2025년 한국생태학회 학술발표대회

The Effects of Garlic mustard (*Alliaria petiolata*) Invasions on Soil Microbiome

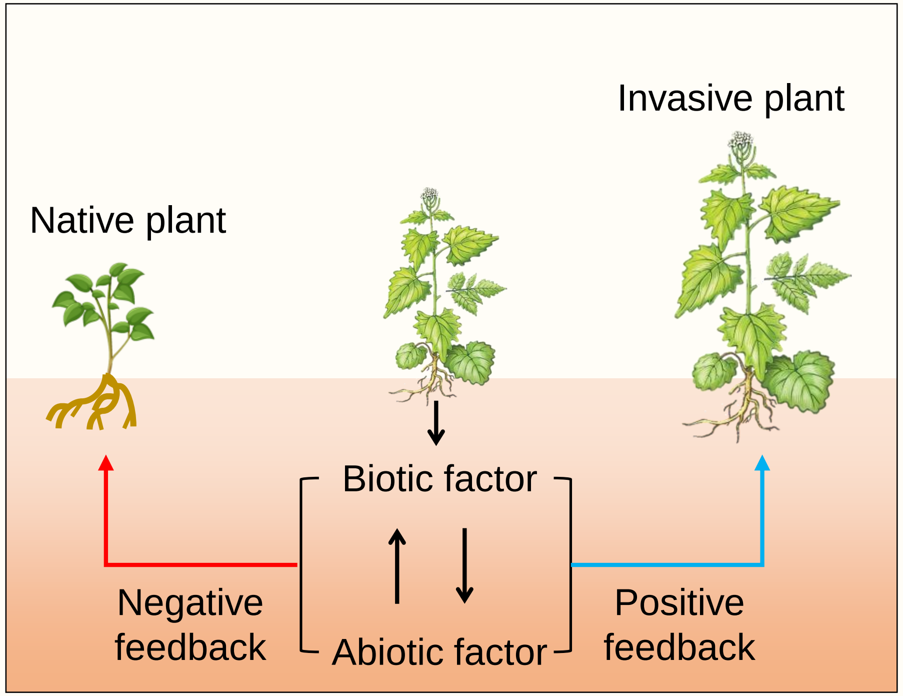
마늘냉이의 침입이 토양 미생물 군집에 미치는 영향

Yousuk Kim[P], Seorin Jeong, Tae-min Kim, Byungwook Choi, Eunsuk Kim[C]

2025.03.20

Evolutionary Ecology Laboratory

Invasive Plants and Plant-Soil Feedback



Mechanism of invasion

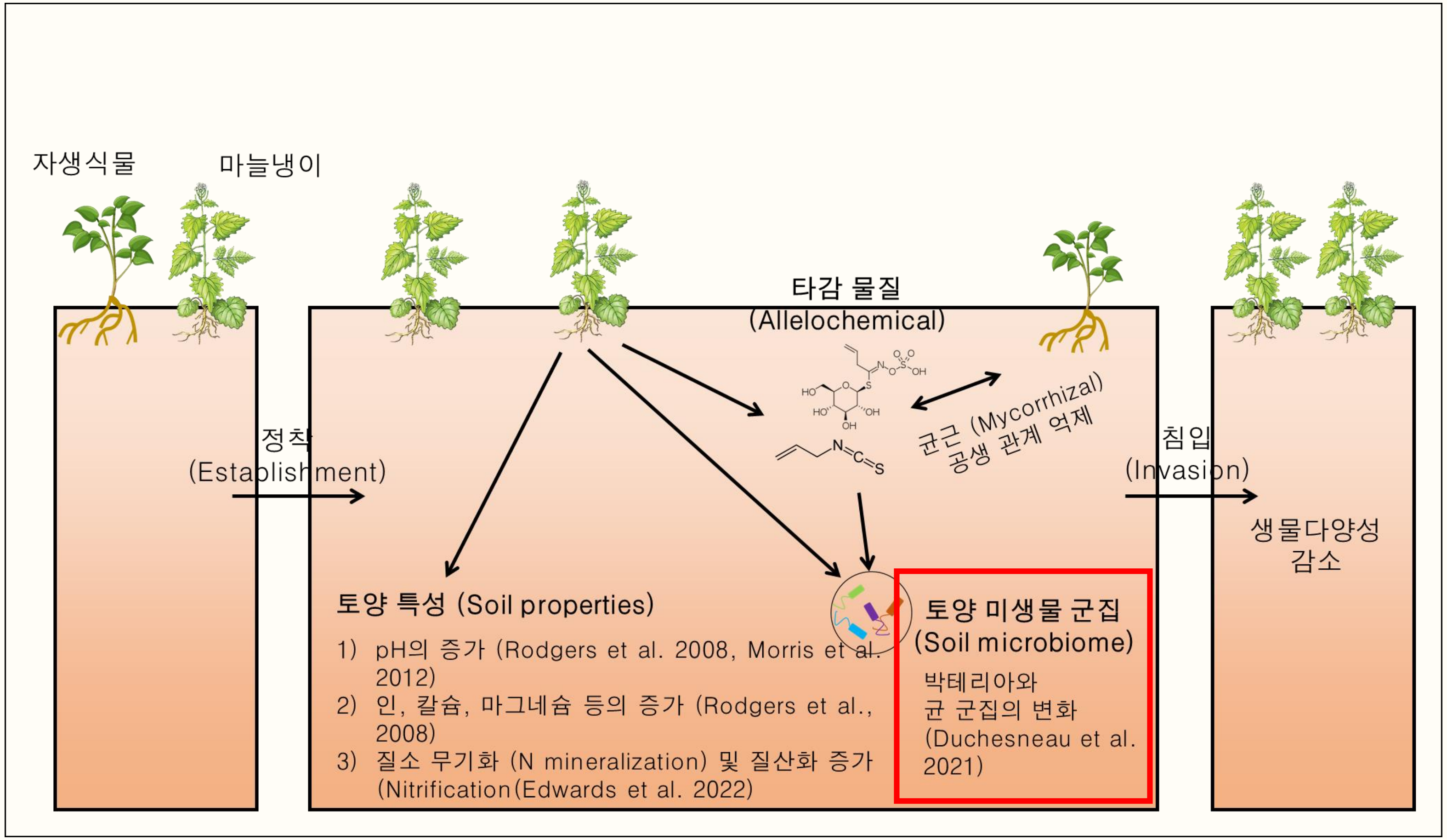
- Niche
- Competitive ability

Successful spread and invasion

Target Species

Garlic mustard (*Alliaria petiolata*)

- Introduced to North America in the mid-1800s, its invasion of forests has led to significant ecosystem changes.
- In South Korea, first discovered in Samcheok (SC) in 2012, later discovered in Suwon (SW) and Incheon (IC).
- Even after 10 years of its introduction, specific information on the impact of the garlic mustard invasion remains unavailable.



자생식물

마늘냉이

정착
(Establishment)

타감 물질
(Allelochemical)

균근 (Mycorrhizal)
공생 관계 억제

침입
(Invasion)

생물다양성
감소

토양 특성 (Soil properties)

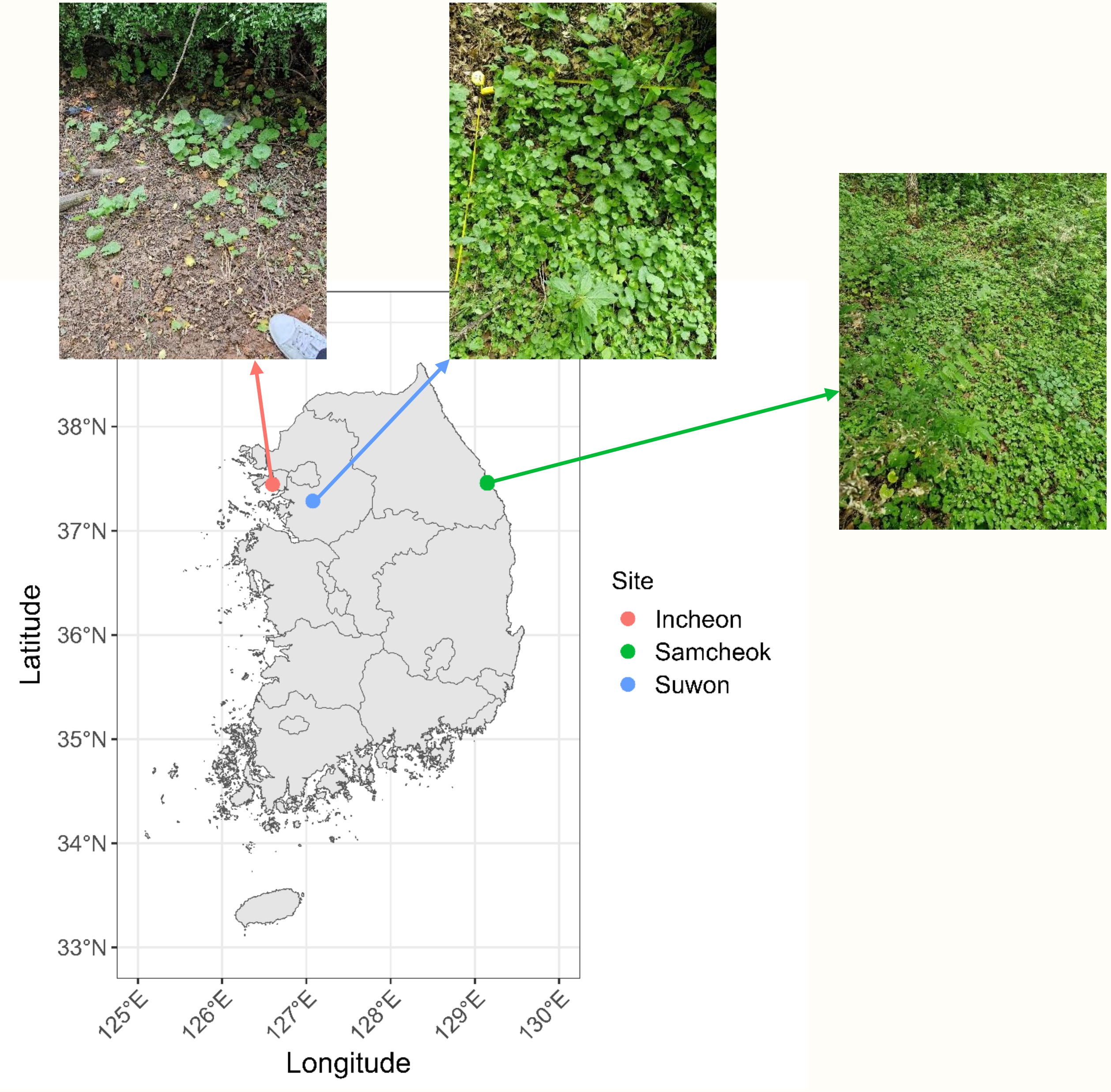
1) pH의 증가 (Rodgers et al. 2008, Morris et al. 2012)
2) 인, 칼슘, 마그네슘 등의 증가 (Rodgers et al., 2008)
3) 질소 무기화 (N mineralization) 및 질산화 증가 (Nitrification(Edwards et al. 2022)

