

Supplemental Figures and Tables

Management and rhizosphere microbial associations modulate genetic-driven nitrogen fate

Mitra Ghotbi ^{1,2*}, Marjan Ghotbi ³, Yakov Kuzyakov ^{4,5}, William R. Horwath ⁶

¹ Department of Natural Resources & Environmental Sciences, University of Illinois at Urbana-Champaign, IL, USA

² Department of Biology, Middle Tennessee State University at Murfreesboro, TN, USA

³ GEOMAR Helmholtz Centre for Ocean Research at Kiel, Germany

⁴ Department of Soil Science of Temperate Ecosystems and Department of Agricultural Soil Science, Georg-August-University of Göttingen at Göttingen, Germany

⁵ Peoples Friendship University of Russia (RUDN University), 117198 Moscow, Russia

⁶ Plant and Environmental Sciences Building, Department of Land, Air & Water Resources, University of California Davis, at Davis, CA, USA

*Corresponding author: Dr. Mitra Ghotbi, Department of Biology, Middle Tennessee State University at Murfreesboro, MTSU Box 0060, SCI 2141, TN, USA. Primary Phone: (615) 898-5307, Cellphone: +1 313 707 6077, mitra.ghotbi@mtsu.edu

ORCID ID 0000-0001-9185-9993

Table S1 Monthly total precipitation and average temperature values during the 2021-2022 farming seasons at Champaign, IL (40° 3'6.61"N, 88°13'52.01"W) and the 30-year average of precipitation and temperature of the region obtained from the Illinois Water Survey.

	Precipitation (inches)			Temperature (°F)			
Month	2021	2022	30-Year Average	2021	2022	30-Year Average	
April	2.1	3.2	4	53	50	53	
May	3.4	3.2	5	61	66	63	
June	7.6	0.8	4.7	75	75	72	
July	4.3	2.4	4.4	74	76	75	
August	4.1	4.9	3.5	76	73	74	
September	3	4.6	3.3	70	67	67	
Total/Average	24.5	19.1	24.9	68	68	67	
Pre-plant soil properties (0-12” depth) and Mehlich 3-extraction-based mineral test results at Champaign, IL							
Soil edaphic properties	[†] OM	^{††} CEC	pH	NO ₃	NH ₄	P	K
Units	%	meq/100g		ppm			
	3.1	17.1	6.8	5.2	2.9	20	104

[†] OM=organic matter; ^{††}CEC= cation exchange capacity

TABLE S2 Individual and interaction effects of genotype, inoculant, mineral N amendment, and GDD on soil prokaryotic richness and alpha diversity indices, evaluated by linear model. F-values are given, and significance is indicated by asterisks

Factors (2021)	Observed	Chao1	Simpson	Shannon
[∞] Genotype	3.02	3.02	0.63	4.10*
^α Inoculant	0.69	0.69	1.41	1.77
^ε N dosage	4.34*	4.34*	1.23	1.39
[¥] Growth stage	20.70***	20.70***	1.09	15.68***
Genotype:Inoculant	0.05	0.05	0.78	0.08
Genotype:N dosage	0.10	0.10	0.29	0.05
Inoculant:N dosage	0.01	0.01	1.03	0.01
Genotype: Growth stage	0.15	0.15	0.56	0.03
Inoculants: Growth stage	1.07	1.07	0.97	0.78
N dosages: Growth stage	1.30	1.30	0.77	1.26
Genotype:Inoculant: Growth stage	0.16	0.16	1.22	0.67
Genotype:N dosages: Growth stage	0.03	0.03	1.10	0.24
Inoculant:N dosages: Growth stage	0.31	0.31	1.50	0.32

Factors (2022)	Observed	Chao1	Simpson	Shannon
Genotype	0.75	0.75	1.15	1.10
Inoculant	1.40	1.40	1.61	2.50
N dosage	2.05	2.05	2.31	3.69
Growth stage	2.57	2.57	0.36	0.38
Genotype:Inoculant	0.82	0.82	0.58	0.76
Genotype:N dosage	0.83	0.83	1.03	0.75
Inoculant:N dosage	1.39	1.39	0.09	0.32
Genotype: Growth stage	0.09	0.09	0.70	0.12
Inoculant: Growth stage	0.01	0.01	1.91	0.49
N dosage: Growth stage	3.59	3.59	0.18	1.73
Genotype:Inoculant:N dosage	2.10	2.10	2.30	2.90
Genotype:Inoculant: Growth stage	0.55	0.55	0.43	0.19
Genotype:N dosage: Growth stage	0.31	0.31	0.75	0.48
Inoculant:N dosage: Growth stage	8.21**	8.21**	2.26	5.56*
Genotype:Inoculant:N dosage: Growth stage	0.65	0.65	0.49	0.19

[∞]Genotype (B73, NIL 1, and NIL 2), ^αInoculant (Proven, None), ^εN dosage= (N:0 kg ha⁻¹, N:67 kg ha⁻¹), [¥] Growth stage= In 2021 maize vegetative growth stages of V5 and V12 and in 2022 the vegetative maize growth stages of V8 and VT (tasseling), were considered. NIL= near isogenic line.

