The 11th EAFES International Congress

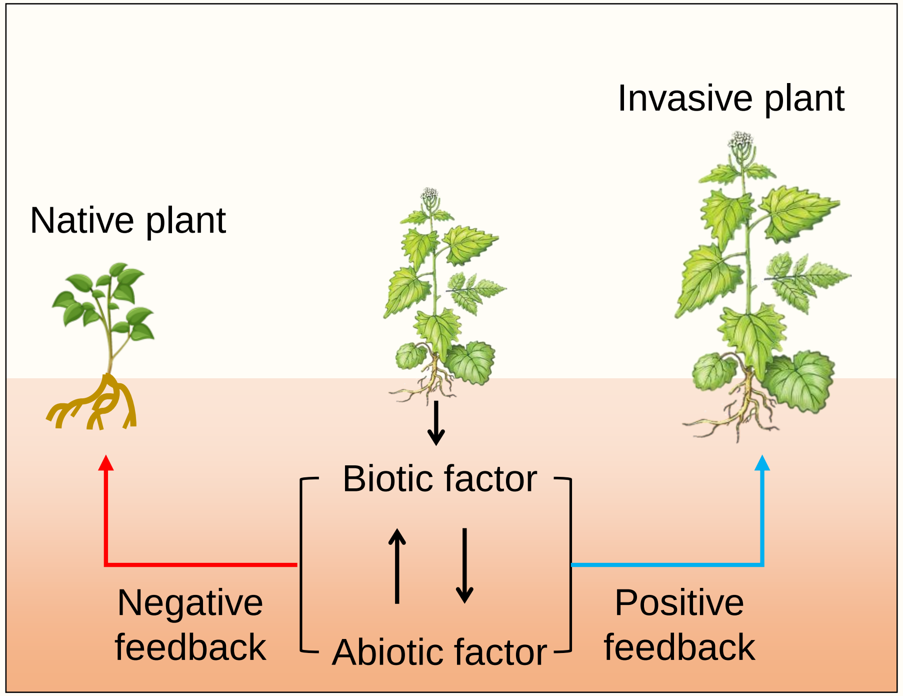
Garlic mustard (*Alliaria petiolata*) Invasion Reshapes Soil Microbial Communities and Network Structures in South Korea

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

2025.07.20

Evolutionary Ecology Laboratory
Gwangju Institute of Science and Technology (GIST)

Invasive Plants and Plant-Soil Feedback



Mechanism of invasion

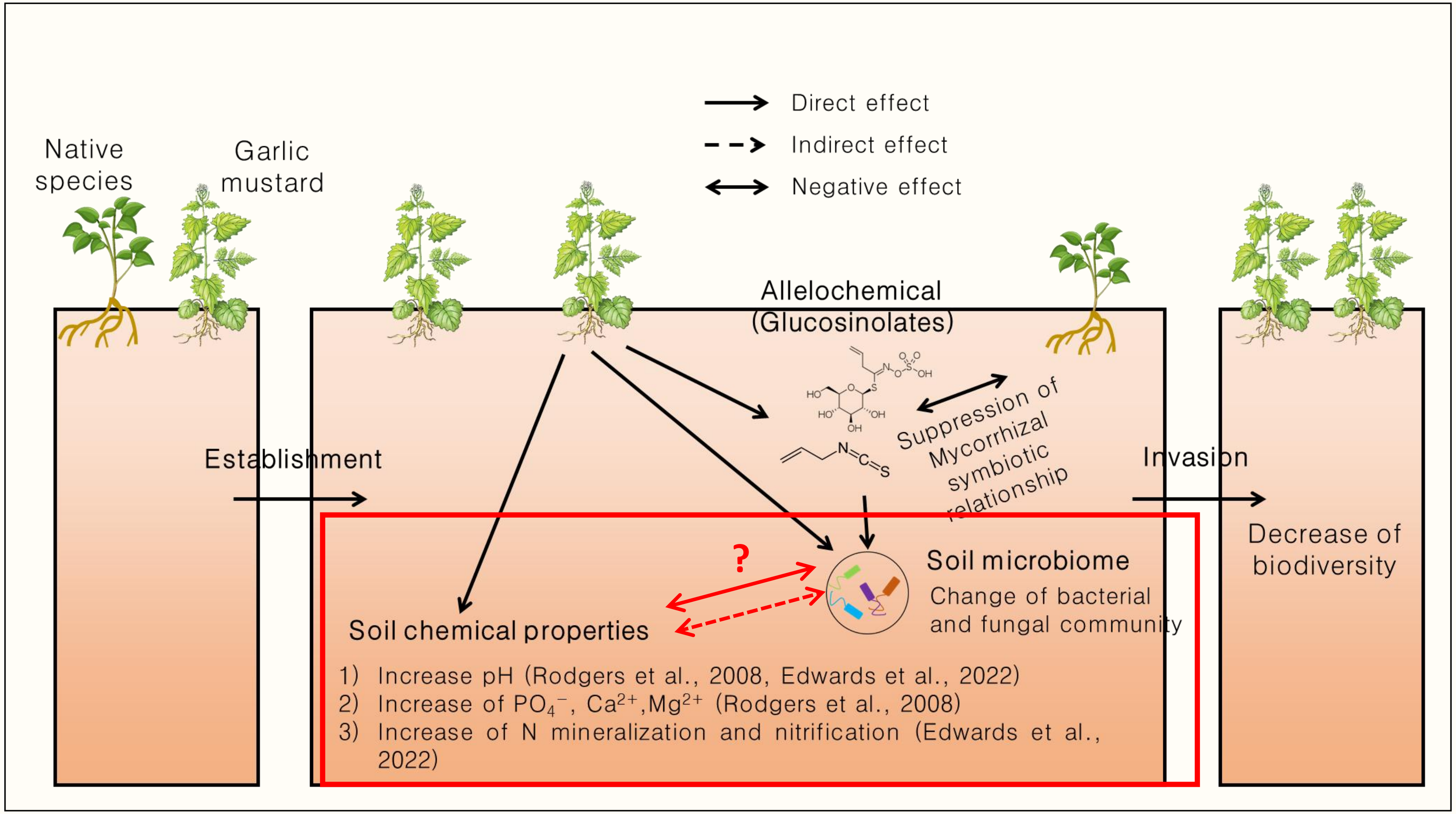
- Niche
- Competitive ability

Successful spread and invasion

Target Species

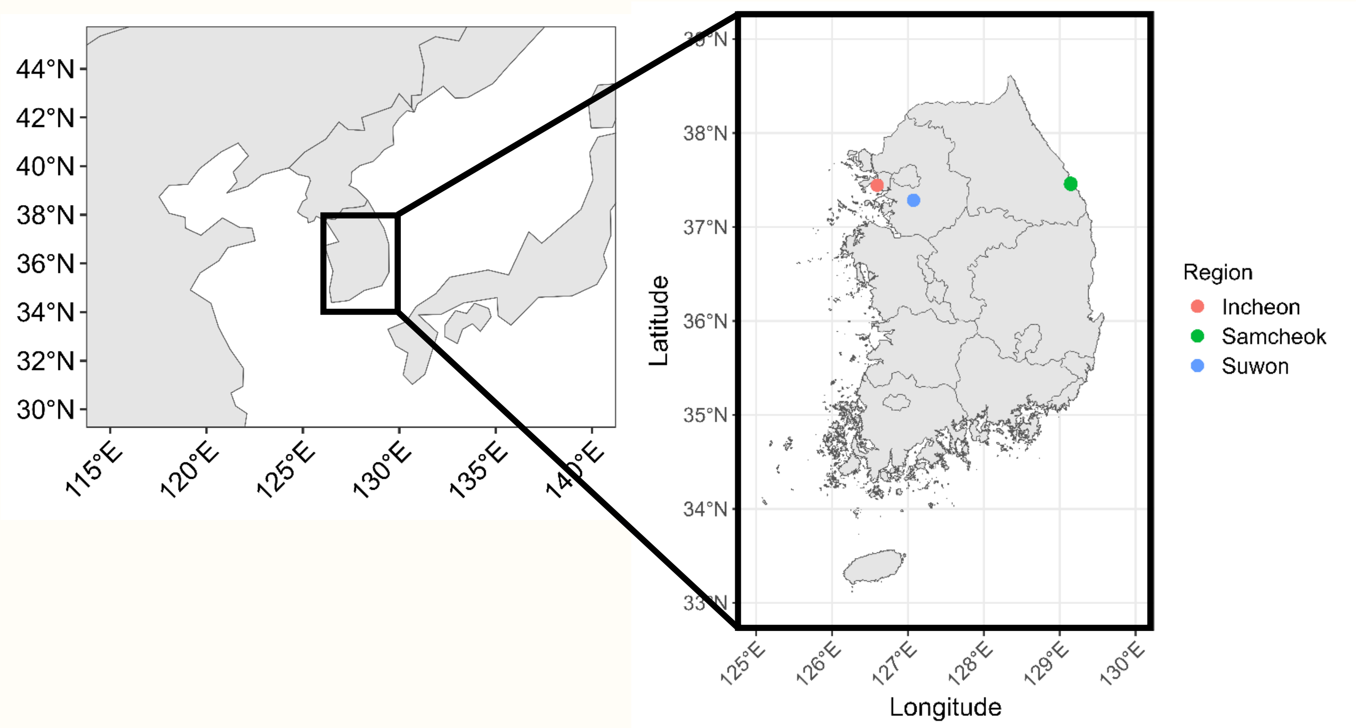
Garlic mustard (*Alliaria petiolata*)

- 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).