토양 미생물 군집
(Soil microbiome)

박테리아와
균 군집의 변화
(Duchesneau et al. 2021)

Experimental Sites and Sampling

- Site : SC, SW, IC
- After assessing the invasion of garlic mustard in each site, randomly selected GM invaded areas and uninvaded areas adjacent to the each invaded areas (SC : 4, SW : 3, IC : 1).
- Establish five 1m x 1m plots (replicates) in each invaded and uninvaded areas.
- Collect soil samples at a depth of 0–10 cm in each plot.
- Total 80 bulk soil samples : SC (4 x 2 x 5 = 40 Samples), SW (3 x 2 x 5 = 30 Samples), IC (1 x 2 x 5 = 10 Samples).
- After sample collection, remove roots using a 2mm sieve and store at -80℃ until DNA extraction.



DNA Extraction and Amplicon Sequencing

DNA extraction

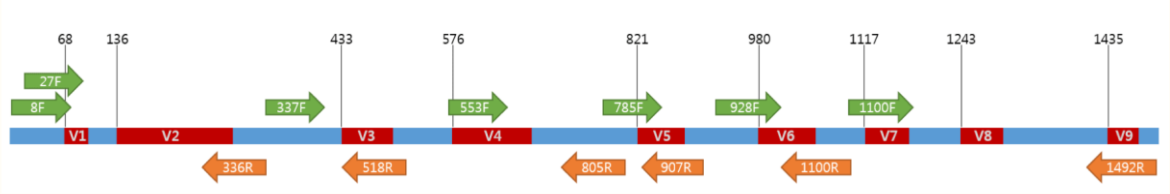
- Using DNeasy PowerSoil Pro Kit (Qiagen, Germany)

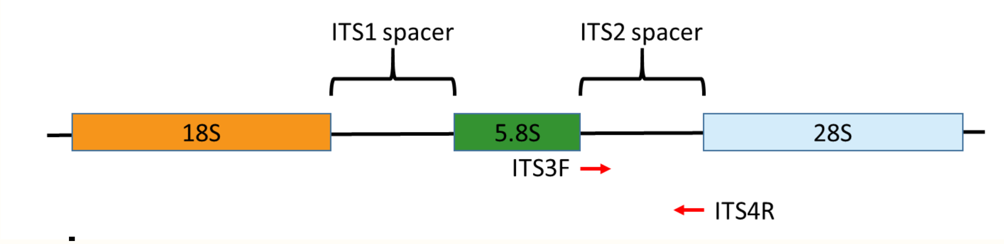
1) Nanopore sequencing for bacterial community

- Long-read sequencing (V1-V9 region; 27F-1492R)

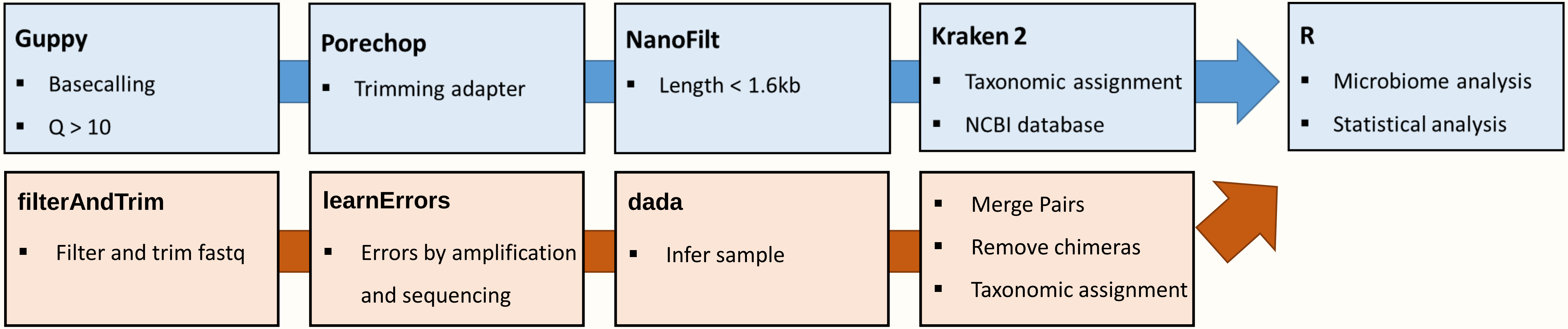
2) Illumina sequencing for fungal community

- ITS2 region (ITS3F-ITS4R)





Post-sequencing pipeline for Nanopore and Illumina sequencing



Guppy
- Basecalling
- Q > 10

Porechop
- Trimming adapter

NanoFilt
- Length < 1.6kb

Kraken 2
- Taxonomic assignment
- NCBI database

R
- Microbiome analysis
- Statistical analysis

filterAndTrim
- Filter and trim fastq

learnErrors
- Errors by amplification and sequencing

dada
- Infer sample

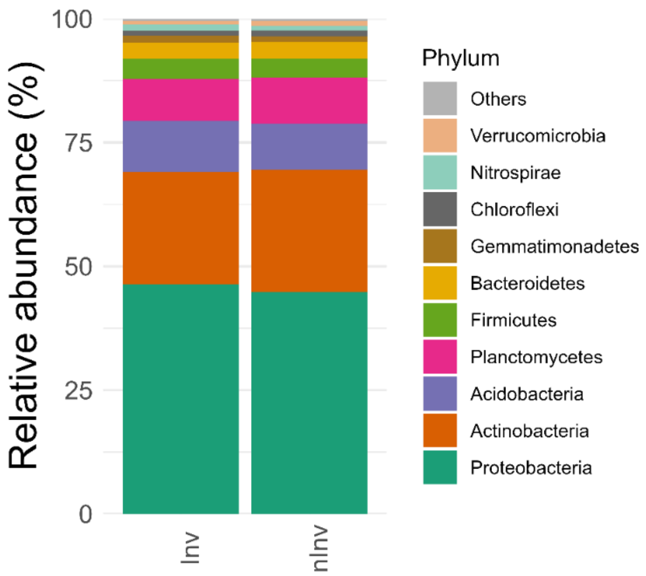
- Merge Pairs
- Remove chimeras
- Taxonomic assignment