Significance codes: P < 0.05 ‘*’, P < 0.01 ‘**’, P < 0.001 ‘***’

TABLE S3 Individual and interaction effects of genotype, inoculant, mineral N amendment, and GDD on soil Fungi richness and alpha diversity indices, evaluated by linear model. F-values are given, and significance is indicated by asterisks

Factors (2021)	Observed	Chao1	Simpson	Shannon
[∞] Genotype	0.21	0.21	0.61	0.26
^α Inoculant	0.14	0.14	0.11	0.01
^ε N dosage	2.28	2.28	5.37*	6.51*
[¥] Growth stage	10.64***	10.64***	1.67	5.88**
Genotype:Inoculant	0.34	0.34	0	1.16
Genotype:N dosage	5.64*	5.64*	0.45	1.89
Inoculant:N dosage	0.38	0.38	0.6	0.05
Genotype: Growth stage	0.85	0.85	0.48	0.9
Inoculants: Growth stage	1.48	1.48	0.26	0.67
N dosages: Growth stage	8.86***	8.86***	0.21	6.23***
Genotype:Inoculant: Growth stage	0.61	0.61	0.85	0.14
Genotype:N dosages: Growth stage	2.34	2.38	0.17	0.25
Inoculant:N dosages: Growth stage	0.09	0.09	1.06	0.41

Factors (2022)	Observed	Chao1	Simpson	Shannon
Genotype	0.69	0.69	1.22	0.29
Inoculant	0.1	0.1	1.36	0.2
N dosage	1.36	1.36	0.24	0.48
Growth stage	0.42**	0.42**	0.67	2.44
Genotype:Inoculant	0.3	0.3	1.31	1.26
Genotype:N dosage	1.07	1.07	0.24	0.29
Inoculant:N dosage	0.09	0.09	1.66	1.61
Genotype: Growth stage	0.05	0.05	1.7	0.79
Inoculant: Growth stage	1.57	1.57	4.07*	3.74
N dosage: Growth stage	3.59	3.59	0.5	0.8
Genotype:Inoculant:N dosage	0.45	0.45	0.17	0.05
Genotype:Inoculant: Growth stage	0.48	0.48	1.41	1.38
Genotype:N dosage: Growth stage	0.59	0.59	0.55	1.03
Inoculant:N dosage: Growth stage	1.57	1.57	0.72	0.04
Genotype:Inoculant:N dosage: Growth stage	0.02	0.02	0.3	0.23

[∞]Genotype (B73, NIL 1, and NIL 2), ^αInoculant (Proven, None), ^εN dosage= (N:0 kg ha⁻¹, N:67 kg ha⁻¹), [¥] Growth stage= In 2021 maize vegetative growth stages of V5 and V12 and in 2022 the vegetative maize growth stages of V8 and VT (tasseling), were considered. NIL= near isogenic line.

Significance codes: P < 0.05 ‘*’, P < 0.01 ‘***’, P < 0.001 ‘****’

TABLE S4 Individual and interactive effects of genotype, inoculant, mineral N dosage, and growth stages as well as their interactions across 2021 on prokaryotic (a) and fungi community (b) structure assessed by ADONIS, applying Bray-Curtis distance matrices with number of permutations=999

Treatment (a)	n	R²	P
All samples (prokaryotes 2021)	56		
[∞] Genotype		0.01	0.26
^ε N dosage		0.01	0.14
^α Inoculant		0.01	0.87
[¥] Growth stage		0.06	0.001 ***
Genotype:N.dosages		0.01	0.14
Genotype:Inoculant		0.01	0.67
N.dosages:Inoculant		0.01	0.29
Genotype: Growth stage		0.03	0.75
N.dosages: Growth stage		0.02	1.00
Inoculant: Growth stage		0.02	0.93
Genotype:N.dosages: Growth stage		0.02	0.99
Genotype:Inoculant: Growth stage		0.02	1.00
N.dosages:Inoculant: Growth stage		0.02	0.98
Residual		0.75	0.26
Total		1.00	0.14
All samples (Fungi 2021)	56		
Genotype		0.01	0.32
N.dosages		0.01	0.75
Inoculant		0.01	0.84
Growth stage		0.06	0.00***
Genotype:N.dosages		0.01	0.19
Genotype:Inoculant		0.01	0.38
N.dosages:Inoculant		0.01	0.36
Genotype: Growth stage		0.03	0.05*
N.dosages: Growth stage		0.03	0.56
Inoculant: Growth stage		0.03	0.48
Genotype:N.dosages: Growth stage		0.03	0.34
Genotype:Inoculant: Growth stage		0.02	0.86
N.dosages:Inoculant: Growth stage		0.02	0.94
Residual		0.73	
Total		1.00	

[∞]Genotype (B73, NIL 1, and NIL 2), ^αInoculant (Proven, None), ^εN dosage= (N:0 kg ha⁻¹, N:67 kg ha⁻¹), [¥]Growth stage= In 2021 maize vegetative growth stages of V5 and V12 and in 2022 the vegetative maize growth stages of V8 and VT (tasseling), were considered. NIL= near isogenic line.

