

# Changes in 16S rRNA bacterial community structures after C and N additions

## - comparison of organic farmed and conventionally farmed soils



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### Introduction

- Green manure legumes are <u>alternatives</u> to chemical fertilizer <u>in organic farming system.</u>
- Green manure legumes supply organic matter to soils as their residues and influence soil microbial community (Mao et al. 2011).
- Microbes can also be sinks for nitrogen (N) and the N within live-microbes (biomass N) cannot be utilized by plants resulting in the competition between plant and microbe for available N.
- The use of green manure legumes may lead to increased available N in soils but it may influence on soil microbial N assimilation (N immobilization) potential.

### **Key Questions**

- 1. Does the long-term legume application change soil microbial community structure? →Exp 1.
- 2. Does soil microbial community with the history of legume application affect microbial N immobilization potential? →Exp 2.

# Experiment 1. Materials and Methods Colony counting method Bacteria • 6 days incubation • Trypticase soy agar Fungi • 3 days incubation • Potato dextrose agar

### Results

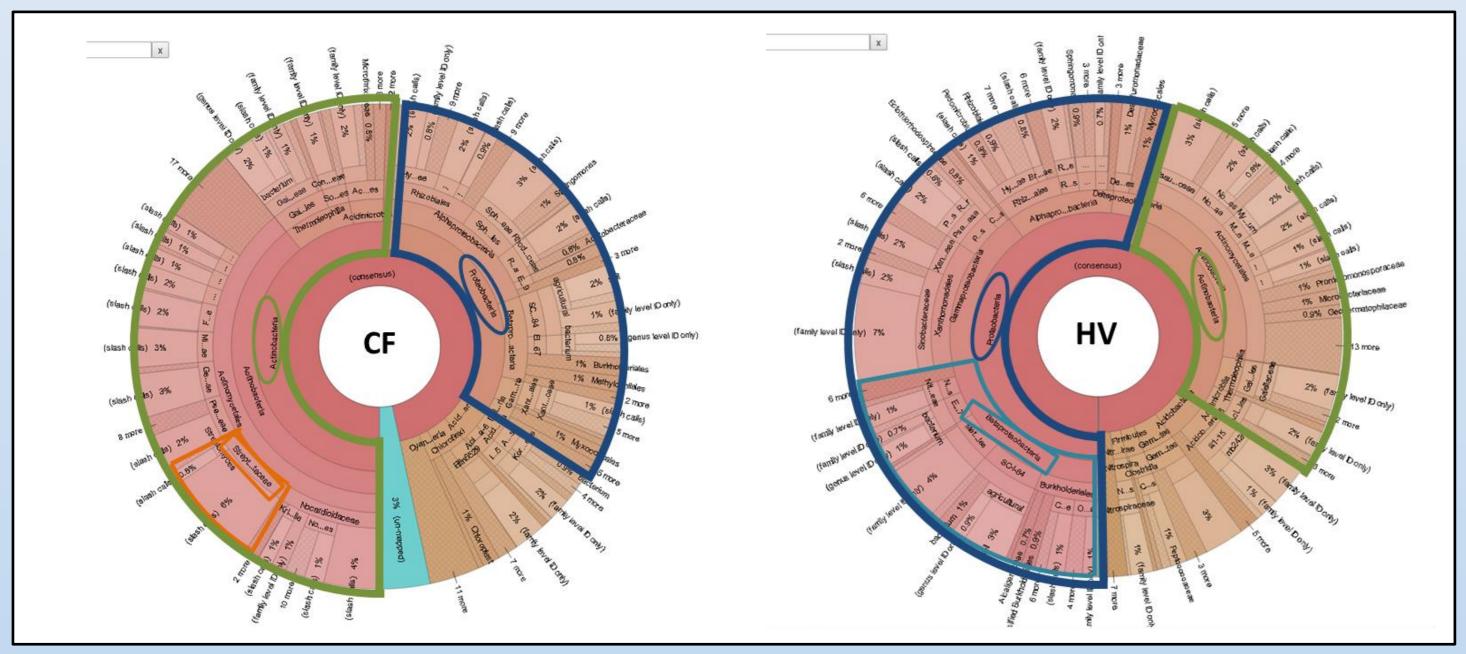
**DNA** extraction

Next generation sequencing