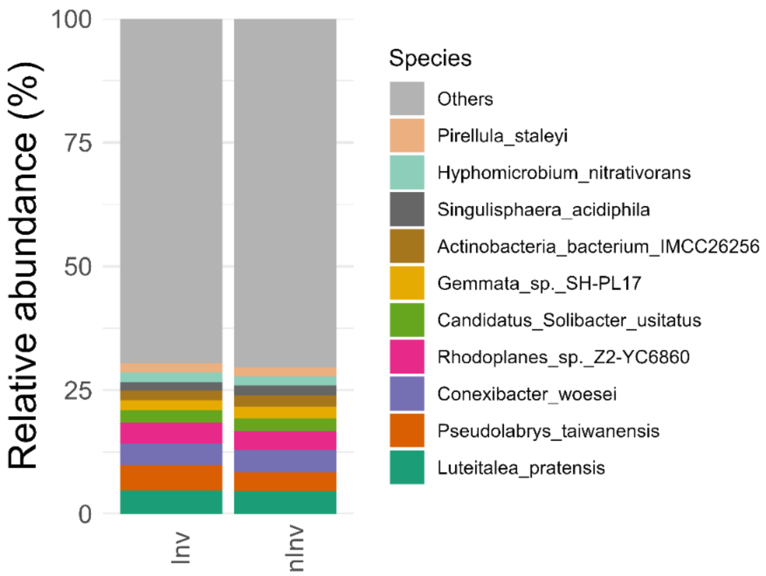
Statistical Analysis

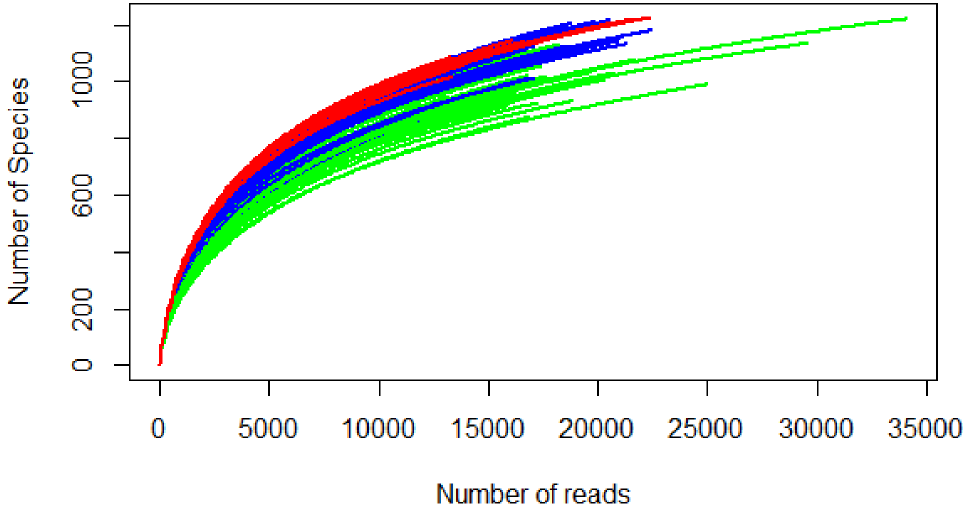
1) **Alpha diversity**
    - Analysis of variance (ANOVA), Pairwise comparison
2) **Beta diversity**
    - Permutational analysis of variance (PERMANOVA), Non-metric multidimensional scaling (nMDS), Analysis of similarities (ANOSIM)
3) **Differential abundance analysis (DA analysis)**
    - ANCOM-BC2
4) **Functional analysis**
    - FAPROTAX (Functional annotation of prokaryotic taxa) for bacterial community
    - FunGuild for fungal community
    - LEfSe (Linear discriminant analysis Effect Size) (DA analysis)
    - Pearson’s Correlation

Bacterial community

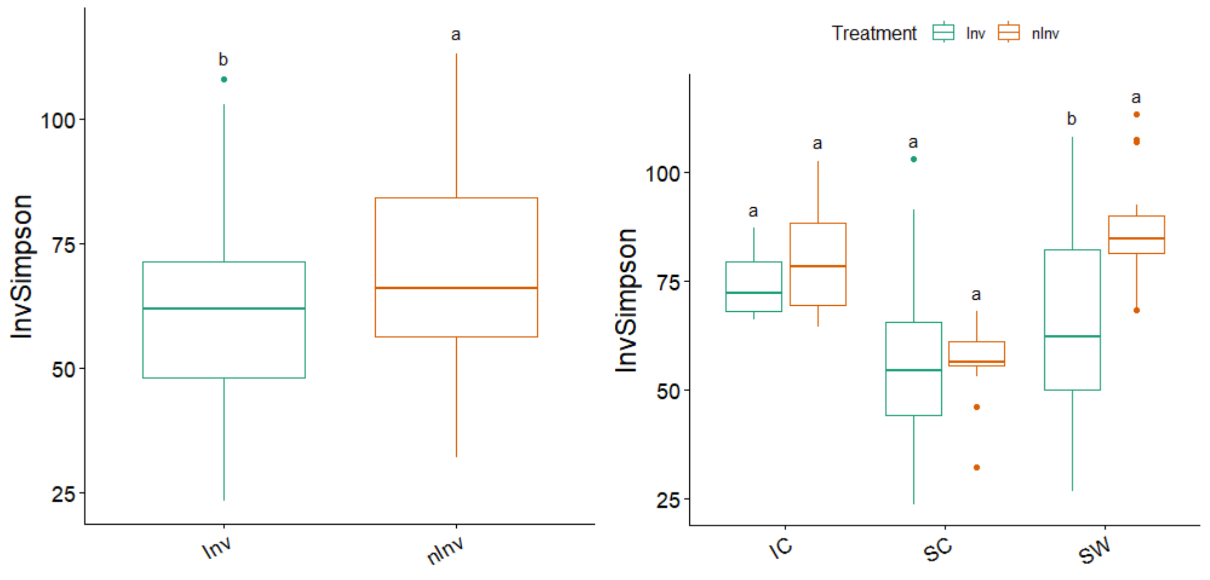
- Total : 80 Samples, 2,443 Species (1,370,937 Reads)
- Samcheok (SC) : 40 Samples, 2,252 Species (695,052 Reads)
- Suwon (SW) : 30 Samples, 2,284 Species (522,747 Reads)
- Incheon (IC) : 10 Samples, 1,913 Species (153,138 Reads)
- Conduct additional analyses at the species level.



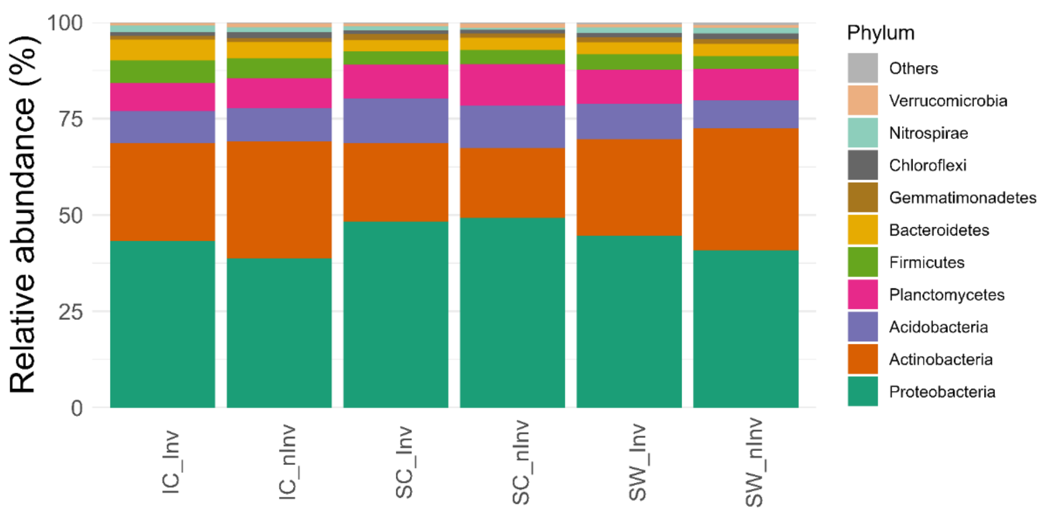


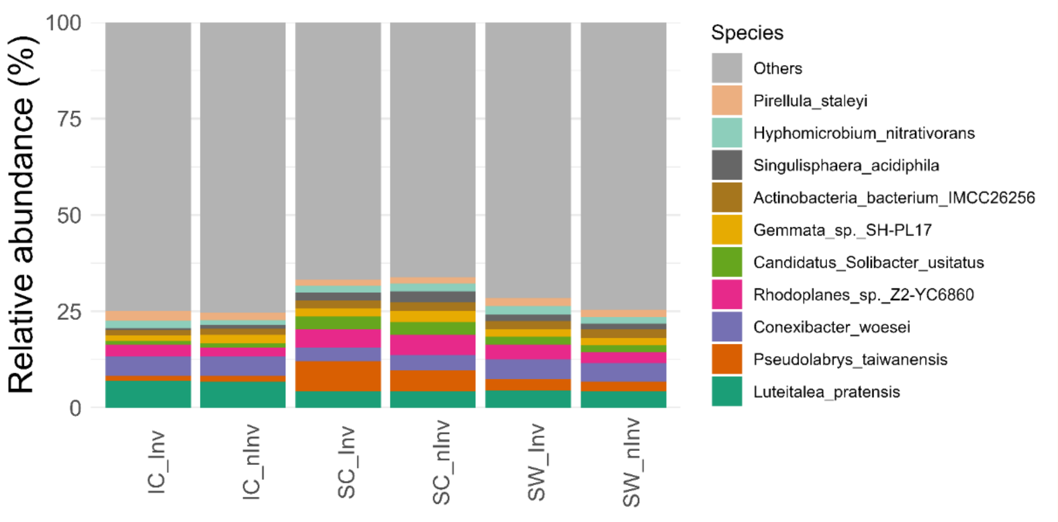


Alpha diversity

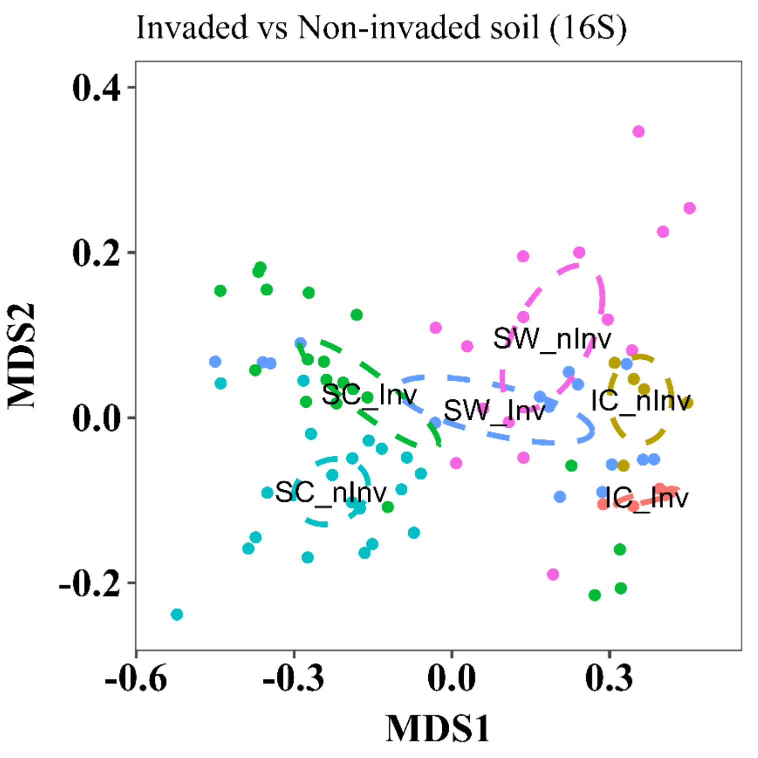


- <u>GM invaded areas showed lower diversity than uninvaded areas</u>.
- Significant differences were observed in SW (71.1 vs. 86.5), and similar trends were observed in IC (73.4 vs. 80.6) and SC (50.9 vs. 56.2).
- At the phylum level, Proteobacteria and Actinobacteria were dominated, and at the species level, *L. pratensis*, *P. taiwanensis*, and *C. woesei* account for the largest proportions.