Significance codes: P < 0.05 ‘*’, P < 0.01 ‘***’, P < 0.001 ‘****’

TABLE S5 Individual and interactive effects of Genotype, Inoculant, mineral N dosage, and growth stage as well as their interactions in 2022 on prokaryotic and fungi community structure assessed by ADONIS, applying Bray-Curtis distance matrices, with number of permutations=999

Treatment (b)	n	R ²	P
All samples (prokaryotes 2022)	96		
^α Genotype		0.02	0.38
^ε N dosage		0.01	0.51
^α Inoculant		0.01	0.13
[¥] Growth stage		0.01	0.00**
Genotype:N.dosage		0.02	0.03*
Genotype:Inoculant		0.02	0.03*
N.dosage:Inoculant		0.01	0.54
Genotype: Growth stage		0.02	0.85
N.dosage: Growth stage		0.01	0.82
Inoculant: Growth stage		0.01	0.46
Genotype:N.dosage:Inoculant		0.02	0.98
Genotype:N.dosage: Growth stage		0.02	0.99
Genotype:Inoculant: Growth stage		0.02	0.73
N.dosage:Inoculant: Growth stage		0.01	0.98
Genotype:N.dosage:Inoculant: Growth stage		0.02	0.93
Residual		0.76	
Total		1.00	
All samples (Fungi 2022)	96		
Genotype		0.02	0.19
N dosage		0.01	0.30
Inoculant		0.01	0.38
Growth stage		0.02	0.00***
Genotype:N dosages		0.02	0.52
Genotype:Inoculant		0.02	0.08
N dosages:Inoculant		0.01	0.47
Genotype: Growth stage		0.02	0.35
N dosages: Growth stage		0.01	0.39
Inoculant: Growth stage		0.01	0.81
Genotype:N.dosages:Inoculant		0.02	0.97
Genotype:N dosages: Growth stage		0.02	0.75
Genotype:Inoculant: Growth stage		0.02	0.95
N dosages:Inoculant: Growth stage		0.01	0.65
Genotype:N dosages:Inoculant: Growth stage		0.02	0.73
Residual		0.76	
Total		1.00	

^αGenotype (B73, NIL 1, and NIL 2), ^εN dosage= (N:0 kg ha⁻¹, N:67 kg ha⁻¹), ^αInoculant (Proven, None), [¥]Growth stage= In 2021 maize vegetative growth stages of V5 and V12 and in 2022 the vegetative maize growth stages of V8 and VT (tasseling), were considered. NIL= near isogenic line.

Significance codes: P < 0.05 ‘*’, P < 0.01 ‘**’; P < 0.001 ‘***’

Table S6 Significant Fungi taxa contributing to differential abundance between treatments

ITS 21	N dosages	Enriched	padj
marker1	k__Fungi p__Ascomycota c__Eurotiomycetes o__Chaetothyriales f__Herpotrichiellaceae g__Exophiala s__Exophiala_alcalophila	N:0	***
	Inoculant		
marker1	k__Fungi p__Basidiomycota c__Agaricomycetes o__Agaricales f__Bolbitiaceae	None	***
marker2	k__Fungi p__Ascomycota c__Dothideomycetes o__Capnodiales f__Mycosphaerellaceae g__Septoria g__Septoria	Proven	***
marker3	k__Fungi p__Chytridiomycota c__Rhizophyidiomycetes o__Rhizophydiales	Proven	***
ITS 22	Genotype		
marker1	k__Fungi p__Basidiomycota c__Tremellomycetes o__Tremellales f__Bulleribasidiaceae g__Hannaella s__Hannaella_luteola	NIL 1	***
marker2	k__Fungi p__Ascomycota c__Eurotiomycetes o__Eurotiales f__Aspergillaceae g__Aspergillus	NIL 1	**
marker3	k__Fungi p__Ascomycota c__Sordariomycetes o__Hypocreales f__Hypocreaceae g__Trichoderma	NIL 2	**
marker4	k__Fungi p__Mortierellomycota c__Mortierellomycetes o__Mortierellales f__Mortierellaceae g__Mortierella	B73	**
marker5	k__Fungi p__Ascomycota c__Sordariomycetes o__Hypocreales f__Hypocreaceae	NIL 2	**
marker6	k__Fungi p__Ascomycota c__Sordariomycetes o__Hypocreales f__Hypocreaceae	B73	**
marker7	k__Fungi p__Ascomycota c__Sordariomycetes o__Sordariales f__Lasiosphaeriaceae g__Apodus s__Apodus_deciduus	NIL 1	*
marker8	k__Fungi p__Ascomycota c__Dothideomycetes o__Pleosporales f__Sporormiaceae g__Preussia s__Preussia_flanaganii	B73	*
marker9	k__Fungi p__Ascomycota c__Pezizomycetes o__Pezizales f__Ascobolaceae	NIL 1	*
marker10	k__Fungi p__Ascomycota c__Eurotiomycetes o__Eurotiales f__Aspergillaceae g__Aspergillus s__Aspergillus_caninus	NIL 1	*
marker11	k__Fungi p__Basidiomycota c__Agaricomycetes o__Hymenochaetales	NIL 1	*
marker12	k__Fungi p__Basidiomycota c__Agaricomycetes o__Hymenochaetales f__Schizoporaceae	NIL 1	*
marker13	k__Fungi p__Ascomycota	NIL 1	*
	Growth stage	Enriched	padj
marker1	k__Fungi p__Basidiomycota c__Agaricomycetes o__Hymenochaetales f__Schizoporaceae g__Lyomyces s__Lyomyces_mascarensis	V8	***
marker2	k__Fungi p__Ascomycota c__Eurotiomycetes o__Chaetothyriales f__Chaetothyriaceae	V8	***
marker3	k__Fungi p__Ascomycota c__Leotiomycetes o__Helotiales f__Myxotrichaceae g__Oidiodendron s__Oidiodendron_cereale	V8	***
marker4	k__Fungi p__Ascomycota c__Leotiomycetes o__Helotiales f__Helotiaceae g__Hymenoscyphus s__Hymenoscyphus_menthae	V8	***
marker5	k__Fungi p__Ascomycota c__Sordariomycetes o__Sordariales f__Lasiosphaeriaceae g__Lasiosphaeris s__Lasiosphaeris_hispida	VT	***
marker6	k__Fungi p__Ascomycota c__Dothideomycetes o__Pleosporales f__Sporormiaceae	V8	***
	N.dosages		
marker1	k__Fungi p__Ascomycota c__Sordariomycetes o__Hypocreales f__Nectriaceae	N.67	***
marker2	k__Fungi p__Ascomycota c__Dothideomycetes o__Pleosporales f__Sporormiaceae	N.0	***
marker3	k__Fungi p__Ascomycota c__Orbiliomycetes o__Orbiliales f__Orbiliaceae g__Arthrobotrys	N.67	***

