.21) b	3.2 (0.33) a	0.17 (0.04) a	21.14 (15.04) a	16.27 (8.38) a	2.35 (0.6) a	2.41 (0.37) b
Pr > F	0.071	**0.018**	0.513	0.194	**0.000**	0.592	0.723	0.748	0.697	0.506	**0.010**
Significant	No	**Yes**	No	No	**Yes**	No	No	No	No	No	**Yes**

Note: For each variable, values followed by different letters are significantly different at p<0.05 (pairwise comparisons of the means using Tukey HSD tests). Standard deviation values are given in brackets.
Abbreviation: OM: organic matter, OC: organic carbon, CEC: cation exchange capacity

In the comparative analysis of agricultural practices, a statistically significant disparity was observed in the percentage of total nitrogen content between intercropping and monocropping systems. The higher nitrogen content in the intercropped soil may enhance nutrient cycling and overall soil fertility compared to monocropping systems (refer to Table 4a). About the chemical factor levels, the low chemical level soil exhibited a significantly greater proportion of sand compared to the high-chemical soil, potentially influencing variations in water and nutrient retention rates. Additionally, the data revealed a greater potassium concentration in soils subjected to high-chemical soil as opposed to those with low-chemical levels (refer to Table 4b).

Table 4. Soil analysis with only one factor (agricultural practices or level of chemicals)

(a)

Agricultural practices	Soil Texture				Soil Characteristics						
	Sand (%)	Slit (%)	Limon (%)	Clay (%)	pH(H2O)	OM (%)	Total Nitrogen (%)	Phospho (mg/100g)	Potassium (mg/100g)	OC (%)	CEC (meg/100g)
Intercrop	27.73 (10.73)a	33.8 (6.77) a	33.94 (10.24) a	4.53 (3.56) a	4.97 (0.36) b	3.23 (0.60) a	0.20 (0.03) a	16.157 (13.43) a	11.698 (8.84) a	2.486 (0.56) a	2.950 (0.40) a
Monocrop	27.28 (9.82)a	29.38 (6.55) b	39.3 (8.80) a	4.05 (2.85) a	5.21 (0.24) a	3.20 (0.46) a	0.17 (0.04) b	22.562 (6.34) a	13.773 (8.19) a	2.362 (0.42) a	2.584 (0.49) b
Pr > F	0.877	0.029	0.080	0.648	0.008	0.890	**0.017**	0.074	0.417	0.450	0.011
Significant	No	Yes	No	No	Yes	No	**Yes**	No	No	No	Yes

(b)

Level of chemical	Soil Texture				Soil Characteristics						
	Sand (%)	Slit (%)	Limon (%)	Clay (%)	pH(H2O)	OM (%)	Total Nitrogen (%)	Phospho (mg/100g)	Potassium (mg/100g)	OC (%)	CEC (meg/100g)
Low	32.24 (7.9) a	29.46 (6.10) b	33.34 (8.33) b	4.95 (2.22) a	5.11 (0.18) a	3.17 (0.45) a	0.19 (0.04) a	22.022 (11.39) a	9.665 (8.75) b	2.492 (0.55) a	2.747 (0.53) a
High	22.76 (10.21) b	33.27 (7.23) a	39.90 (10.28) a	3.82 (3.89) a	5.07 (0.43) a	3.26 (0.60) a	0.2 (0.03) a	18.497 (10.49) a	15.705 (7.28) a	2.356 (0.44) a	2.777 (0.43) a
Pr > F	**0.002**	0.035	**0.034**	0.204	0.686	0.619	0.908	0.624	**0.029**	0.408	0.884
Significant	**Yes**	Yes	**Yes**	No	No	No	No	No	**Yes**	No	No

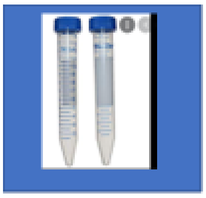
Note: For each variable, values followed by different letters are significantly different p<0.05 (pairwise comparisons of the means using Tukey HSD tests). Standard deviation values are given in brackets.
Abbreviation: OM: organic matter, OC: organic carbon, CEC: cation exchange capacity

3. Next steps

- Receive metabarcoding sequencing data of WP3 around early January 2023.
- Detailed comparative analysis of microbial diversity between different agricultural practices and levels of chemicals, integrating with soil physicochemical characteristics.

Appendix 1. Sampling protocol

- 3 crops:
  - Rice
  - Maize
  - Vegetables (target the same species for all the farms / Home gardens
- 2 Villages
  - Thanh Binh village(Village Lech Dao village)
  - Muong Hoa village(Hoa Su Pan village)
- 10 farms per village and per crop: TOTAL OF 10 x 3 = 30 samples / village x 2 = total of 60 samples



Material:

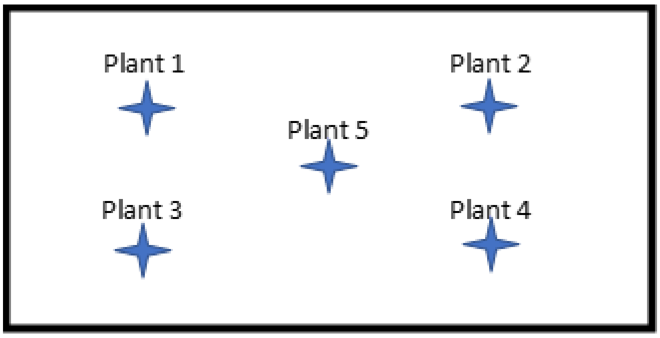
- Labels
- Tubes: Falcon 15 ml
- Paper bags (for roots)  
- Bucket/basin:  
- Large Ziplock bags (for soil analysis) => size will depend on the required quantity of Large Ziplock bags (for sample grouping for the falcon tubes)
- Shovel/spade: 
- Marker pens
- Paper clips (to hold the envelopes together)
- Tape
- GPS
- Dettol 
- Hand towel/ piece of cloth
- Water to clean the tools

- Cool box (polystyrene)

- Ice packs
- Ice cubes
-

Methods:

- 1 composite sample will be taken from each crop/field.
- Record the GPS coordinates of the field (in the middle of the field).
- Select 5 plants per field in different locations (see figure below). Avoid edges and big trees if any.