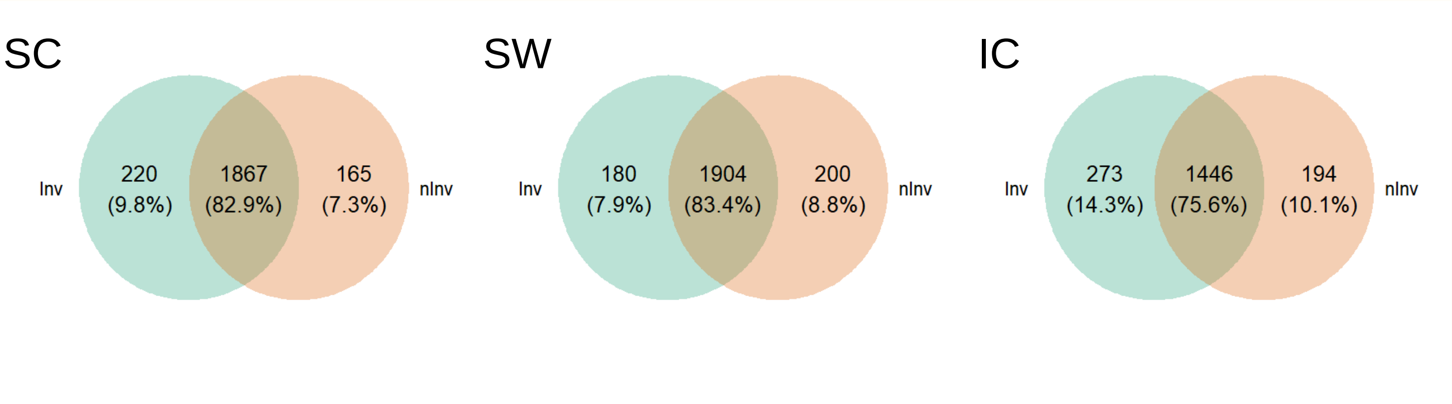




Beta diversity

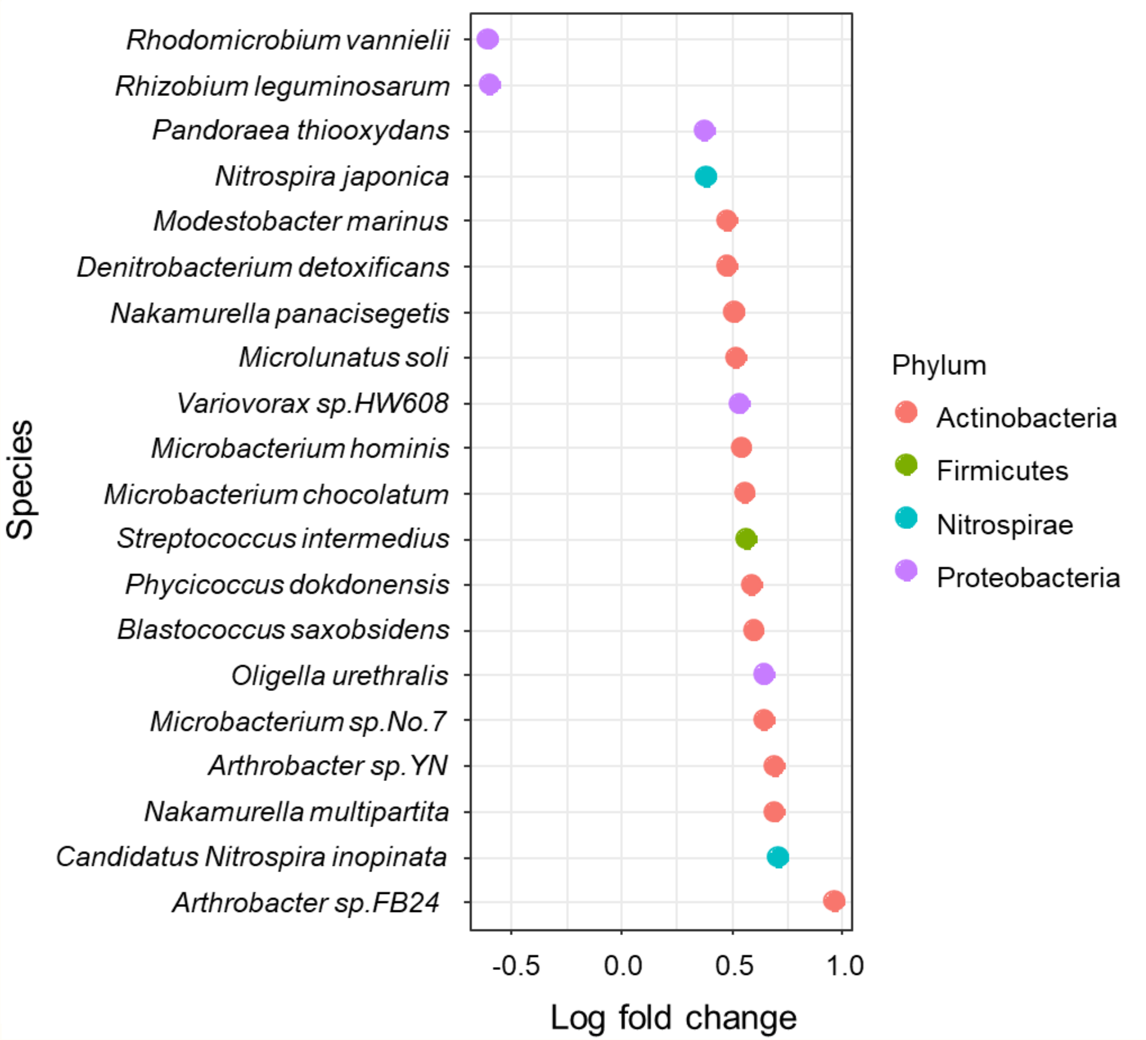


	Df	SS	R^2	F	Pr(>F)
Site	2	1.34	0.31	18.23	0.0001***
Treatment	1	0.11	0.03	2.99	0.0224*
Site:Treatment	2	0.20	0.05	2.73	0.0098**
Residual	74	2.72	0.62		
Total	79	4.37	1.00		



- Similar with alpha diversity, there were differences between sites.
- Differences in bacterial communities between GM invaded and uninvaded areas were identified.
- The differences in bacterial communities seemed to result from changes in relative abundance rather than shifts in community member.

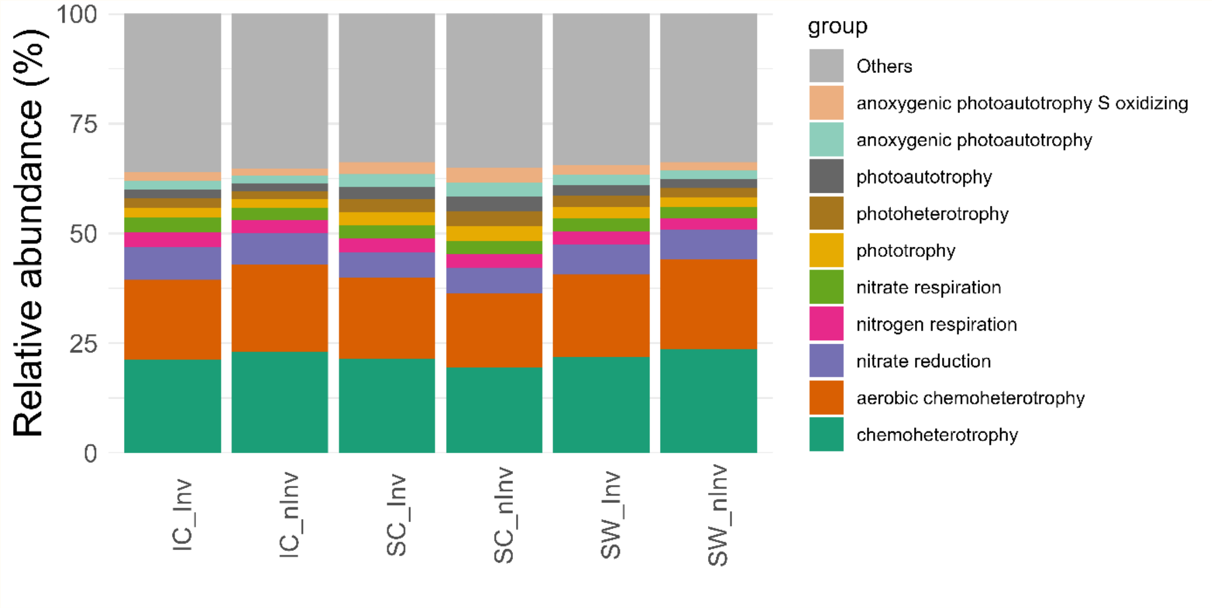
Differential abundant Species after Garlic mustard Invasion