***Significant with P < 0.001.

Table S7 significant Prokaryotes taxa contributing to differential abundance between treatments

Prokaryotes21	Genotype	Enriched	padj
marker1	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Burkholderiales f__Oxalobacteraceae g__Noviherbaspirillum s__Noviherbaspirillum_malthae	NIL.1	***
	Growth stage		
marker1	d__Bacteria p__Acidobacteriota c__Acidobacteriae o__Acidobacteriales_f__uncultured_g__uncultured s__uncultured_Acidobacteria	V12	***
marker2	d__Bacteria p__Actinobacteriota c__Actinobacteria o__Frankiales f__Acidothermaceae g__Acidothermus g__Acidothermus	V12	***
marker5	d__Bacteria p__Actinobacteriota c__Thermoleophila o__Gaiellales_f__uncultured_g__uncultured s__uncultured_Conexibacteraceae	V12	***
marker6	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Enterobacterales f__Enterobacteriaceae	V12	***
marker7	d__Bacteria p__Acidobacteriota c__Acidobacteriae o__Acidobacteriales o__Acidobacteriales	V12	***
marker8	d__Bacteria p__Proteobacteria c__Alphaproteobacteria_o__uncultured_f__uncultured_g__uncultured_s__uncultured_Alphaproteobacteria	V12	***
marker9	d__Bacteria p__Firmicutes c__Bacilli o__Bacillales f__Planococcaceae	V12	***
marker10	d__Bacteria p__Acidobacteriota c__Acidobacteriae o__Acidobacteriales	V12	***
marker11	d__Bacteria p__Chloroflexi c__Ktedonobacteria o__Ktedonobacterales f__JG30-KF-AS9 g__JG30-KF-AS9 g__JG30-KF-AS9	V12	**
marker12	d__Bacteria p__Chloroflexi c__AD3 o__AD3 f__AD3 g__AD3	V12	**
marker14	d__Bacteria p__Proteobacteria c__Alphaproteobacteria c__Alphaproteobacteria_o__uncultured c__Alphaproteobacteria_o__uncultured_f__uncultured	V12	***
marker15	d__Bacteria p__Proteobacteria__Alphaproteobacteria	V12	***
marker16	d__Bacteria p__Acidobacteriota c__Acidobacteriae o__Subgroup_2 f__Subgroup_2 g__Subgroup_2	V12	**
marker17	d__Bacteria p__Firmicutes c__Bacilli o__Bacillales f__Planococcaceae g__Sporosarcina	V12	**
marker18	d__Bacteria p__Actinobacteriota c__Thermoleophila o__Gaiellales f__Gaiellaceae g__Gaiella g__Gaiella	V12	**
marker19	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Burkholderiales f__Rhodocyclaceae	V12	**
marker20	d__Bacteria p__Chloroflexi c__AD3 o__AD3 f__AD3 g__AD3	V12	**
marker21	d__Bacteria p__Firmicutes c__Bacilli o__Bacillales f__Planococcaceae g__Sporosarcina g__Sporosarcina	V12	**
marker22	d__Bacteria p__Methylomirabilota c__Methylomirabilia o__Rokubacteriales f__Rokubacteriales g__Rokubacteriales	V12	**
marker23	d__Bacteria p__Acidobacteriota c__Subgroup_25 o__Subgroup_25 f__Subgroup_25 g__Subgroup_25	V12	*
marker24	d__Bacteria p__Gemmatimonadota c__Gemmatimonadetes o__Gemmatimonadales f__Gemmatimonadaceae g__uncultured s__uncultured_Gemmatimonas	V12	*
marker25	d__Bacteria p__Acidobacteriota c__Acidobacteriae o__Acidobacteriales	V12	*
marker26	d__Bacteria p__Latescibacterota c__Latescibacterota o__Latescibacterota f__Latescibacterota g__Latescibacterota	V12	*
marker27	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Enterobacterales f__Enterobacteriaceae g__Escherichia-Shigella	V12	*
marker28	d__Bacteria p__Chloroflexi c__AD3 o__AD3 f__AD3 g__AD3 g__AD3_s__uncultured_Thermomicrobia	V12	*
marker29	d__Bacteria p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Xanthobacteraceae g__Xanthobacteraceae s__uncultured_Alphaproteobacteria	V12	*
marker30	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Xanthomonadales f__Rhodanobacteraceae g__Ahniella g__Ahniella	V12	*
marker31	d__Bacteria p__Verrucomicrobiota c__Verrucomicrobiae o__Opitutales f__Opitutaceae g__Opitutus	V5	***
marker32	d__Bacteria p__Myxococcota c__Polyangia o__Polyangiales f__BIRi41 g__BIRi41	V5	***
marker33	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Xanthomonadales f__Xanthomonadaceae g__Arenimonas s__uncultured_Arenimonas	V5	***
marker34	d__Bacteria p__Myxococcota c__Polyangia o__Polyangiales f__BIRi41 g__BIRi41	V5	***
marker35	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Xanthomonadales f__Xanthomonadaceae g__Arenimonas	V5	***
marker36	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales	V5	***
marker37	d__Bacteria p__Bacteroidota c__Bacteroidia o__Cytophagales f__Microscillaceae f__Microscillaceae	V5	***
marker38	d__Bacteria p__Verrucomicrobiota c__Verrucomicrobiae o__Pedosphaerales f__Pedosphaeraceae f__Pedosphaeraceae	V5	***
marker39	d__Bacteria p__Bacteroidota c__Bacteroidia o__Cytophagales	V5	***
marker40	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Xanthomonadales f__Xanthomonadaceae	V5	***