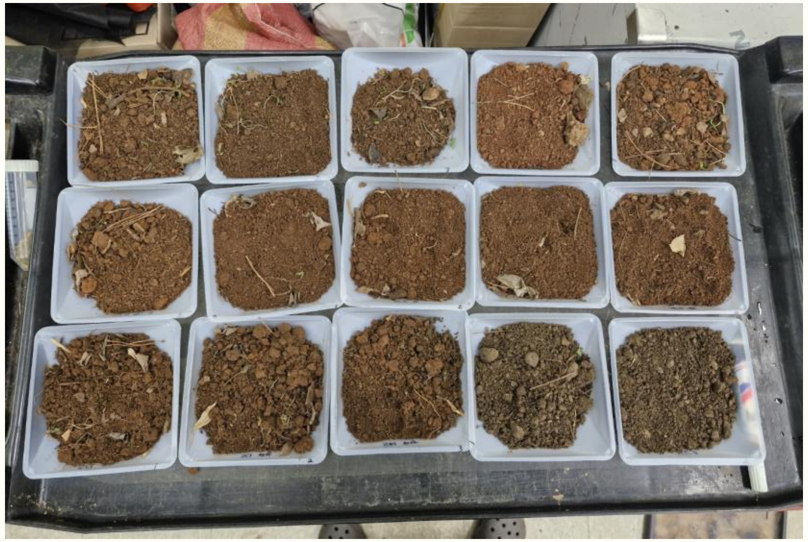


1) Increase pH (Rodgers et al., 2008, Edwards et al., 2022)
2) Increase of PO_4^-, Ca^{2+}, Mg^{2+} (Rodgers et al., 2008)
3) Increase of N mineralization and nitrification (Edwards et al., 2022)

Experimental Sites and Sampling



- Period : June, 2024
- Region : IC, SC, SW
- Site : IC (1), SC (4), SW (3)
- Treatment : Invasion vs. Control
- Establish five 1m x 1m plots in each treatment
- Collect soil samples at a depth of 0–10 cm in each plot
- Total 80 bulk soil samples
- Remove roots using a 2-mm sieve



Measurement of Soil Chemical Properties

- 5 g of each soil sample with 25 mL of sterilized distilled water
- After an incubation for 30 min in a shaking incubator at 25°C, 180 rpm
- K^+, Ca^{2+}, Mg^{2+}, NH_4^+, PO_4^{3-}, NO_3^-, Cl^-, SO_4^{2-}, pH

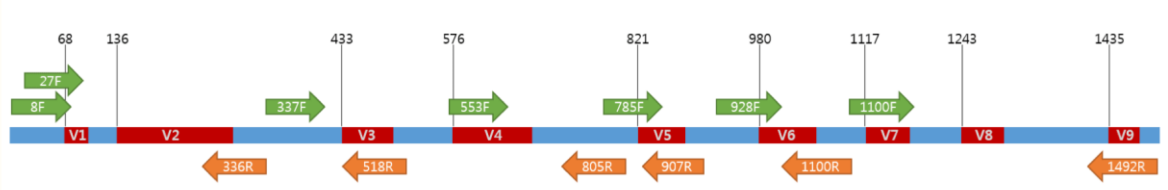
DNA Extraction and Amplicon Sequencing

DNA extraction

- Using 250mg bulk soil in each treatment (Total 80 Samples)

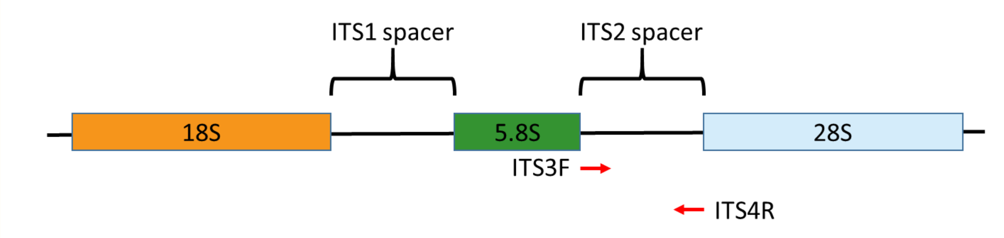
1) Nanopore sequencing for bacterial community

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

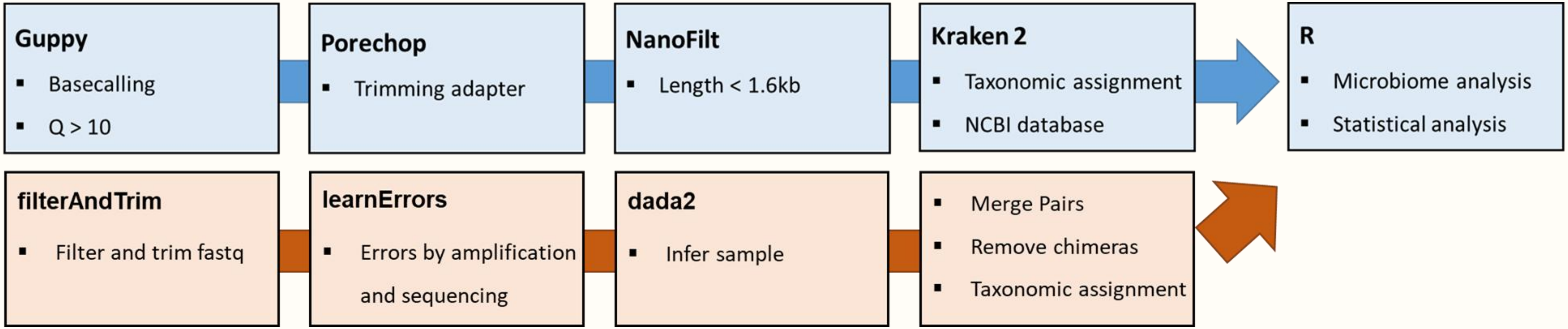


2) Illumina sequencing for fungal community

- ITS2 region (ITS3F-ITS4R)



Post-sequencing pipeline for each community



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

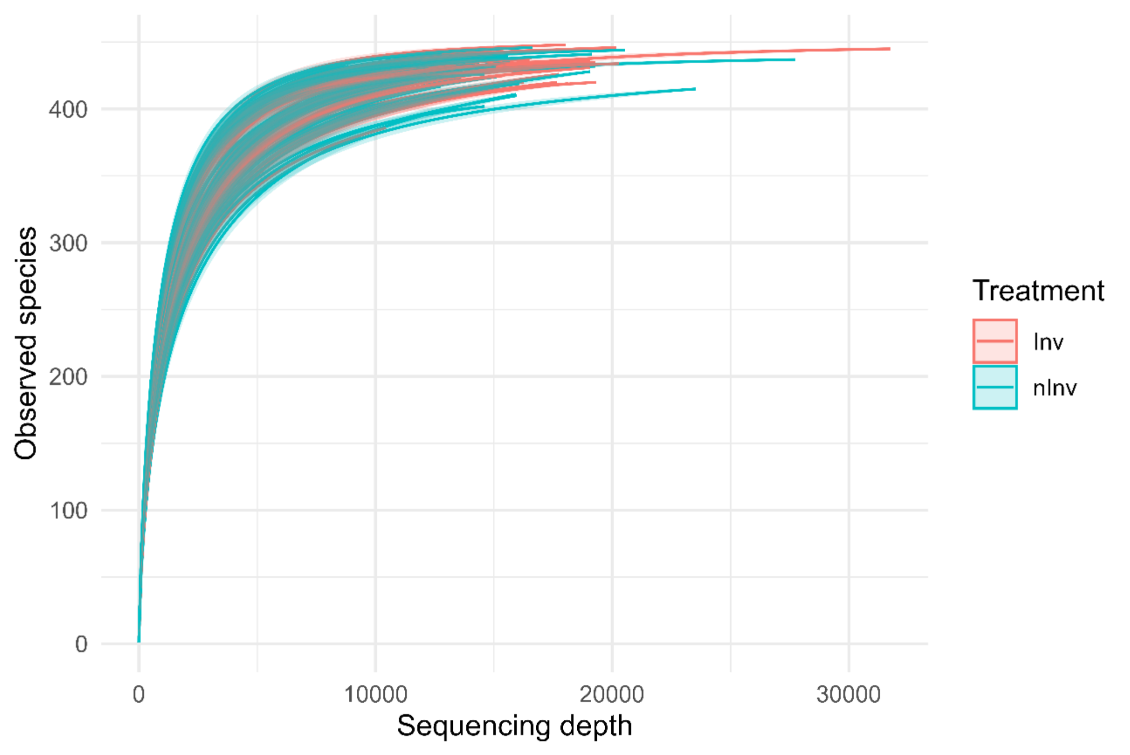
dada2
- Infer sample

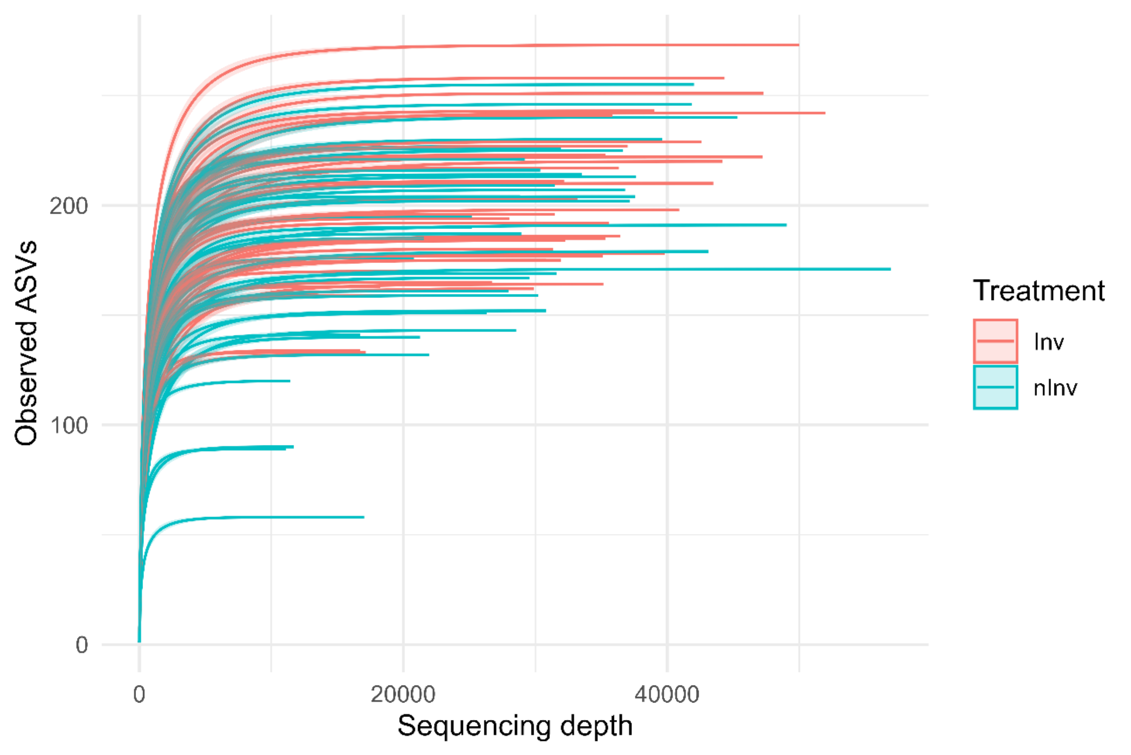
- Merge Pairs
- Remove chimeras
- Taxonomic assignment