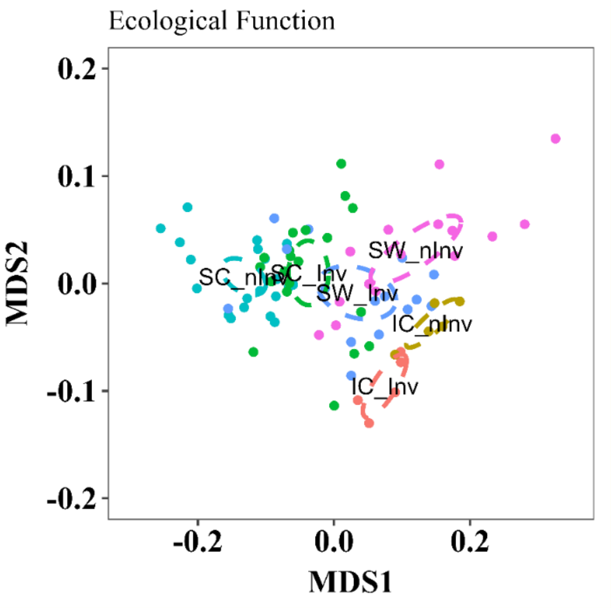


- This is the result of dividing the bacterial communities by site and conducting ANCOM-BC2 analysis.
- A total of 20 bacterial species showed differences due to garlic mustard invasion.
- Most species that showed differences after garlic mustard invasion showed increases in the invaded area.
- However, the bacterial species that showed differences were only identified at the SC.

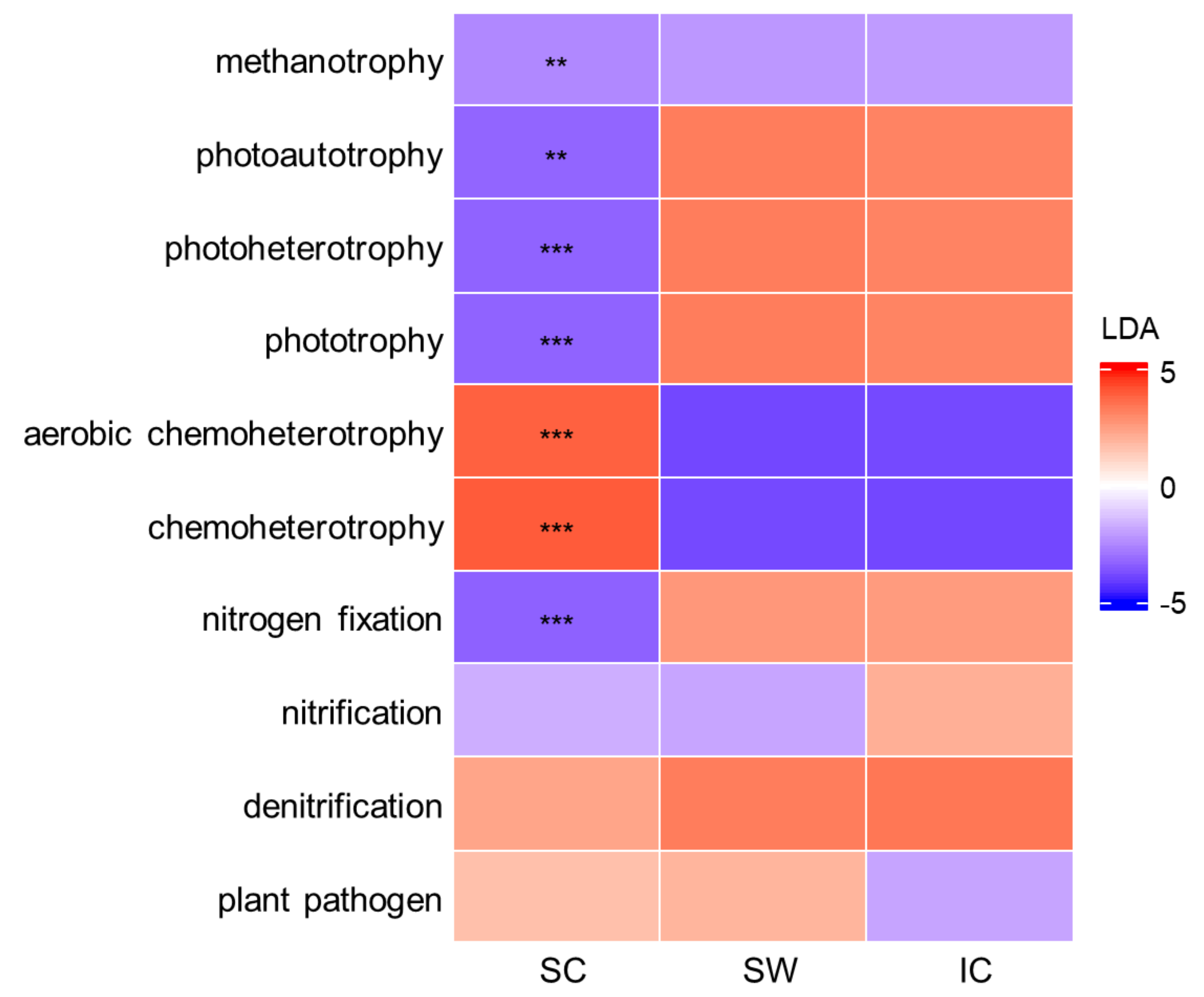
Functional Diversity of Bacterial community

- 1,688 out of 2,443 records (69.1%) were assigned to at least one group
- A total of 80 functions were derived from FAPROTAX.
- Chemoheterotrophy and aerobic chemoheterotrophy functions accounted for the largest proportion.
- Nitrate reduction, nitrogen respiration, and nitrate respiration accounted for a significant portion of nitrogen cycle-related functions, following chemoheterotrophy following previous functions.
- Across all sites, we observed <u>differences in functional diversity due to GM invasion</u>.





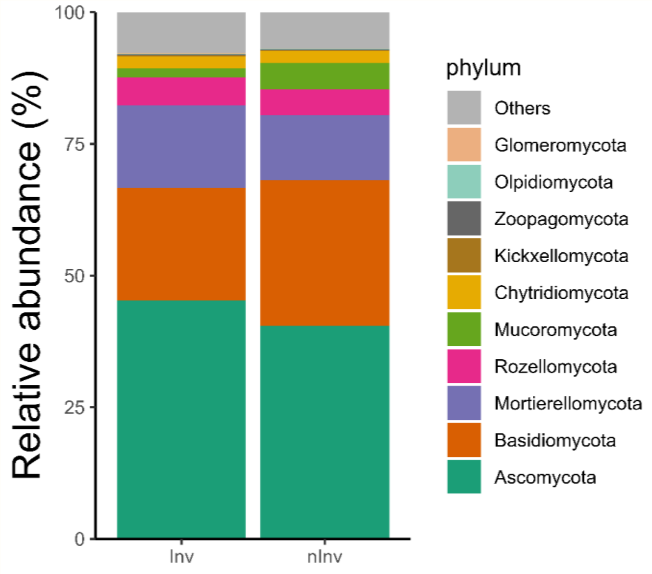
Functional Diversity of Bacterial community

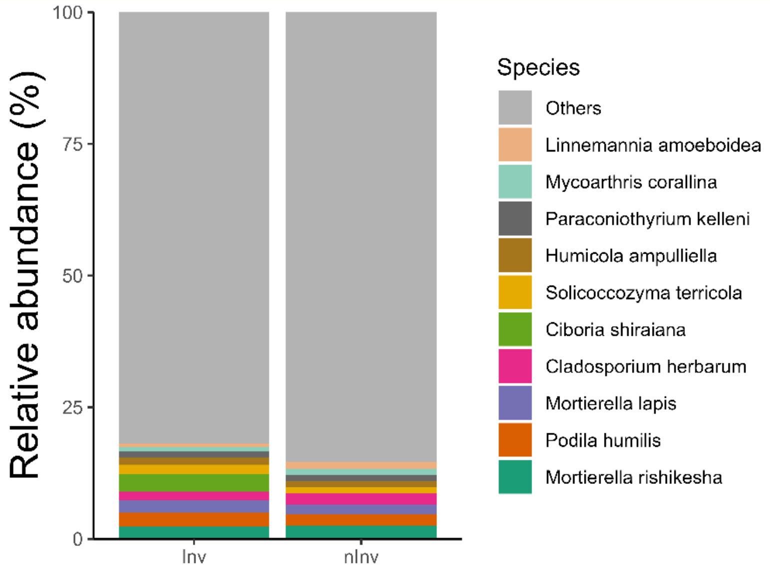


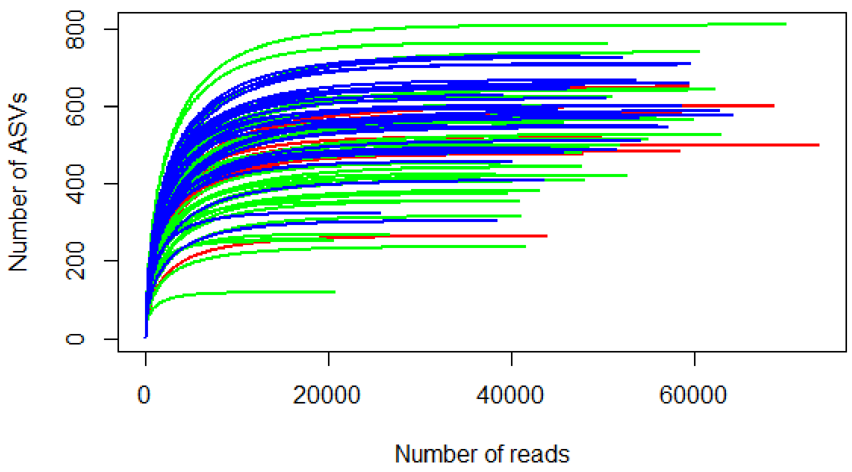
- LEfSe analysis to explore functional changes due to GM invasion at each site.
- A total of 27 ecological functions showed significant differences.
- This difference was confirmed <u>only in SC</u>.
- <u>Phototrophic bacteria decreased, while heterotrophic bacteria increased</u>.
- <u>Bacteria that fix nitrogen were found to be decreasing</u>.