- Colony forming unit of Bacteria (×10<sup>7</sup>): HV (13± 5.2) > CF (5.7 ± 2.7) (p < 0.05)
- Colony forming unit of Fungi ( $\times 10^5$ ): CF (7.5  $\pm$  1.9) > HV (3.2  $\pm$  3.6) (p < 0.05)
- Shannon diversity index at family revel:  $HV (5.94 \pm 0.1) > CF (5.54 \pm 0.24) (p < 0.05)$

9 **Years** management (2006 – 2014)

• <u>Different soil microbial structures</u> after 9 years of organic vs conventional farming



**Figure 1**. Community composition of soil with the history of chemical fertilizer application (CF) and soil with the history of hairy vetch application (HV) for 9 years (one of representatives) The most dominant phylum and the second dominant phylum (*Actinobacteria* and *Proteobacteria*) were opposite between two soils.

### Discussion

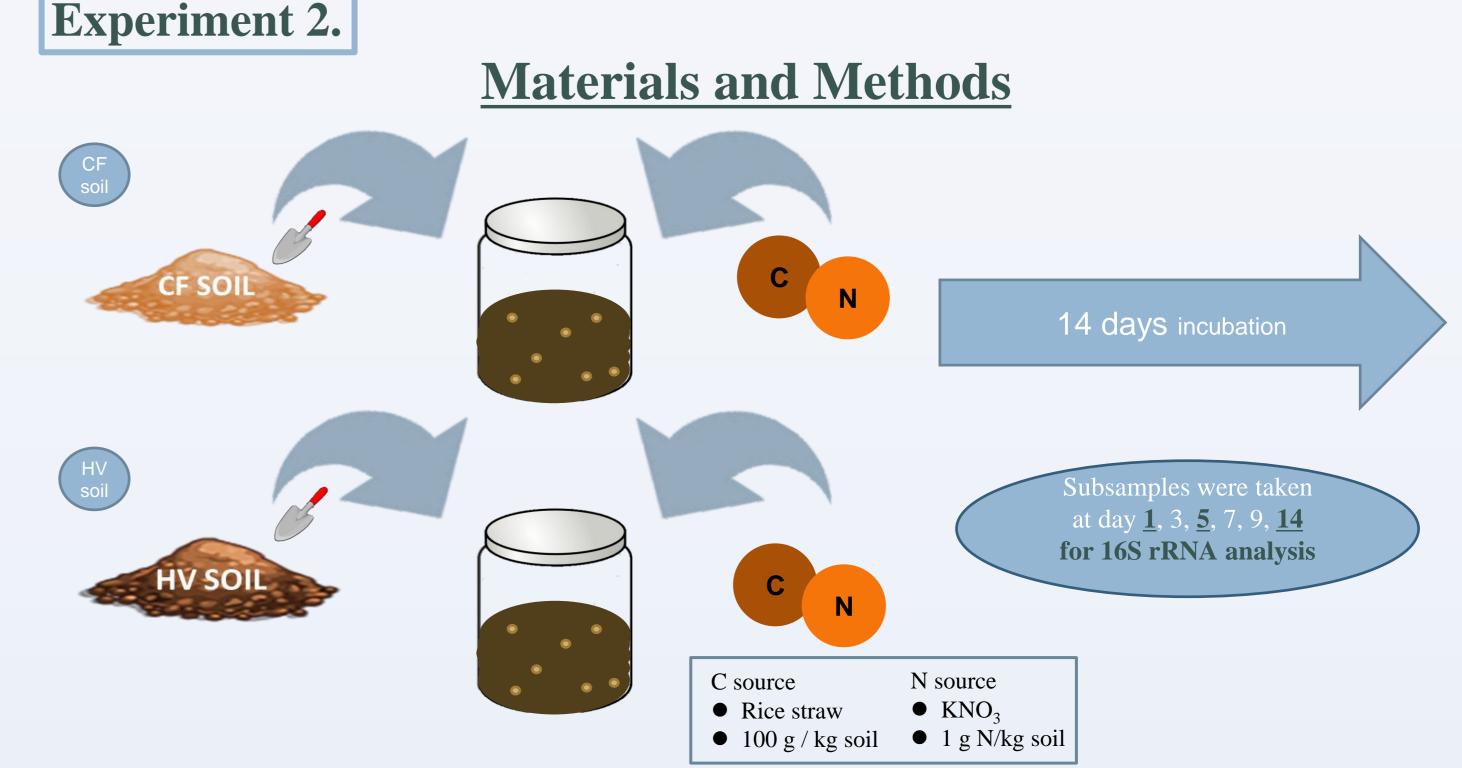
- The ratio of  $\beta$ -Proteobacteria was significantly higher in the HV soil compared to CF soil
- The abundance of β-Proteobacteria was positively correlated with C mineralization rate thus the phylum is considered to be more favorable to high C condition (Fierer et al. 2007).
   →the application of HV into soil increased C source in soil and might have
- The ratio of family *Streptomycetaceae* (family *Actinobacteria*, genus *Streptomyces*) was significantly higher in the CF soil compared to HV soil.
- Genus *Streptomyces* utilizes sulfate as sulfur sources (Fischer et al. 2012).