marker41	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales f__Pseudomonadaceae g__Pseudomonas	V5	***
marker42	d__Bacteria p__Proteobacteria c__ Betaproteobacteria o__ Nitrosomonadales f__Nitrosomonadaceae g__MND1	V5	**
marker43	d__Bacteria p__Bacteroidota c__Bacteroidia o__Flavobacteriales f__Flavobacteriaceae g__Flavobacterium	V5	**
marker44	d__Bacteria p__Myxococcota c__Polyangia o__Polyangiales	V5	**
marker45	d__Bacteria p__Bacteroidota c__Bacteroidia o__Flavobacteriales	V5	**
marker46	d__Bacteria p__Actinobacteriota c__Actinobacteria o__Micrococcales f__Microbacteriaceae	V5	**
marker48	d__Bacteria p__Armatimonadota c__Fimbriimonadia o__Fimbriimonadales	V5	**
marker49	d__Bacteria p__Proteobacteria c__ Betaproteobacteria o__ Nitrosomonadales f__Nitrosomonadaceae	V5	**
marker50	d__Bacteria p__Proteobacteria c__ Gammaproteobacteria o__Xanthomonadales	V5	**
marker51	d__Bacteria p__Chloroflexi c__Anaerolineae o__SBR1031	V5	**
marker52	d__Bacteria p__Armatimonadota c__Fimbriimonadia o__Fimbriimonadales f__Fimbriimonadaceae g__Fimbriimonadaceae	V5	*
marker53	d__Bacteria p__Proteobacteria c__Alphaproteobacteria o__Dongiales f__Dongiaceae g__Dongia	V5	*
marker54	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Burkholderiales f__Oxalobacteraceae g__Massilia s__Massilia_brevitalea	V5	*
marker55	d__Bacteria p__Firmicutes c__Bacilli o__Paenibacillales f__Paenibacillaceae g__Ammoniphilus g__Ammoniphilus	V5	*
marker56	d__Bacteria p__Bacteroidota c__Bacteroidia o__Cytophagales f__Microscillaceae	V5	*
marker57	d__Bacteria p__Armatimonadota c__Fimbriimonadia o__Fimbriimonadales f__Fimbriimonadaceae g__Fimbriimonadaceae	V5	*
marker58	d__Bacteria p__Verrucomicrobiota c__Verrucomicrobiae o__Opitutales f__Opitutaceae g__Lacunisphaera	V5	*
marker59	d__Bacteria p__Proteobacteria c__ Gammaproteobacteria o__Oceanospirillales f__Pseudohongiellaceae g__BIyi10	V5	*
marker60	d__Bacteria p__Chloroflexi c__Anaerolineae	V5	*
marker61	d__Bacteria p__Planctomycetota c__OM190 o__OM190 f__OM190 g__OM190	V5	*
marker62	d__Bacteria p__Proteobacteria c__ Gammaproteobacteria o__R7C24 f__R7C24 g__R7C24	V5	*

Significance codes: P < 0.05 '**', P < 0.01 '***'; P < 0.001 '****'

TABLE S8 Individual and interactive effects of Genotype, Inoculant, mineral N dosage, and growth stages as well as their interactions on nitrifiers community structure assessed by ADONIS, applying a Bray-Curtis distance matrix; number of permutations=999

Treatment	n	R²	P
Nitrifiers 2021	56		
[∞] Genotype		0.00	0.77
^ε N dosage		0.03	0.05
^α Inoculant		0.00	0.71
[¥] Growth stage		0.14	0.001 ***
Genotype:N.dosages		0.00	0.75
Genotype:Inoculant		0.00	0.81
N.dosages:Inoculant		0.01	0.23
Genotype: Growth stage		0.01	0.98
N.dosages: Growth stage		0.02	0.51
Inoculant: Growth stage		0.01	0.78
Genotype:N.dosages: Growth stage		0.02	0.50
Genotype:Inoculant: Growth stage		0.01	0.89
N.dosages:Inoculant: Growth stage		0.01	0.89
Residual		0.72	
Total		1.00	
	n	R²	P
Nitrifiers 2022	96		
Genotype		0.01	0.67
N.dosages		0.00	0.99
Inoculant		0.01	0.38
Growth stage		0.02	0.18
Genotype:N.dosages		0.03	0.15
Genotype:Inoculant		0.05	0.03*
N.dosages:Inoculant		0.01	0.46
Genotype: Growth stage		0.02	0.54
N.dosages: Growth stage		0.00	0.94
Inoculant: Growth stage		0.00	1.00
Genotype:N.dosages:Inoculant		0.01	0.77
Genotype:N.dosages: Growth stage		0.02	0.46
Genotype:Inoculant: Growth stage		0.02	0.48
N.dosages:Inoculant: Growth stage		0.00	0.91
Genotype:N.dosages:Inoculant: Growth stage		0.01	0.93
Residual		0.78	
Total		1.00	

[∞]Genotype (B73, NIL 1, and NIL 2), ^αInoculant (Proven, None), ^εN dosage= (N:0 kg ha⁻¹, N:67 kg ha⁻¹), [¥] Growth stage= In 2021 maize vegetative growth stages of V5 and V12 and in 2022 the vegetative maize growth stages of V8 and VT (tasseling), were considered. NIL= near isogenic line.