Fungal community

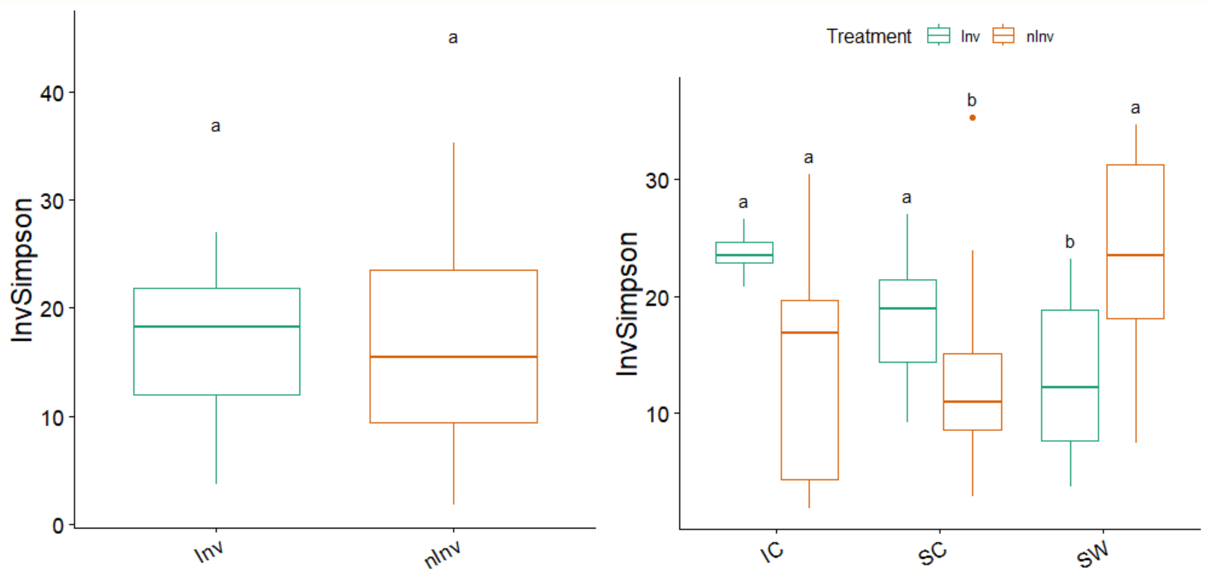
- Total (80 Samples) : 14,717 ASVs (3,833,771 reads), 1,546 Species (1,882,257 reads)
- SC (40 Samples) : 8,079 ASVs (1,810,123 reads), 1,211 Species (857,470 reads)
- SW (30 Samples) : 6,964 ASVs (1,465,553 reads), 581 Species (663,312 reads)
- IC (10 Samples) : 2,329 ASVs (558,095 reads), 771 Species (361,475 reads)
- Conduct additional analyses at the species level.



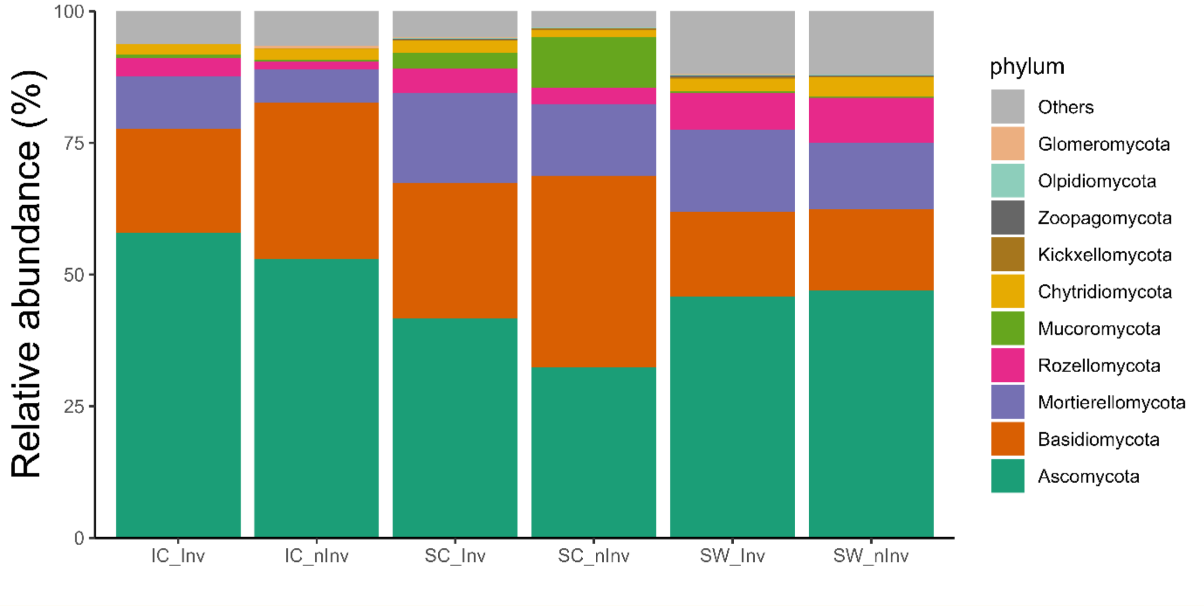


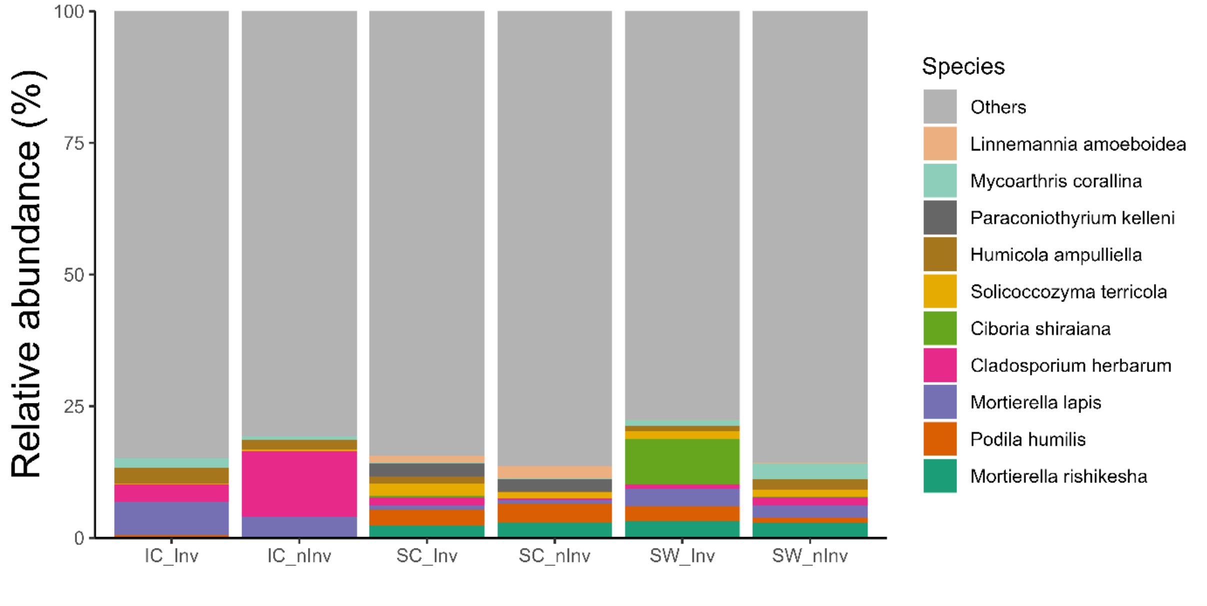


Alpha diversity

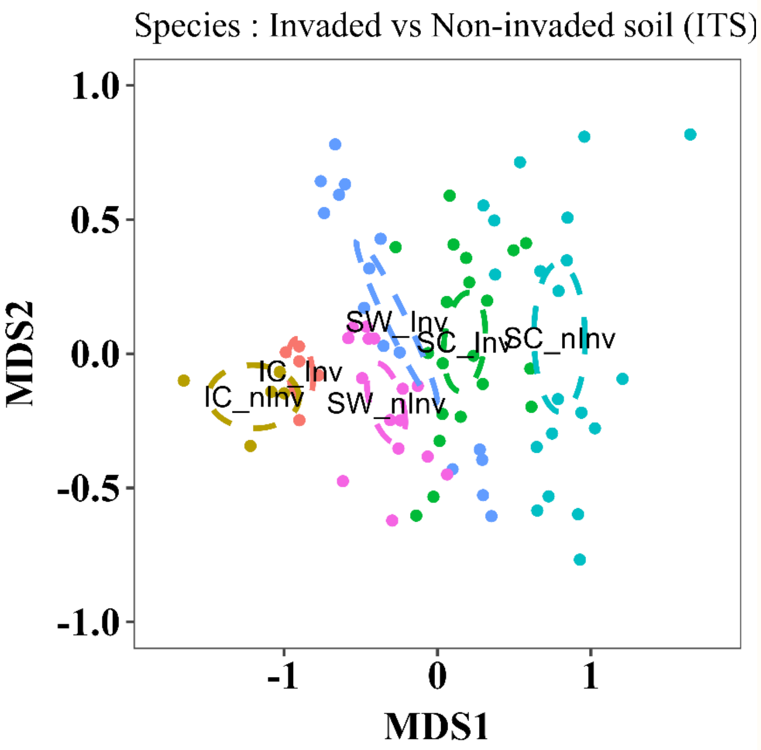


- Unlike the bacterial community, no differences in alpha diversity were observed between the overall invaded and uninvaded areas.
- SW showed lower diversity in the invaded area than in the uninvaded area, and SC showed the opposite trend.
- Among the entire community, Ascomycota (자낭균), Basidomycota (담자균) are dominant at the phylum level.