Statistical Analysis

1) Bacteria : Species level; Fungi : ASV level
2) Bacterial species and fungal ASVs present with fewer than 10 reads in fewer than 10% of the total samples were excluded from downstream analyses.
3) Diversity analysis
4) Differential abundance analysis
5) Functional annotation
6) Network analysis
7) Canonical correspondence analysis (CCA)
    - Using relative abundance of microbial community and z-score of soil properties
    - Using soil properties only VIF < 10
    - Forward selection
8) Linear regression
    - Per region (SC, SW, IC), compute Bray–Curtis dissimilarity for microbial community
    - Compute Euclidean distance for each soil property
    - Select only invaded vs. non-invaded sample pairs

Rarefaction Curve of Microbial Community



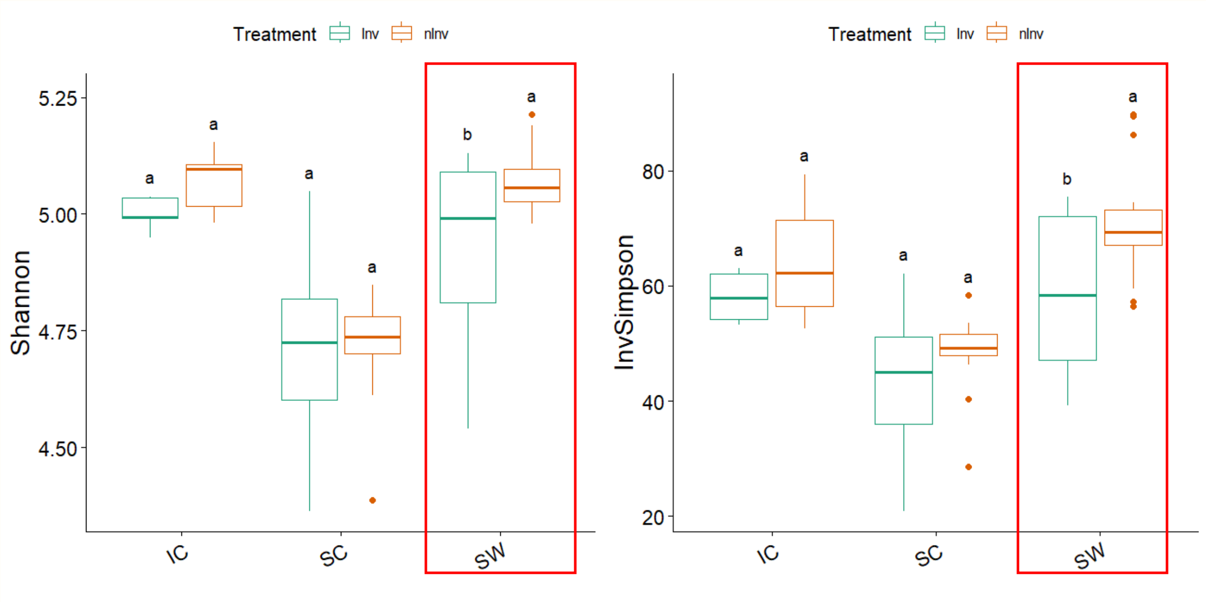


- Curves plateau beyond ~10 K reads in all samples, confirming adequate sampling depth after filtering.

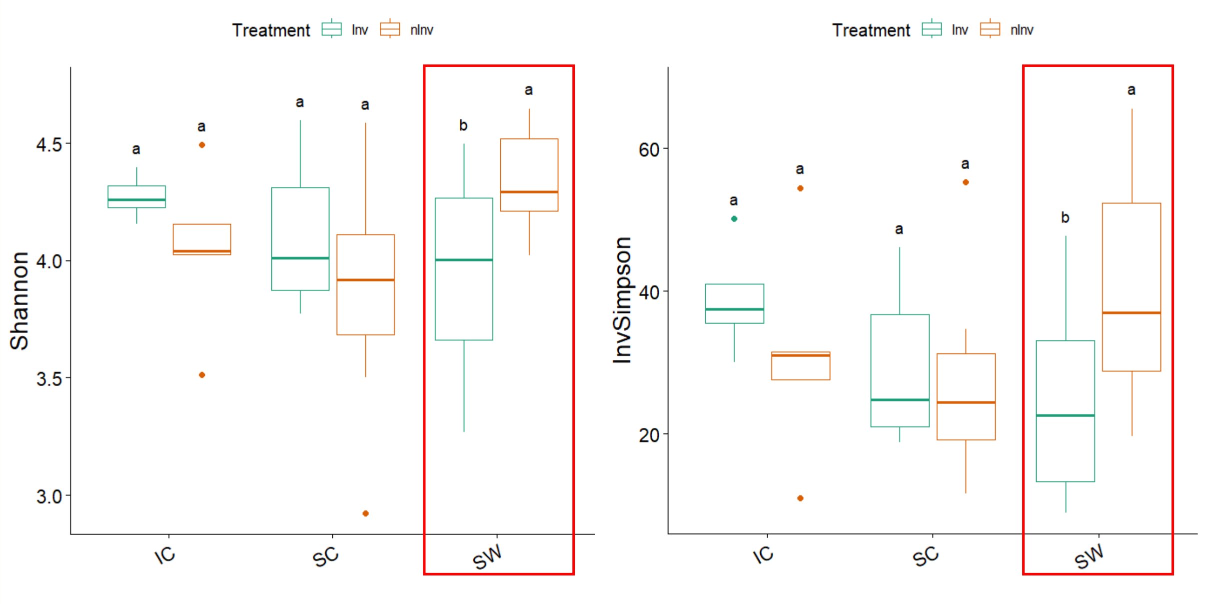
	Raw	Removal Singleton	Additional Filter
Bacteria	2,589 Species	2,443 Species	450 Species
	1,371,083 Reads	1,370,937 Reads	1,257,039 Reads
Fungi	14,721 ASVs	14,717 ASVs	810 ASVs
	3,833,775 Reads	3,883,771 Reads	2,574,949 Reads