Significance codes: P < 0.05 ‘*’, P < 0.01 ‘***’, P < 0.001 ‘****’

Table S9 significant nitrifier taxa contributing to differential abundance between treatments

Nitrifiers 2021	N dosage	Enriched	padj
marker1	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Burkholderiales f__Nitrosomonadaceae g__Nitrospira	N:67	***
marker2	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae g__Nitrospira s__Nitrospira tenuis	N:67	***
	Growth stage		
marker1	d__Bacteria p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Xanthobacteraceae g__Nitrobacter s__uncultured_Nitrobacter	V5	***
marker3	d__Archaea p__Crenarchaeota c__Nitrososphaeria o__Nitrososphaerales f__Nitrososphaeraceae g__Candidatus_Nitrososphaera s__Thaumarchaeota_archaeon	V12	***
marker4	d__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Nitrosococcales f__Nitrosococcaceae g__wb1-P19	V5	***
marker5	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae g__Nitrosomonas	V5	***
marker7	d__Archaea p__Crenarchaeota c__Nitrososphaeria o__Nitrosotaleales f__Nitrosotaleaceae g__Candidatus_Nitrosotalea s__uncultured_archaeon	V5	***
marker8	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae	V5	***
marker10	d__Archaea p__Crenarchaeota c__Nitrososphaeria o__Nitrososphaerales f__Nitrososphaeraceae g__Candidatus_Nitrososphaera	V5	***
marker11	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae g__Nitrosomonas	V12	***
marker12	d__Archaea p__Crenarchaeota c__Nitrososphaeria o__Nitrosotaleales f__Nitrosotaleaceae g__Nitrosotaleaceae	V12	***
marker13	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae	V5	***
marker15	d__Bacteria p__Proteobacteria c__Alphaproteobacteria o__Rhizobiales f__Bradyrhizobiaceae g__Nitrobacter	V5	***
Nitrifiers 2022	Growth stage	Enriched	padj
marker1	d__Bacteria p__Proteobacteria c__Betaproteobacteria o__Nitrosomonadales f__Nitrosomonadaceae g__Nitrospira	V8	***
marker2	d__Archaea p__Crenarchaeota c__Nitrososphaeria o__Nitrososphaerales f__Nitrososphaeraceae	VT	***

Significance codes: P < 0.001 '***'

Table S10 comparative and interactive effects of genotype and management practices on potential nitrification rate, plant N content and grain protein%. Letters show the pairwise Tukey HSD results. Ismean ± standard error is shown in the upper part of the table. The lower part shows the fixed factors results of linear mixed-effect models. The output is summarized by “anova” function. The significance of each factor and their interactions are shown by F-values followed by asterisks as defined in footnote.