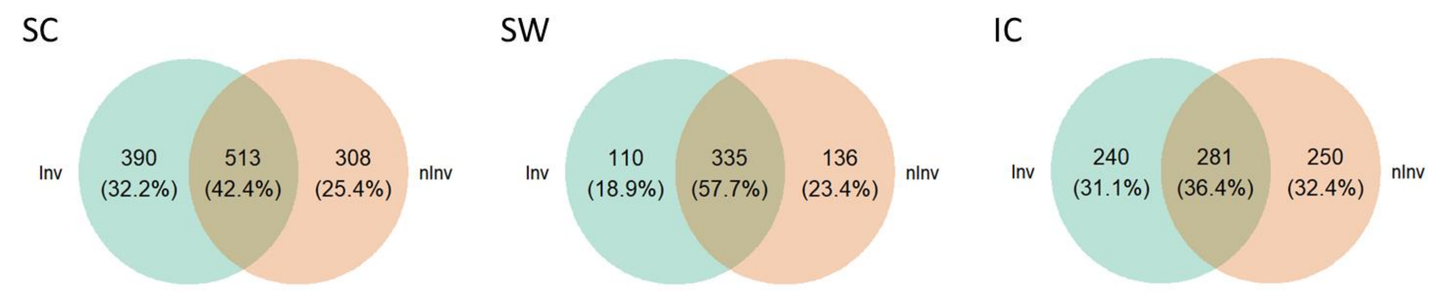




Beta diversity

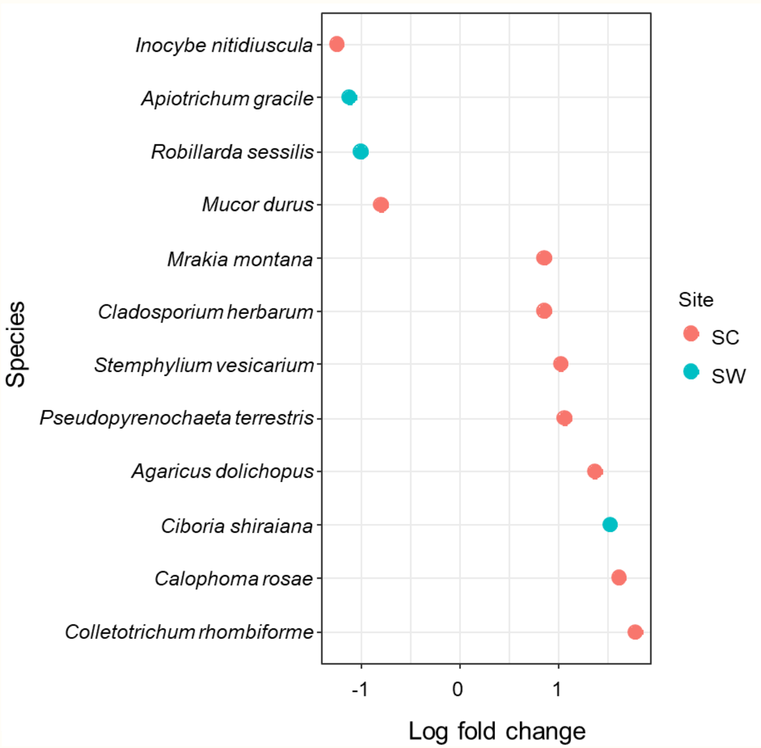


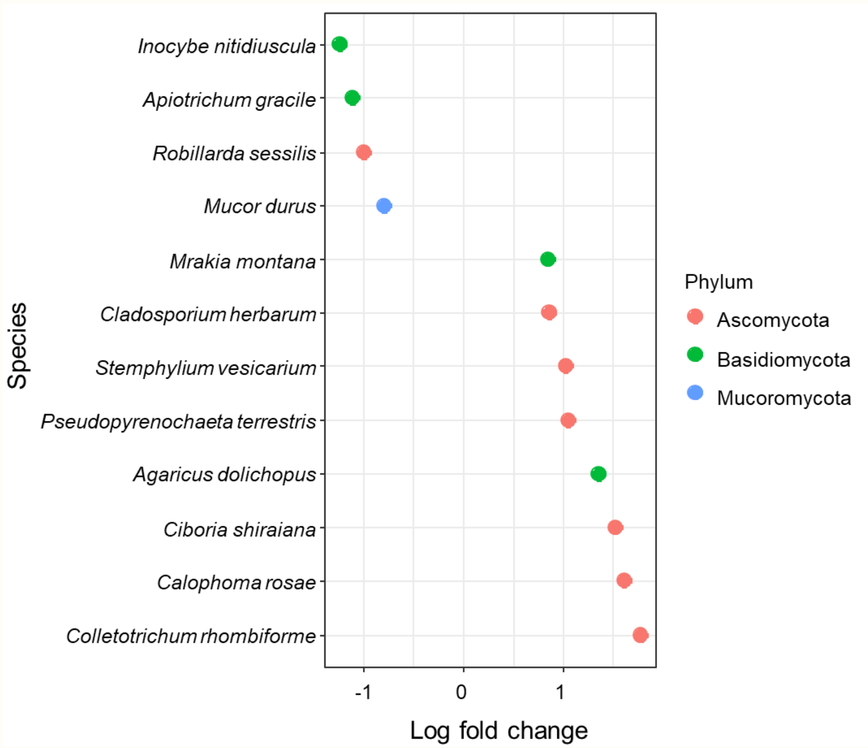
	Df	SS	R^2	F	Pr(>F)	
Site	2	4.77	0.21	10.82	0.0001	***
Treatment	1	0.69	0.03	3.13	0.0001	***
Site:Treatment	2	1.42	0.06	3.22	0.0001	***
Residual	74	16.30	0.70			
Total	79	23.18	1.00			

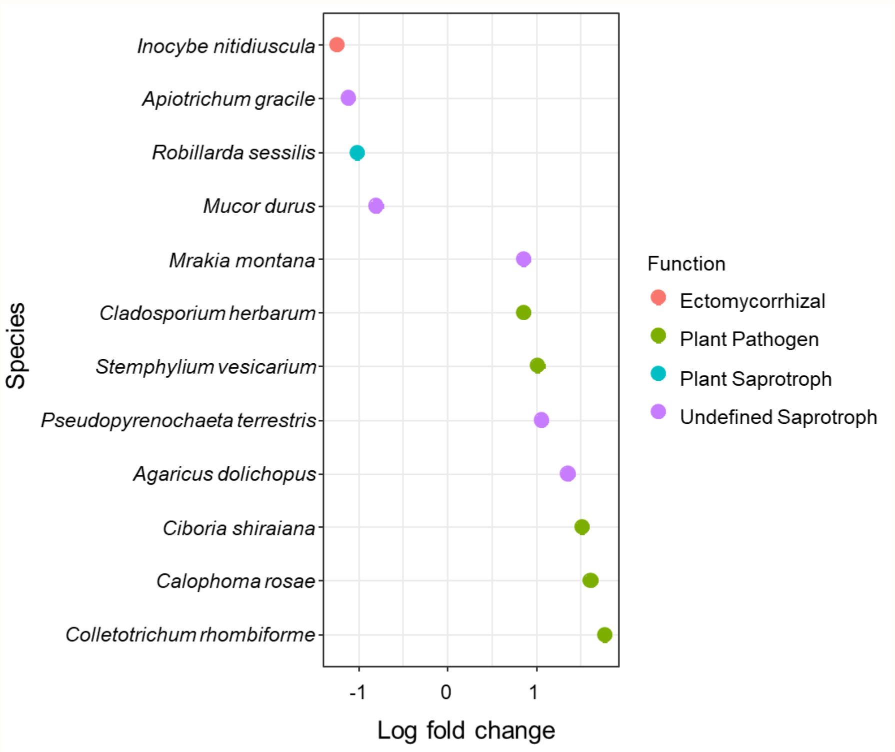


- As with the bacterial communities, <u>differences in fungal communities were identified between invaded and uninvaded areas</u>, with differences observed between sites.
- Fungal communities have a <u>relatively lower proportion of species in common</u> between invaded and uninvaded areas than bacterial communities.
- This suggests that the changes in the community due to invasion by GM occurred through <u>a process of selection for specific fungi</u>.