Alpha diversity of Microbial Community

Bacteria

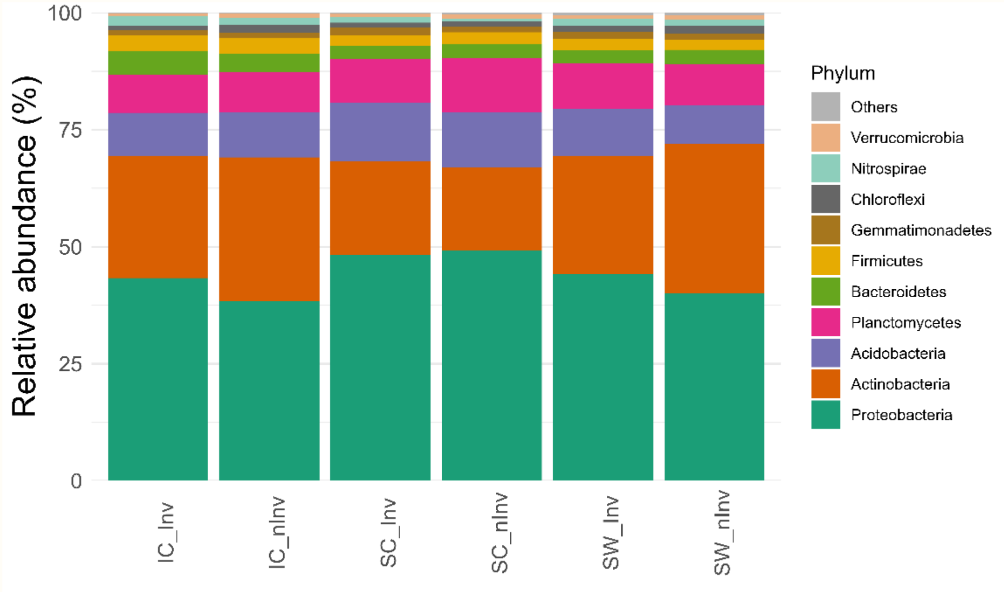


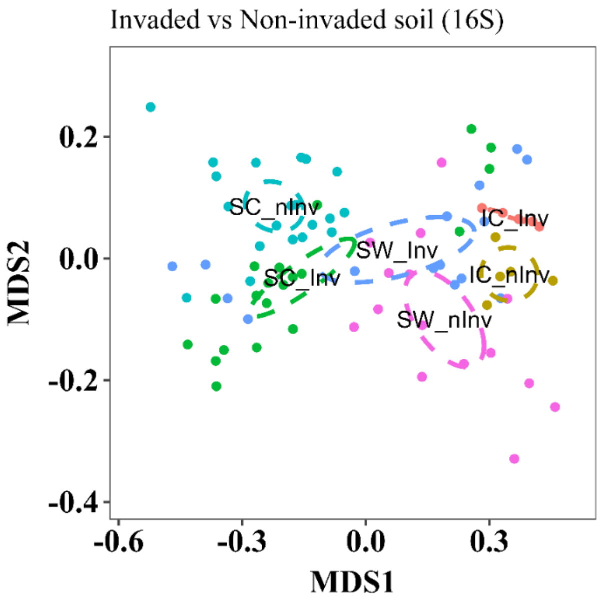
Fungi

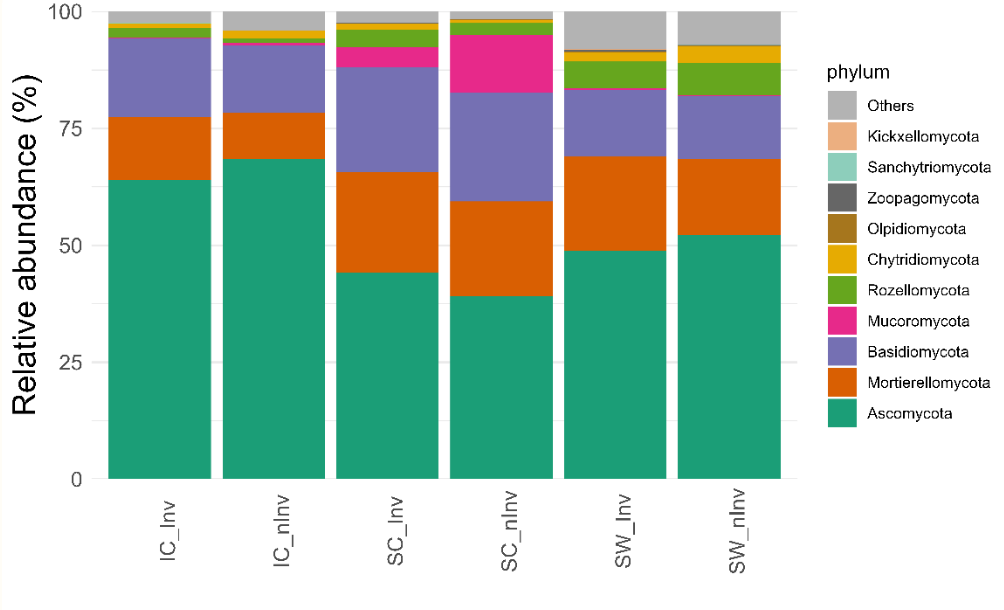


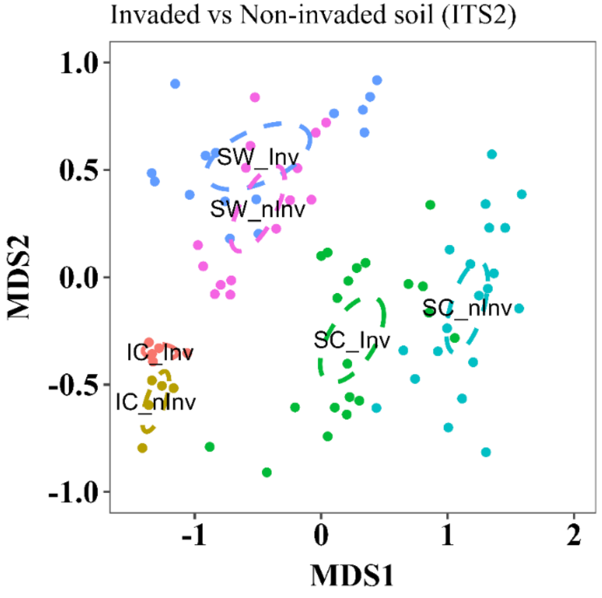
- In SW, alpha diversity of microbial community was significantly lower in the invaded soils than in the non-invaded soils.
- In IC and SC, there was no difference between invaded and non-invaded soils in alpha diversity.

Beta diversity of Microbial Community





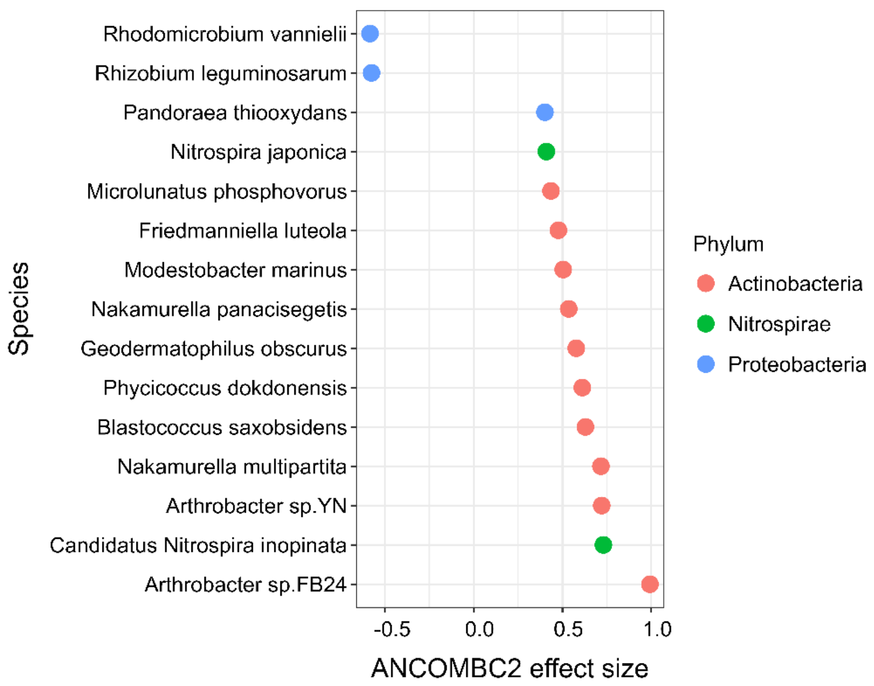




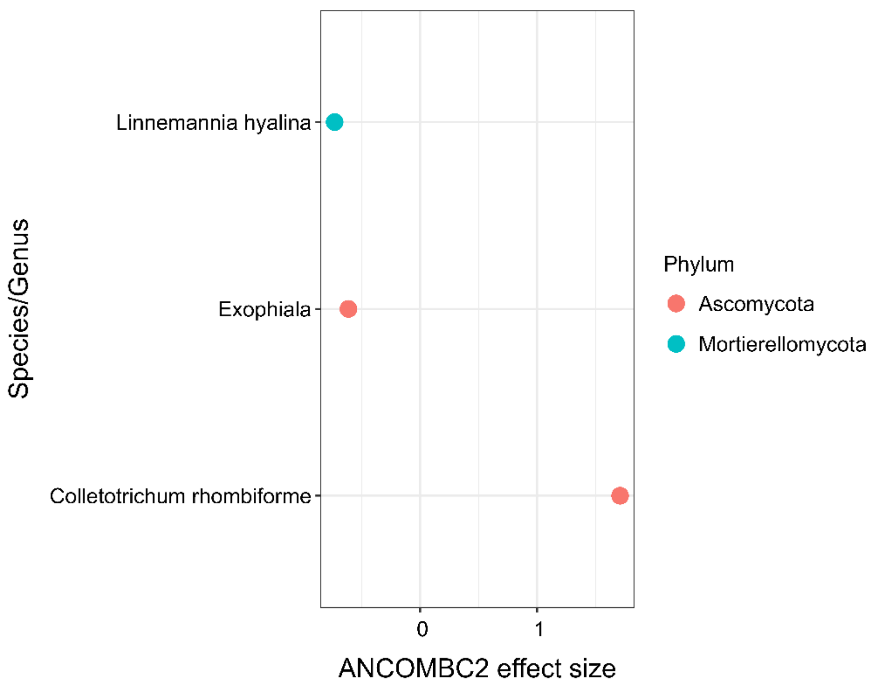
- Similar with alpha diversity, there were differences between regions.
- Differences in microbial communities between treatments were identified in both bacterial and fungal communities.
- These shifts suggest that garlic mustard invasion alters belowground microbial communities.