Year 2021			†Gen		‡GS		#Inoc		‡N (kg ha ⁻¹)					
	Potential		B73	NIL 1	V5	V12	None	Proven	N:0		N:67			
	Nitrification rate (ngN g ⁻¹ soil)		1901± 110.0	1724 ± 133.0	1536 ± 137.0 a	2089 ± 137.0 b	1674 ± 147.0	1951 ± 127.0	1523 ± 133.0 a		2120 ± 110.0 b			
			†Gen		‡GS		#Inoc		‡N (kg ha ⁻¹)					
	Plant data		B73	NIL 1			None	Proven	N:0		N:67			
	N content/V8		15.1 ± 0.7		15.0 ± 0.8		14.8 ± 0.6	15.4 ± 0.9	14.6 ± 0.8		15.6 ± 0.6			
	N content/VT		76.8 ± 4.50		78.4 ± 5.1		77.3 ± 4.3	78.0 ± 5.8	67.5 ± 5.4 a		87.7 ± 4.2 b			
	Grain protein %		7.3 ± 0.1		8.0 ± 0.2		7.7 ± 0.1	7.6 ± 0.2	7.3 ± 0.2 a		8.0 ± 0.1 b			
	‡Plant biomass		5506 ± 203		5142 ± 211		5281 ± 194	5406 ± 238	5241 ± 226		5402 ± 201			
			†Gen		‡GS		#Inoc		‡N (kg ha ⁻¹)					
Potential		B73	NIL 1	NIL 2	V8	VT	None	Proven	N:0		N:67			
Nitrification rate (ngN g ⁻¹ soil)		1127 ± 58.8	1145 ± 59.3	1153 ± 55.3	1176 ± 46.9	1108 ± 44.5	1156 ± 45.3	1128 ± 48.5	990± 47.2 a		1294 ± 44.4 b			
Year 2022			†Ge		‡GS		#Inoc		‡N (kg ha ⁻¹)					
	Plant data		B73	NIL 1	NIL 2		None	Proven	N:0		N:67			
	N content/V8		29.0 ± 0.9 a	29.3 ± 0.9 a	33.8 ± 0.9 b		31.0 ± 0.7	30.3 ± 0.7	29.2 ± 0.7 a		32.1 ± 0.7 b			
	N content/VT		63.5 ± 2.4	65.0 ± 2.4 a	72.3 ± 2.4 b		66.2 ± 2.0	67.7 ± 2.0	62.4 ± 2.0 a		71.5 ± 2.0 b			
	Grain protein %		7.5 ± 0.1 a	8.4 ± 0.1 b	7.8 ± 0.1 a		7.8 ± 0.1	7.9 ± 0.1	7.3 ± 0.2 a		8.4 ± 0.2 b			
	Plant biomass kg ha ⁻¹		4653 ± 126 a	4751 ± 126 b	5459 ± 126 c		5045 ± 118	4865 ± 118	5036 ± 118		4873 ± 118			
	Kernel weight mg kernel ⁻¹		187 ± 2.26 b	179 ± 2.23 a	190 ± 2.25 c		183 ± 1.92 a	188 ± 1.92 b	181 ± 1.86 a		190 ± 1.86 b			
			†Ge		‡GS		#Inoc		‡N (kg ha ⁻¹)					
2021														
Main effects	Nitrification rate	Main effects	Plant N content		Grain protein	Plant biomass	Main effects	Nitrification rate	Main effects	Plant N content		Grain protein	Plant biomass	Kernel weight
			V5	V12						V8	VT			
Units			(kg ha ⁻¹)	%	%	%		ng N g ⁻¹ soil		(kg ha ⁻¹)		%	(kg ha ⁻¹)	(kg kernel ⁻¹)
Ge	1.72	Ge	0.00	1.12	3.69	2.16	Ge	1.83	Ge	5.4**	3.91*	4.57*	13.59***	0.73
N	18.21***	N	1.21	11.82**	16.28***	0.00	N	31.99***	N	9.58**	12.89***	66.11***	1.41	12.89***
GS	3.37	Inoc	0.35	0.01	0.38	0.71	Inoc	1.34	Inoc	0.15	1.25	0.18	1.71	3.93*
Inoc	2.5	Ge: N	0.03	1.82	1.32	0.46	GS	1.64	Ge:N	1.08	0.22	3.70*	0.05	2.91
Ge: N	0.6					0.17	Ge: N	2.82	Ge: Inoc	1.86	0.37	1.19	0.43	2.63
Ge: GS	0.02						Ge: Inoc	0.72	N: Inoc	0.03	0.97	0.08	0.15	0.00
N: GS	0.81						N: Inoc	0.90	Ge: N: Inoc	2.71	0.19	1.23	0.32	1.59
Ge: N: GS	0.15						Ge: GS	0.87						
							N: GS	0.44						
							Inoc: GS	0.35						
							Ge: N: Inoc	0.94						
							Ge: N: GS	0.89						
							Ge: Inoc: GS	7.82**						
							N: Inoc: GS	9.78**						
							Ge: N: Inoc: GS	0.95						

†Ge= Genotype (B73, NIL_1, and NIL_2), ‡GS= Growth stage (2021 (vegetative growth stages V5 and V12), 2022 (vegetative growth stages V8 and VT (tasseling)), #Inoc=Inoculant (Proven, None), ‡N= N dosages (N:0 kg ha⁻¹, N:67 kg ha⁻¹), ‡Grain N conc= Grain N concentration %.

* Significant at the 0.05 level. ** Significant at the 0.01 level. *** Significant at the 0.001 level. P values were adjusted, using the Hochberg method.

Table S11 Topological properties of microbial networks. The F-values, accompanied by asterisks in the lower table, depict the results of the generalized linear model (GLM) assessing the impact of genotype and the interaction between genotype and management on network topological properties. Topological properties Z-scale normalized, if required polynomial applied to capture nonlinear relationships.

Treatments	Module connectivity		Modularity	Modules No.	Weight		Edge_ betweenness	Node Betweenness	Closeness	Transitivity	Harmonic centrality	Edge density	Mean degree	Size	
	Among	With n× 10 ⁻¹¹			Negative	Positive								Nodes	Edges
Descriptions	Connectedness within a niche and among microbial niches		Network structure	No. of microbial niches	Interactions/associations the frequency of co-occurrence among the connected nodes		No. of the shortest paths that go through an edge (revealing network connectivity & resilience)	Crucial microbial taxa facilitating information flow in a community	Length of path from a node to all others (communication within the community)	Interference and resource exploitation within a community	Organization level around central nodes (highly influential microbes) within a network.	Edge proportion in a network relative to max edges (density of connections between microbes)	Avg. No. of edges/connections per node	Co-occurring ASVs	Co-occurring interactions
	(Schmidt et al., 2019; Ma et al., 2022)				(Fletcher et al., 2013; Zhang et al., 2017)		(Zamkovaya et al., 2021)			(Verdú et al., 2023)	(Freeman, 1978; Nasirian et al., 2020)	(Brisson et al., 2019; Schmidt et al., 2019; Ma et al., 2022)			
Genotype															
B73	0.74	-1.3	0.682	21	137(41.4%)	194(58.61%)	376.169	534.041	0.010	0.064	865.092	0.017	3.360	198	332
NIL 1	0.71	-2.2	0.692	20	198(50.6%)	193(49.36%)	372.422	596.582	0.013	0.100	1742.703	0.018	3.760	209	392
NIL 2	0.69	-2.1	0.651	18	201(46.4%)	232(53.58%)	330.097	561.407	0.013	0.112	1087.956	0.019	4.047	215	434
δ Genotype: management															
B73 : mang	0.74	2.3	0.595	19	277(44.4%)	347(55.6%)	557.021	794.884	0.018	0.172	1366.105	0.026	5.558	220	625
NIL 1 : mang	0.72	8.3	0.638	18	211(53.4%)	184(46.6%)	362.765	574.109	0.011	0.085	767.688	0.018	3.744	202	396
NIL 2 : mang	0.75	2.3	0.668	20	204(45.0%)	249(55.0%)	341.523	596.691	0.013	0.114	1013.500	0.019	4.137	220	454
Variables	Module connectivity				Modularity		Node Betweenness			Closeness		Harmonic centrality		Degree	
	Among			Within											
Treatments (GLM results)															
Genotype	2.7		0.0		2.7		3.4*		155.0***		4.8**		41.3***		
mang	0.01		0.0		1.8		24.6***		590.03***		4.2*		111.0***		
Genotype: mang	4.6*		0.0		4.7***		13.8***		394.2***		4.3*		103.0***		