- Gently dig the first plant out and place it in a basin. We will dig a depth inside the soil from 0 to 15cm
- Cut the above-ground biomass => You can give the farmer the biomass.
- Gently shake the roots on top of the basin to eliminate the soil attached to the roots (the soil will fall into the basin).
- Place the roots in a paper envelope (make sure it contains the label).
- Add some soil to the basin from around the hole where the plant was uprooted (approx. 2 handfuls => to be adjusted if more soil is needed for soil characteristics).
- Gently dig the 2nd plant out. Place it in the basin and separate the roots from the above-ground biomass.
- Shake the roots to remove the soil and store the roots in the paper envelope (same as for plant 1)
- Add the same quantity of soil as for the 1st plant, and mix.
- Repeat for plants 3, 4 and 5.
- Mix the soil in the basin very well.
- Fill soil in 1 Falcon tube and the large Ziplock.

- Store the envelope and the large Ziplock with soil at room temperature.
- Store the Falcon tube in a large Ziplock in the cool box with ice pack/ice cubes). Make sure no water gets in the samples => close the Ziplock very well.
- Dispose of the soil left in the basin.

BETWEEN TWO CROP PLOTS/FIELDS AND BETWEEN TWO FARMS: Clean the basin and spade with water and Dettol.

For each crop/plot, you should have:

- 1 large Ziplock filled with soil (stored at room temperature)
- 1 paper envelop with roots from 5 plants (stored at room temperature)
- 1 Falcon tube filled with soil (stored in the cold box fridge or freezer if possible. The samples should be kept as cold as possible at all times. Store them in a cool box with ice cubes while in the field, in a fridge when back in the hotel (if staying overnight in the field), and in a freezer once back in NOMAFSI.

SEND THE SAMPLES (Tubes and roots) TO HANOI AS SOON AS POSSIBLE (ESPECIALLY IF THE TUBES CANNOT BE KEPT IN A FREEZER). The soil characterization will be done at NOMAFSI HQs.

Appendix 2. Farms information and fertilizer amount in Muong Hoa and Thanh Binh villages

Muong Hoa		**Rice**				**Maize**				**Home Garden**
#	Farmer	Area (ha)	NPK (kg/ha)	Urea (kg/ha)	Herbicide/ Pesticide	Area (ha)	NPK (kg/ha)	Urea (kg/ha)	Herbicide / Pesticide	Manure
1	Tấn A Sinh	0.1	100	0	No	0.05	100	5	No	Manure
2	Tấn A Giàng	0.067	25	25	No	0.1	50	30	2 times prepare the land, once after sowing 1 month	Manure
3	Tấn A Lử	0.067	50	0	No	0.05	20	0	Once, prepare the land	Manure
4	Châu A Sử	0.23	150	70	No	0.2	100	50	Once, prepare the land	Manure
5	Châu A Quả	0.133	100	30	No	0.15	100	30	No	Manure
6	Tấn A Đỏa	0.233	50	50	4 times	0.1	30	60	Once, prepare the land	Manure
7	Châu A Chờ	0.233	100	50	No	0.15	70	30	Once, prepare the land	Manure
8	Tấn A Thào	0.267	75	0	No	0.2	100	70	No	Manure
9	Châu A Chăng	0.25	100	20	Once, prepare the land	0.15	70	20	2 times prepare the land, once after sowing 1 month	Manure
10	Châu A Su	0.4	100	100	No	0.2	100	50	Once, prepare the land	Manure

Appendix 3. Farms information and fertilizer amount in Thanh Binh

Thanh Binh		Rice				Maize				Home Garden
#	Farmer	Area (ha)	NPK (kg/ha)	Urea (kg/ha)	Herbicide/ Pesticide	Area (ha)	NPK (kg/ha)	Urea (kg/ha)	Herbicide /Pesticide	Manure
1	Lý Nhục Nhàn	0.6	350	0	No	0.1	75	40	No	Manure
2	Lý Nhụt Mềnh	0.2	100	25	No	0.4	100	50	No	Manure
3	Lý Sành Siệu	0.25	120	30	No	0.1	50	50	2 times prepare the land, once after sowing 1 month	Manure
4	Lý Nhụt Chẳn	0.167	150	0	No	0.05	50	0	Once, prepare the land	Manure
5	Lý Ông Lai	0.267	250	0	Once, prepare the land	0.3	150	75	Once, prepare the land	Manure
6	Lý Ông Phin	0.267	200	0	No	0.2	100	50	Once, prepare the land	Manure
7	Lý Nhụt Chìu	0.267	400	0	No	0.15	70	40	Once, prepare the land	Manure
8	Lý Nhụt Quáng	0.267	350	0	No	0.2	80	35	No	Manure
9	Lý Cáo Cáo	0.33	400	0	No	0.2	100	35	Once, prepare the land	Manure
10	Lý Ông San	0.33	350	0	No	0.2	80	30	Once, prepare the land	Manure