Identification of Differential Abundant Taxa after Garlic mustard Invasion

Bacterial community

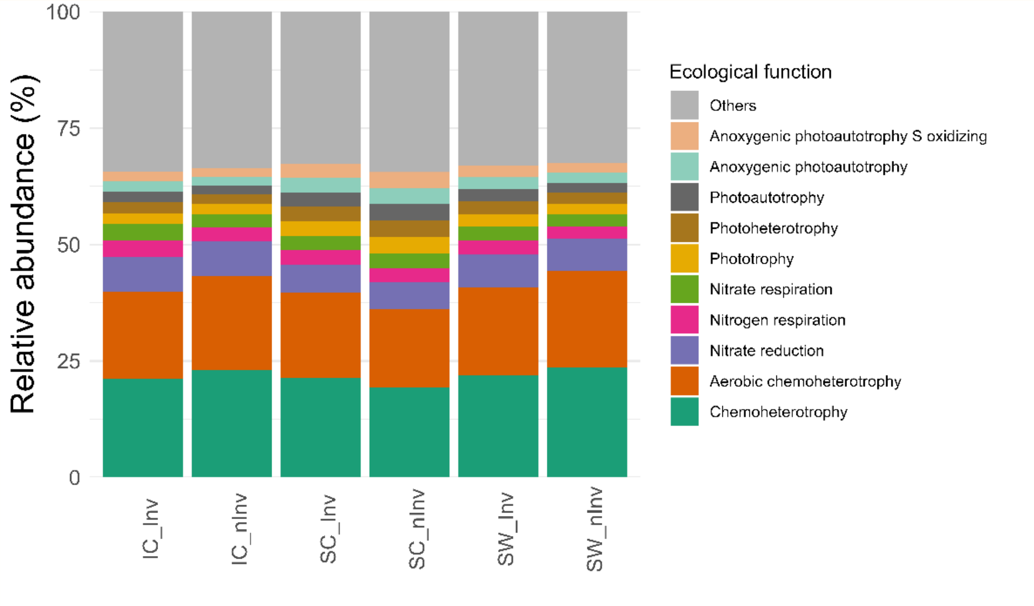


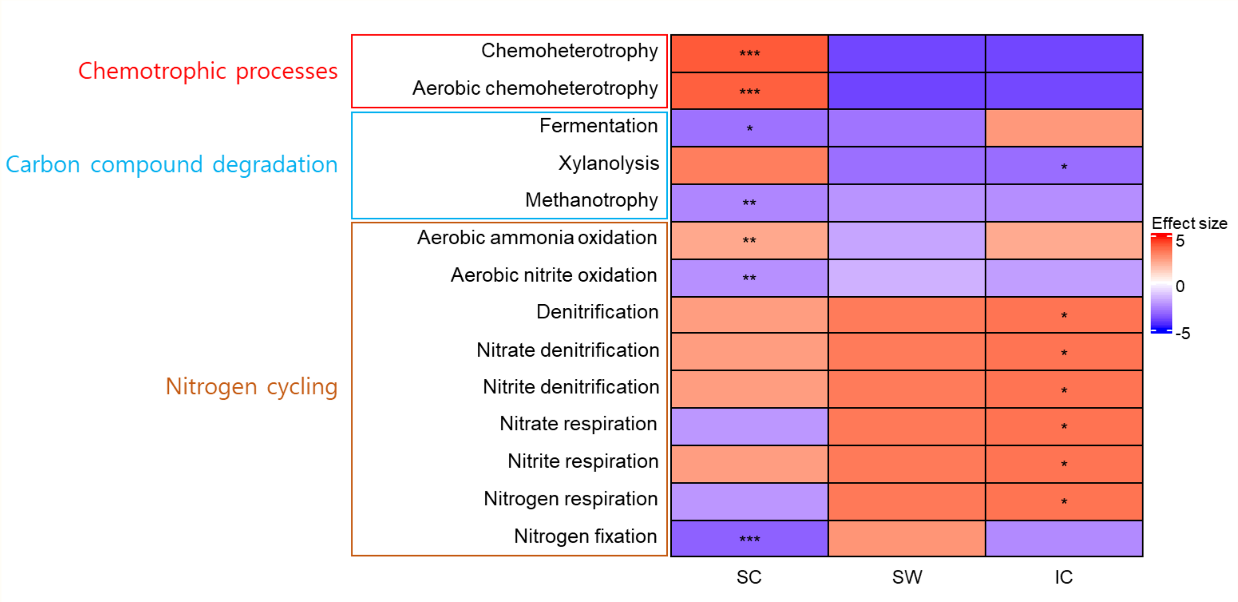
Fungal community



- Only in SC, a total of 15 bacterial species and 3 fungal ASVs showed differences due to garlic mustard invasion.
- Most of bacterial species tend to increase after invasion.
- In particular, bacterial species corresponding to Actinobacteria and Nitrospirae showed a tendency to increase after invasion.
- Same with bacteria, in SC, *Collectotrichum rhombiforme* are increased after invasion.

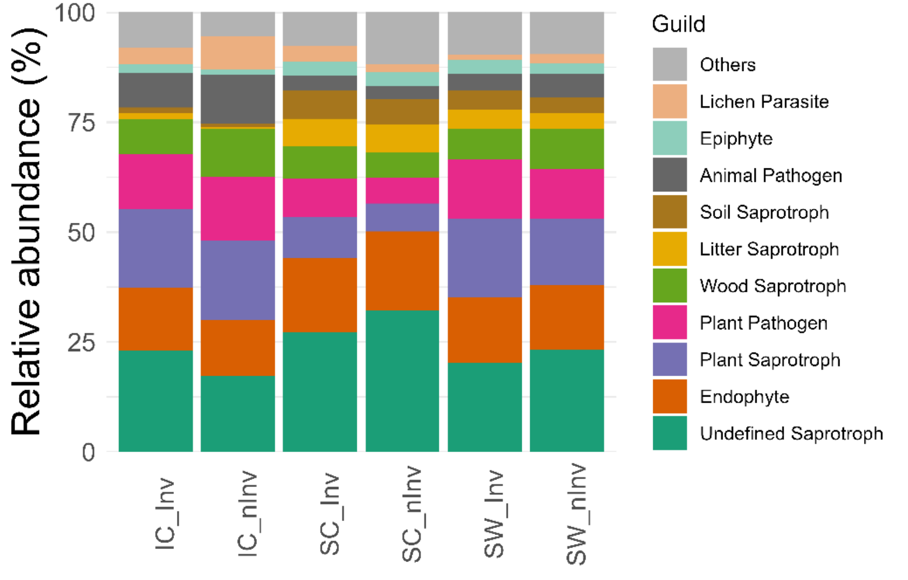
LEfSe-Based Biomarker Discovery for Functional Groups (Bacteria)

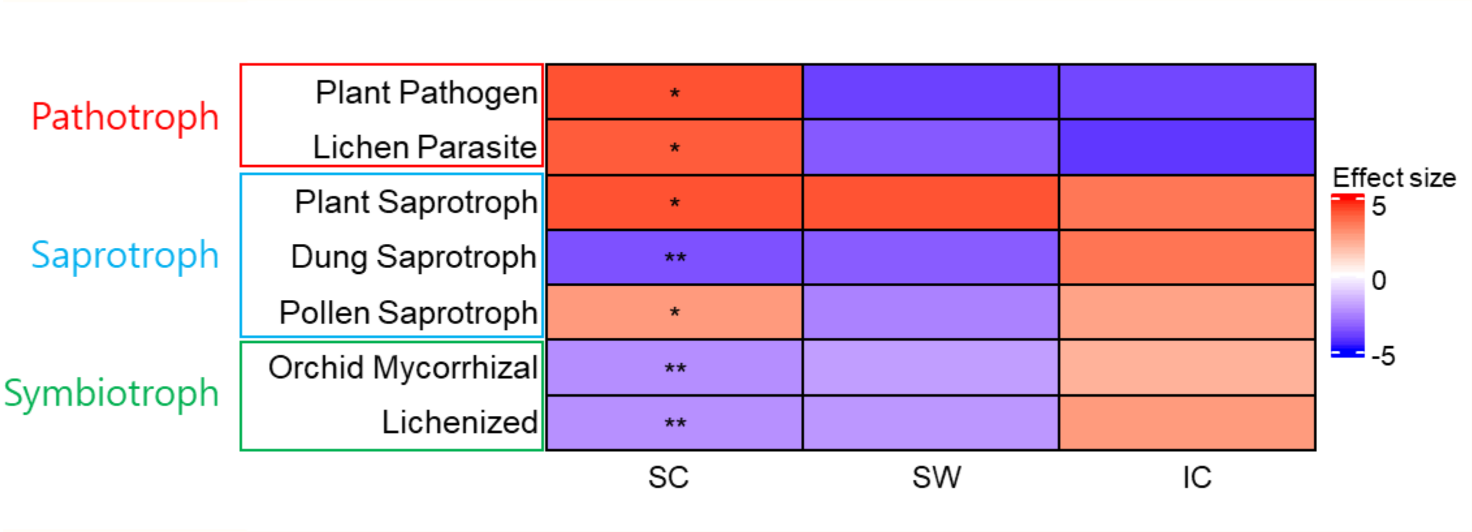




- 280 of 450 bacterial species (62 %) were assigned to at least one function.
- A total of 68 groups were derived from FAPROTAX.
- In SC, invasion significantly enriched chemoheterotrophy (and aerobic chemoheterotrophy), while it significantly suppressed nitrogen fixation.
- In IC, invasion significantly enriched nitrogen cycling steps.
- The results indicate functional restructuring of soil microbial communities following invasion, especially in carbon and nitrogen cycling.

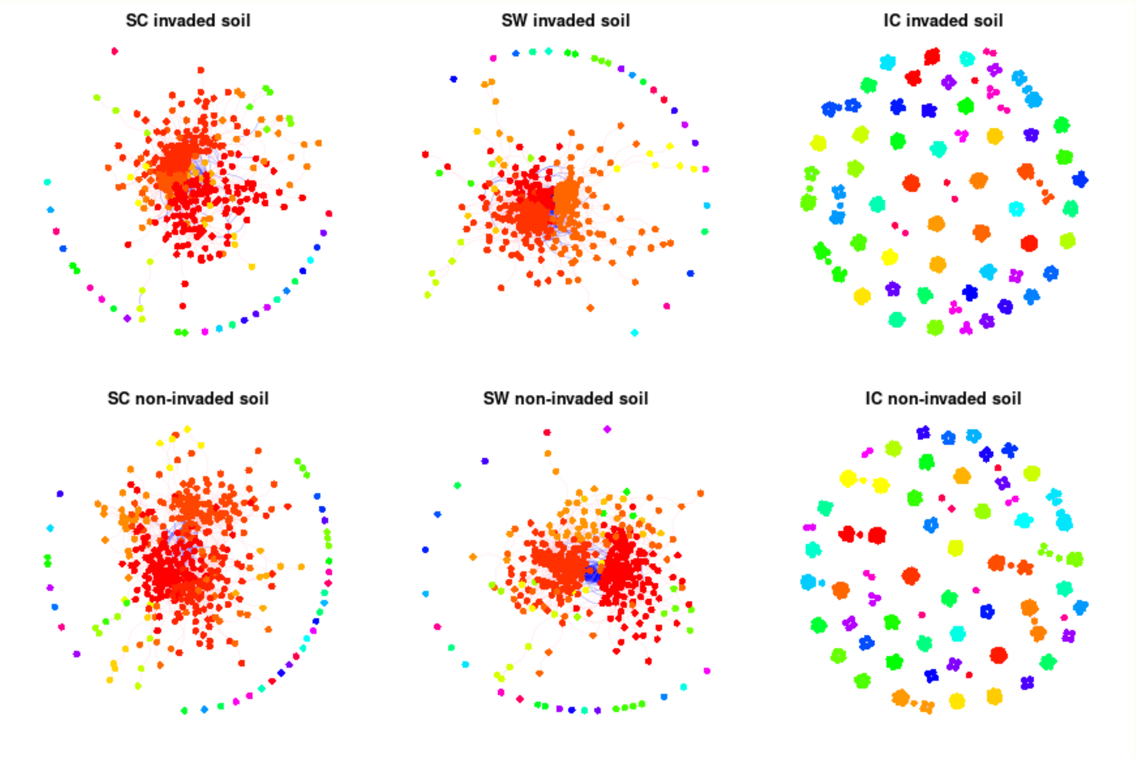
LEfSe-Based Biomarker Discovery for Functional Groups (Fungi)





- 586 of 810 ASVs (72 %) were assigned to at least one guild.
- A total of 29 guilds were derived from FUNGuild.
- In SC, invasion significantly increased plant-pathogen, while decreasing symbiotic guilds (orchid mycorrhizal and lichenized).
- In SW and IC, no functional guild changed significantly.
- These results indicate that garlic mustard invasion reduces beneficial fungal guilds while promoting plant pathogens—particularly in the SC region.