increased the abundance of  $\beta$ -Proteobacteria.

→The application of chemical fertilizer (Ammonium sulfate) into CF soil increased available S source and might have increased the abundance of *Streptomycetaceae*.

### Conclusion

- Exp1.
- Long term use of legume plants in organic farming system <u>changed the structures</u> of bacterial community and increased their diversity when compared to those of conventionally farmed system.
- Exp2.
- Microbial community structures became similar within 14 days with addition of excess C and N.
- No difference was observed in decrease rates of N and changes in microbial biomass C during 14 days incubation.
- The long term use of hairy vetch in soils might not change the soil function in terms of short-term N immobilization process when C and N source is applied into soil.



	CF	HV	P value
Water content (%)	14 ± 2	$17 \pm 0.5$	0.02*
pH (H <sub>2</sub> O)	$6.4 \pm 0.8$	$6.9 \pm 0.2$	NS
WFPS %	$0.23 \pm 0.04$	$0.27 \pm 0.04$	NS
Total C g kg <sup>-1</sup>	$39 \pm 3$	$39 \pm 2$	NS
Total N g kg <sup>-1</sup>	$3.6 \pm 0.3$	$3.4 \pm 0.3$	0.03*
NO <sub>3</sub> N mg kg <sup>-1</sup>	$279 \pm 45$	$201 \pm 167$	NS
NH4 +-N mg kg-1	$34 \pm 47$	$4.0 \pm 2$	NS

- Decreasing NO<sub>3</sub> -- N concentration
- > KCl extraction with colorimetry
- Increasing microbial biomass carbon
- > Chloroform fumigation method
- 16S rRNA gene analyses

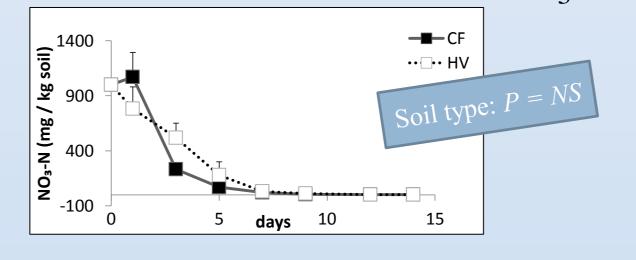
> Same as Exp 1.