Differential abundant Species after Garlic mustard Invasion



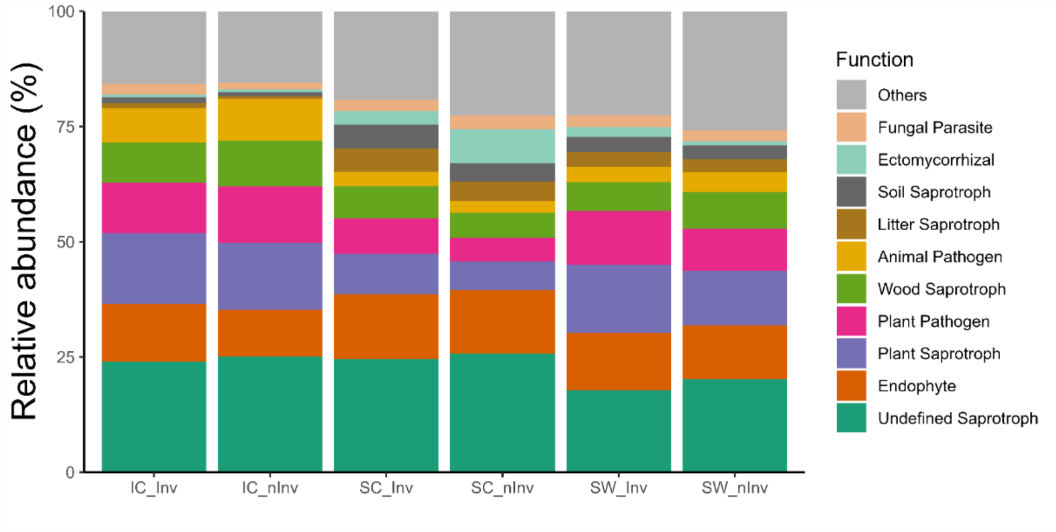


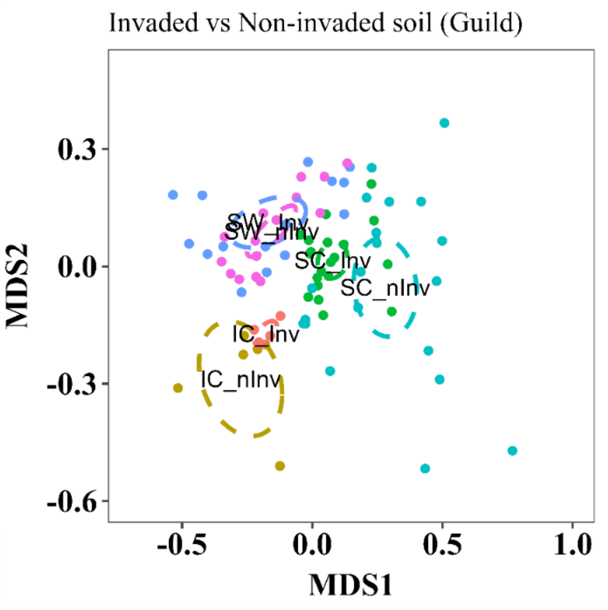


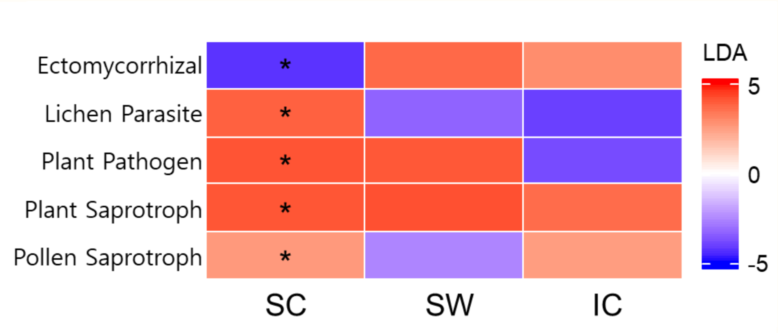
- A total of 12 species showed significant differences due to GM invasion.
- Nine species were identified in SC, three species in SW, and no species with significant differences were identified in IC.
- Most of the increased species were plant pathogen, on the contrary, among the reduced species, ectomycorrhizal.

Functional diversity of Fungal community

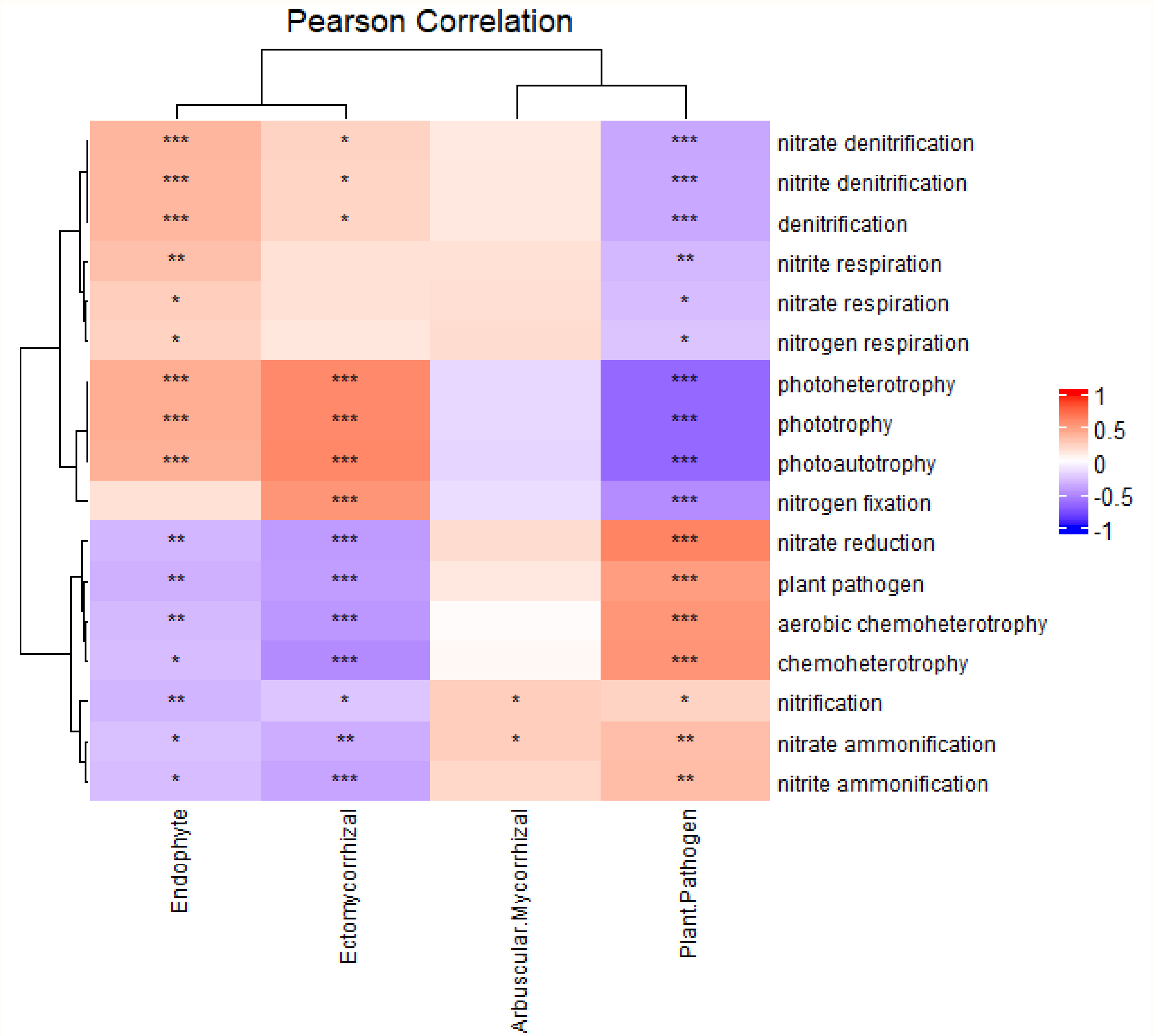
- All : 41 functions / SC : 41 functions / SW : 35 functions / IC : 38 functions
- Undefined saprotroph, endophyte, plant saprotroph and plant pathogen are dominant within the fungal community.
- In terms of functional diversity, <u>only SC exhibits a significant difference, similar to bacterial community</u>.







Correlation between Bacterial function and Fungal function



	Endophyte	Ectomycorrhizal	Arbuscular.Mycorrhizal	Plant.Pathogen
nitrate denitrification	***	*		***
nitrite denitrification	***	*		***
denitrification	***	*		***
nitrite respiration	**			**
nitrate respiration	*			*
nitrogen respiration	*			*
photoheterotrophy	***	***		***
phototrophy	***	***		***
photoautotrophy	***	***		***
nitrogen fixation		***		***
nitrate reduction	**	***		***
plant pathogen	**	***		***
aerobic chemoheterotrophy	**	***		***
chemoheterotrophy	*	***		***
nitrification	**	*	*	*
nitrate ammonification	*	**	*	**
nitrite ammonification	*	***		**

- To identify correlations between functions that showed significant differences in each bacterial and fungal community and functions generally related to plant growth or nutrient cycling.
- In fungal function, <u>endophytes and ectomycorrhizal showed similar trends, while plant pathogens showed opposite trends</u>.

Conclusion

- We observed changes in soil bacterial and fungal communities in domestic GM habitats.
- However, it was only in SC that differences in communities were confirmed to lead to differences in specific microorganisms and differences in function.
- This study confirmed the decrease of ectomycorrhizal after GM invasion, which may cause changes in the nitrogen cycling, which is consistent with the results of previous studies.
- However, since this study only confirmed the changes in biotic factors due to GM invasion, it is not known what factors caused the differences in microbial communities and functions.
- Therefore, additional analysis should be conducted through further studies to measure soil abiotic factors.

Thank you for listening

Q&A