Co-occurrence Network Analysis of Bacterial Community



- Invaded soils in SC and SW showed:

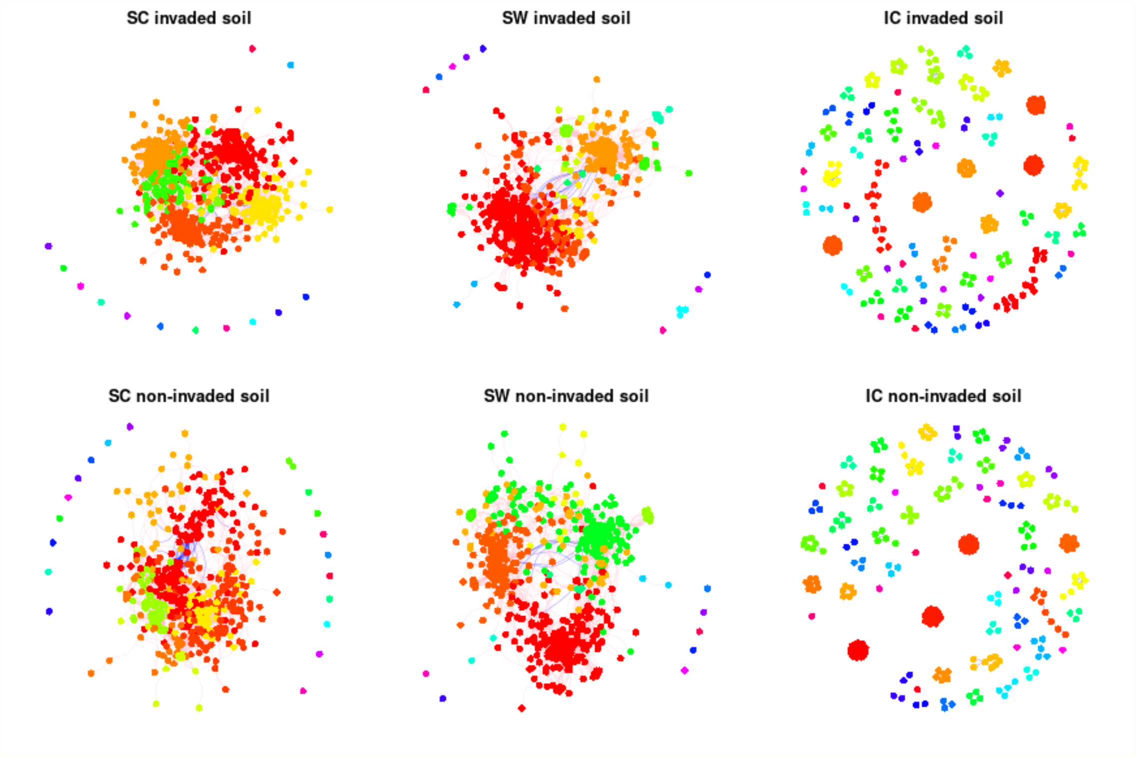
  ↑ : Clustering, average degree, edge density

  ↓ : Path length, modularity

  More connected but less compartmentalized networks
- IC's invaded and non-invaded soils had nearly identical network properties, indicating minimal structural impact there.
- In SC and SW, garlic mustard invasion led to more uniform microbial networks, potentially reducing community complexity and functional balance.

Region	Treatment	Clustering coefficient	Clustering coefficient of random networks	Modularity	Positive ratio	Average degree	Average path length	Normalized degree	Normalized betweenness	Edge density	Number cluster	Diameter
SC	Inv	0.50	0.057	0.29	0.89	26.0	3.1	0.06	0.0042	0.058	26	11
SC	nInv	0.37	0.024	0.46	0.98	10.7	3.6	0.02	0.0049	0.024	33	10
SW	Inv	0.50	0.087	0.36	0.90	38.7	2.8	0.09	0.0035	0.086	22	11
SW	nInv	0.49	0.044	0.46	0.96	19.7	3.3	0.04	0.0045	0.044	22	9
IC	Inv	0.98	0.012	0.97	0.54	6.9	1.3	0.02	0.0000	0.016	60	4
IC	nInv	0.97	0.018	0.96	0.56	7.6	1.6	0.02	0.0000	0.017	59	4

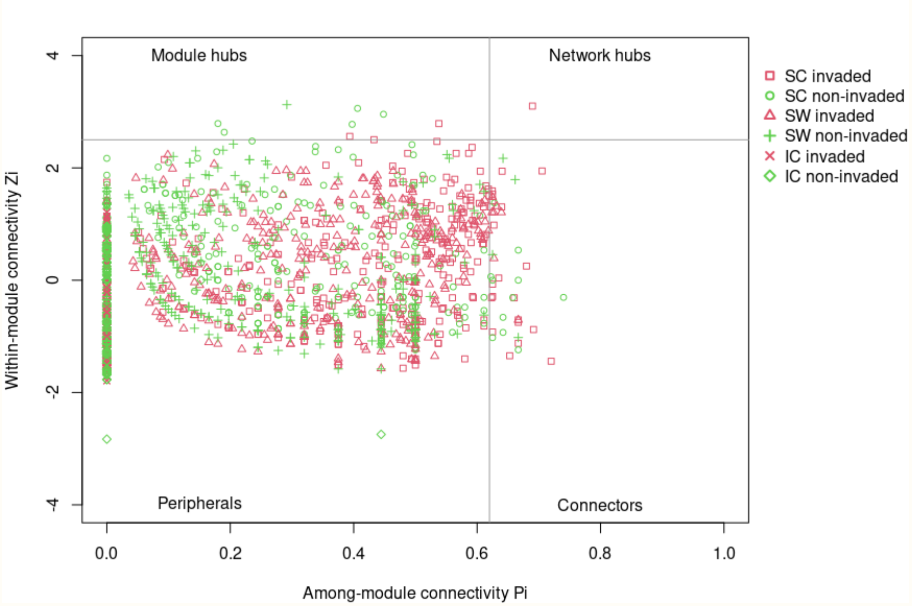
Co-occurrence Network Analysis of Fungal Community



- Invaded soils in SC showed:
  - ↑ : Modularity, average degree
  - ↓ : Clustering
  - <u>Enhancing functional compartmentalization</u>
- Invaded soils in SW showed:
  - ↑ : Clustering, average degree
  - ↓ : Modularity
  - <u>Promoting homogenized microbial interactions</u>
- The IC network appears fragmented, with small, tightly connected clusters but low overall connectivity.

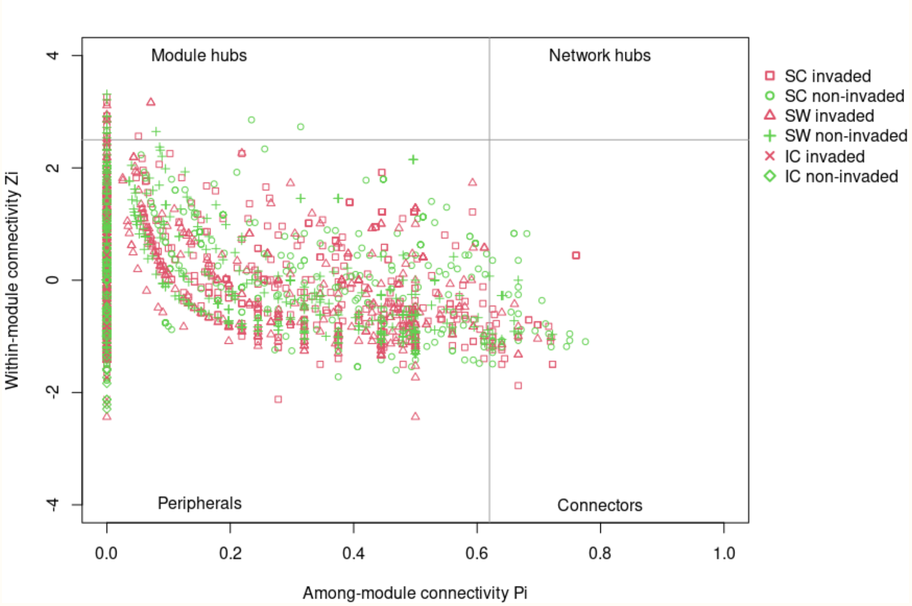
Region	Treatment	Clustering coefficient	Clustering coefficient of random networks	Modularity	Positive ratio	Average degree	Average path length	Normalized degree	Normalized betweenness	Edge density	Number cluster	Diameter
SC	Inv	0.46	0.026	0.62	1.00	17.8	3.7	0.03	0.0039	0.026	14	9
SC	nInv	0.59	0.032	0.59	0.99	16.1	3.7	0.03	0.0048	0.030	20	9
SW	Inv	0.55	0.035	0.55	1.00	19.7	3.6	0.03	0.0042	0.033	10	9
SW	nInv	0.52	0.027	0.61	1.00	17.0	3.9	0.03	0.0048	0.028	8	9
IC	Inv	0.98	0.022	0.92	0.90	5.5	1.6	0.02	0.0001	0.019	72	8
IC	nInv	1.00	0.031	0.82	0.97	8.2	1.1	0.03	0.0000	0.028	64	5