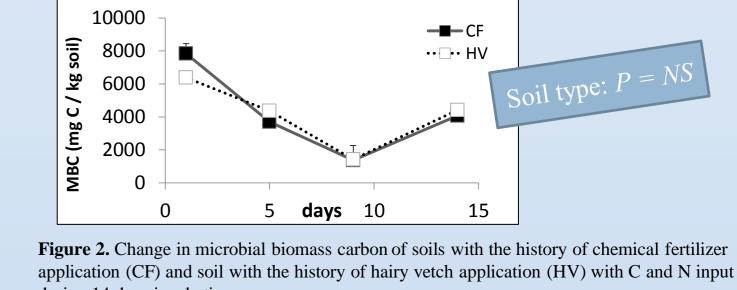
**Table 1.** Basic soil properties for soils sampled at chemical fertilizer (CF) and hairy

vetch (HV) soils from a tomato trial (n=3, error = s.d.).

### Results

■ No difference in decrease of NO<sub>3</sub><sup>-</sup> and increase of microbial biomass carbon

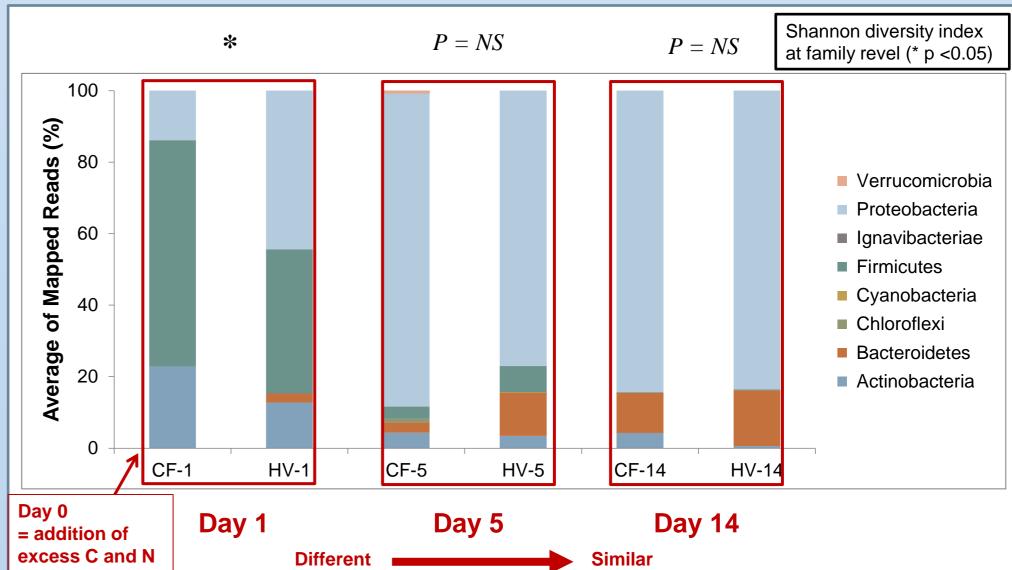




**Figure 2.** Change in NO<sub>3</sub> of soils with the history of chemical fertilizer application (CF) and soil with the history of hairy vetch application (HV) with C and N input during 14 days incubation.

during 14 days incubation.

• Microbial structure getting similar over time with added excess C and N



**Figure 4.** Change of bacterial community composition with the history of chemical fertilizer application (CF) and soil with the history of hairy vetch application (HV) with C and N input during 14 days incubation. The changing pattern seemed to be similar between two soils.

### **Discussion**

- In both soil, *Proteobactreria* and *Bacteroidetes* dominated in the end whereas *Firmicutes* disappeared over time.
- Firmicutes was dominant 3 days after organic matter addition but it was replaced by proteobacteria at 14 and 60 days (Pascault et al. (2013).
  - →changing pattern looked very similar in two soils
- ➤ Proteobacteria and Firmicutes increased with organic matter addition (Van et al. 2014) and Bacteroidetes increased its abundance with high C availability (Fierer et al. 2007).
  - →Bacterial community structure was affected by C addition.