Keystone Roles in Microbial Co-occurrence Networks



Region	Treatment	Network hub	Module hub	Connector	Peripheral
SC	Inv	1	3	21	425
SC	nInv	0	5	11	434
SW	Inv	0	0	7	443
SW	nInv	0	1	4	445
IC	Inv	0	0	0	449
IC	nInv	0	0	0	447

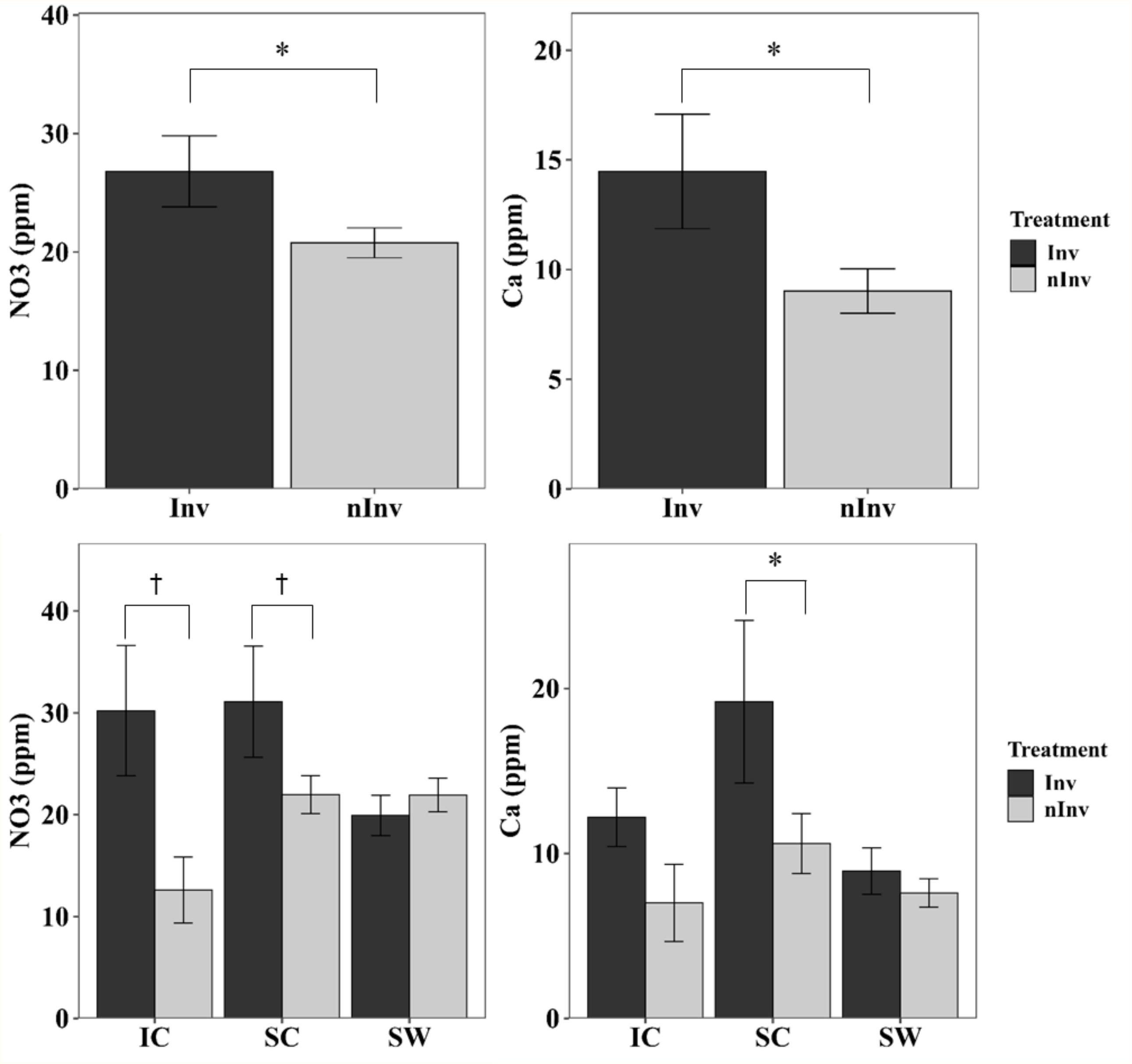
Pi	Zi	Species	Role	Region	Treatment
0.69	3.10	*Niabella ginsenosidivorans*	Network hub	SC	Inv
0.54	2.79	*Candidatus Koribacter versatilis*	Module hub	SC	Inv
0.39	2.56	*Agromyces aureus*	Module hub	SC	Inv
0.43	2.50	*Ottowia sp.oral taxon 894*	Module hub	SC	Inv



Region	Treatment	Network hub	Module hub	Connector	Peripheral
SC	Inv	0	9	32	632
SC	nInv	0	4	32	494
SW	Inv	0	7	9	582
SW	nInv	0	10	15	582
IC	Inv	0	1	0	297
IC	nInv	0	0	0	291

Pi	Zi	ASV	Role	Region	Treatment
0	2.93	*Linnemannia amoeboidea*	Module hub	SC	Inv
0	2.55	*Podila humilis*	Module hub	SC	Inv
0.07	3.16	*Leptodiscella africana*	Module hub	SW	Inv
0	2.83	*Hymenoscyphus imberbis*	Module hub	IC	Inv

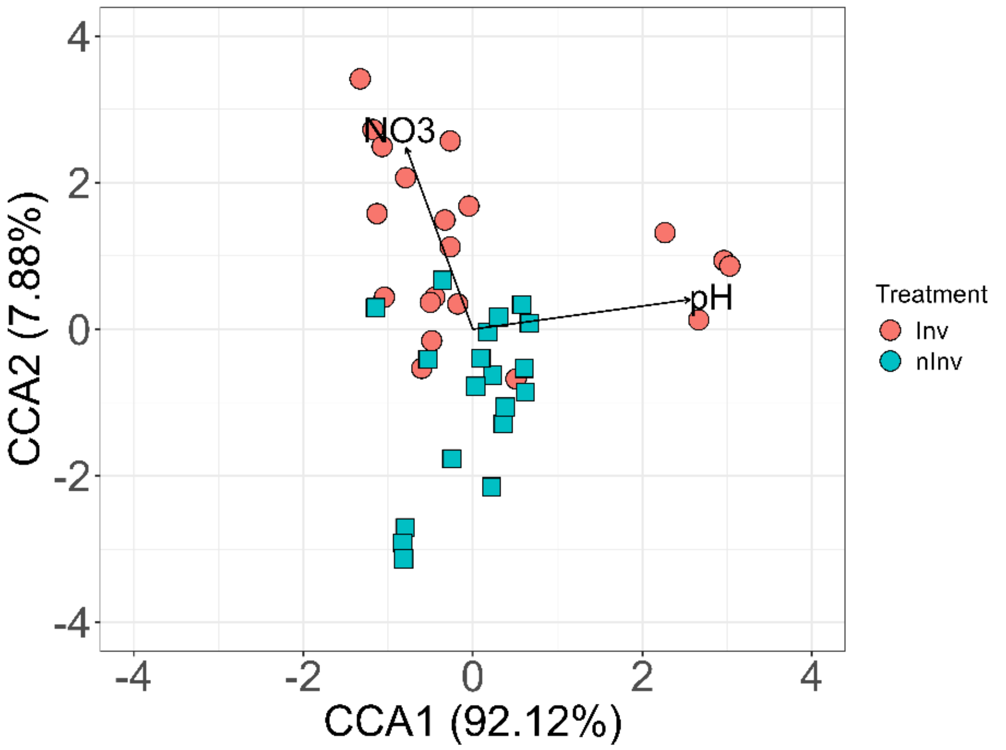
Concentration of Soil Properties



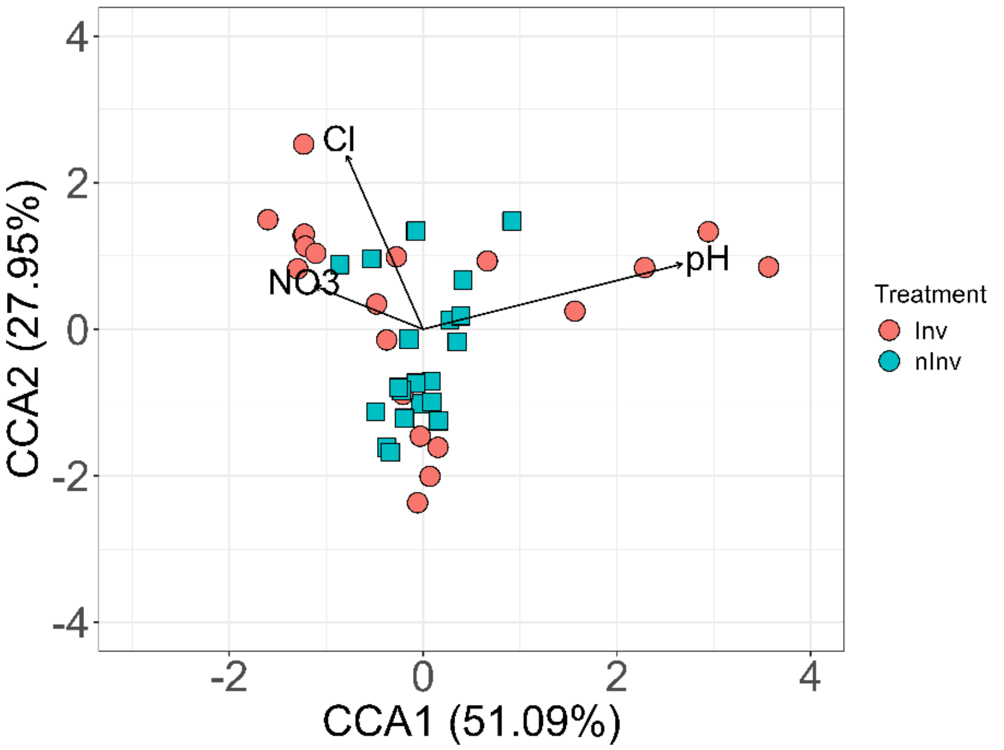
- <u>Nitrate (NO_3^-) and calcium ion (Ca^{2+}) levels were higher in invaded soils</u> compared to non-invaded soils.
- By region, nitrate marginally increased in IC and SC, while calcium ion showed a significant increase in SC.

Correlation of Microbial Community and Soil Properties

Bacterial community (SC)

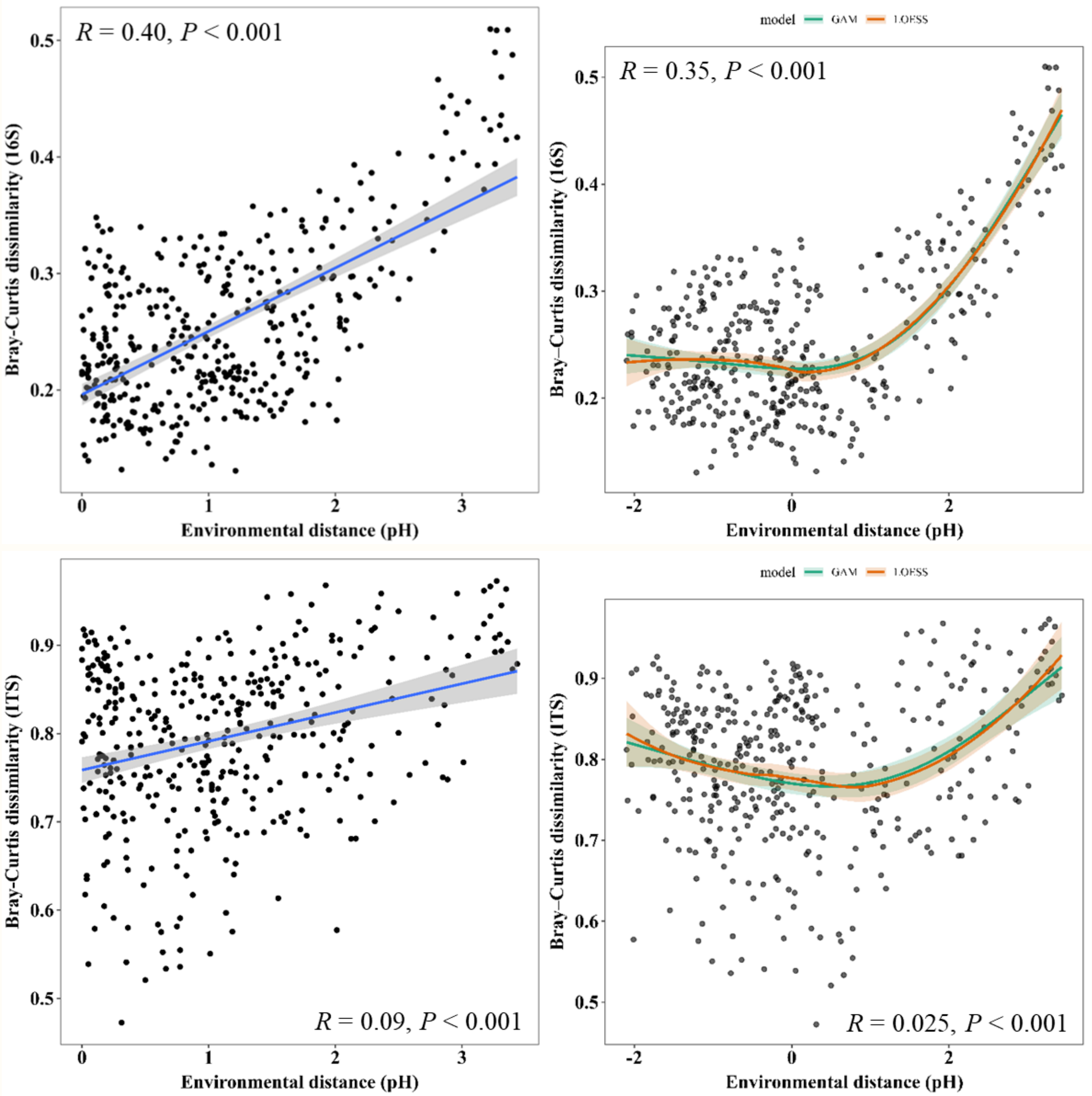


Fungal community (SC)



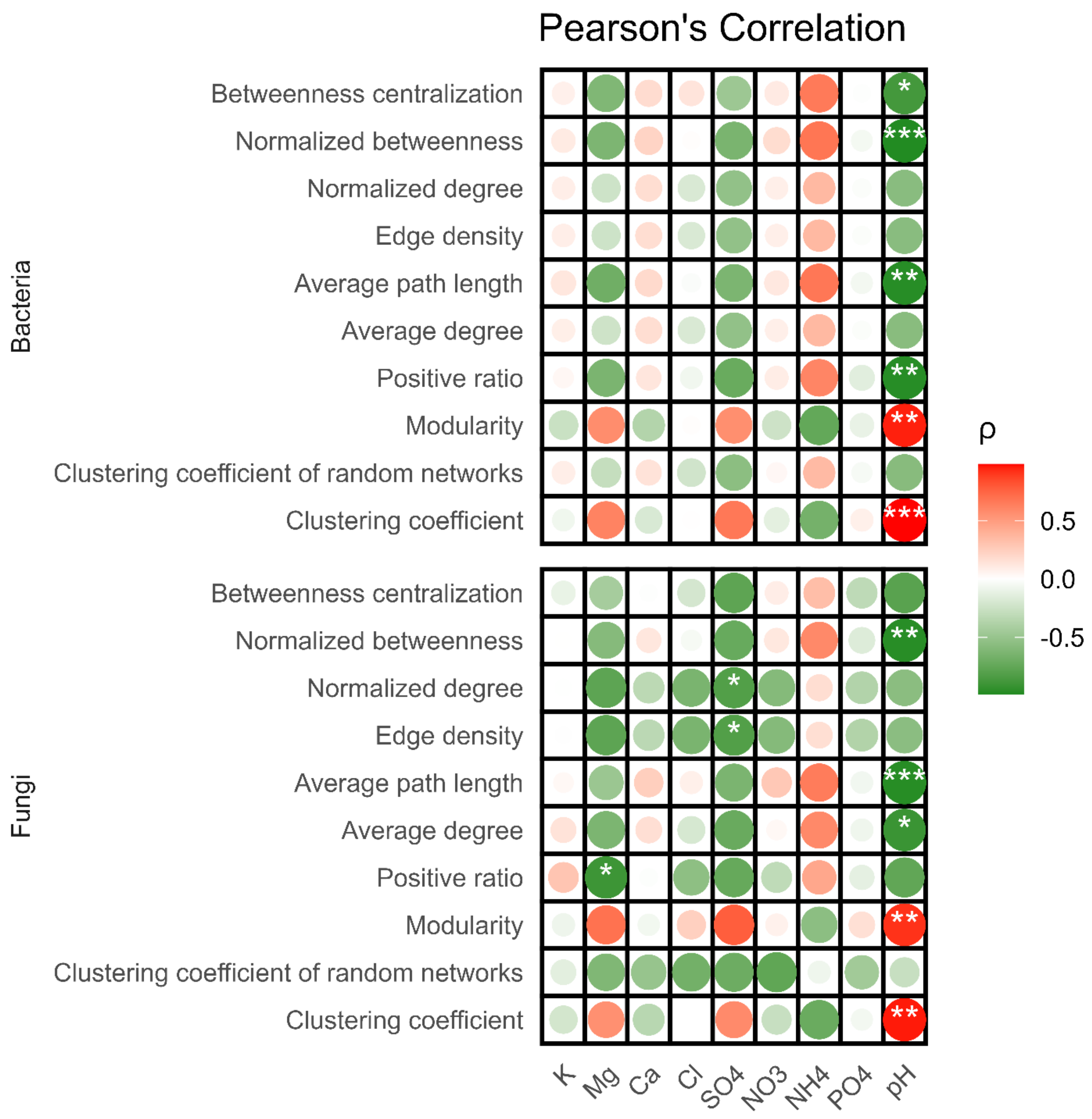
- <u>Only in SC, there was a significant correlation between soil properties and microbial communities</u>.
- Environmental variables like <u>pH and nitrate</u> strongly shaped bacterial communities, especially in invaded soils.
- Fungal communities responded more weakly, with less clear separation along environmental gradients.
- This suggests that <u>invasion-related soil changes may influence microbial communities more strongly in SC</u>, but not in SW or IC.

Correlation of Microbial Community and pH in SC



- In SC, bray-Curtis dissimilarity increased linearly with the absolute pH difference in both bacteria and fungi.
- When directionality was considered, bacterial communities showed stronger compositional shifts under pH increase.
- Fungal communities exhibited weaker and more variable responses regardless of pH change direction.
- This suggests that bacterial communities are more sensitive to pH shifts and likely structured by environmental filtering under higher pH.

Correlation of Network Characters and Soil Properties



- There were consistent correlations between pH and multiple network characteristics in both bacteria and fungi.
- Higher pH was associated with:
  - ↑ Modularity, clustering coefficient
  - ↓ Average path length, normalized betweenness

Conclusion

- Garlic mustard invasion reshaped soil microbial communities and network structures, with regional variation matching invasion history.
- SC, where the invasion is most established, showed the strongest changes across diversity, taxa, functions, networks, and soil properties.
- SW exhibited moderate shifts, while IC, the most recent invasion site, showed little to no response.
- These patterns suggest a cumulative impact of invasion over time, especially in altering microbial networks.
- Soil pH emerged as a key environmental driver influencing network structure regardless of invasion status.

Thank you for listening

Q&A