^δ Genotype: management (mang) (interactive effect of Genotype and management with an N-fixing inoculant (Proven), with 67 kg ha⁻¹ mineral N), P values were adjusted using ("Hochberg") method.

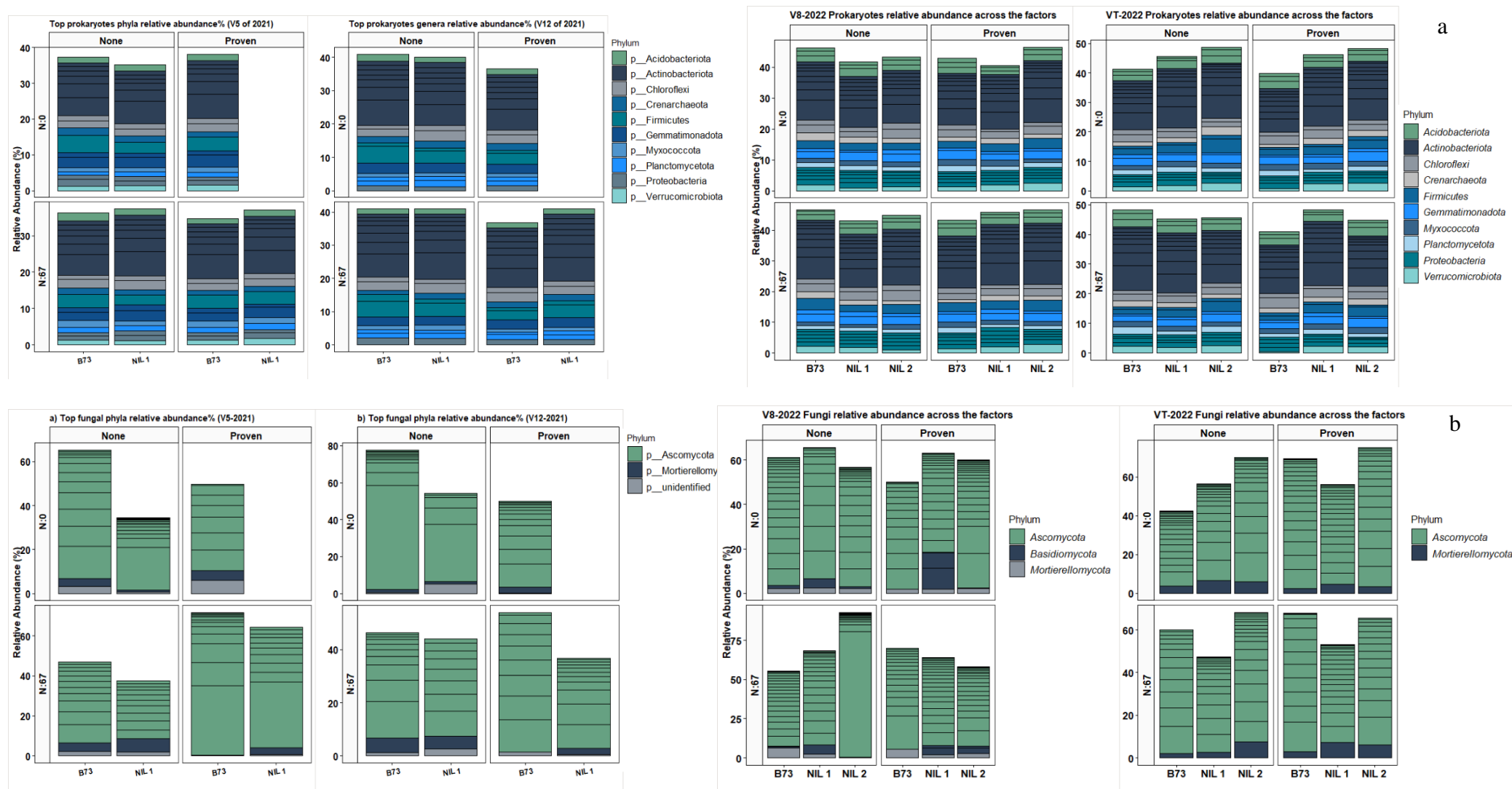


Figure S1 Taxonomic distributions of top dominant phyla among prokaryotes (a) at V8 and VT growth stages of 2022 and 2021 and fungi (b) in V5 and V12 growth stages of maize in 2021. Facets grouping the relative abundance of prokaryotes and fungi at phylum level based on treatments \times management \times Inoculant (Proven, None), mineral N dosages amendment (N:0 kg ha⁻¹, N:67 kg ha⁻¹).

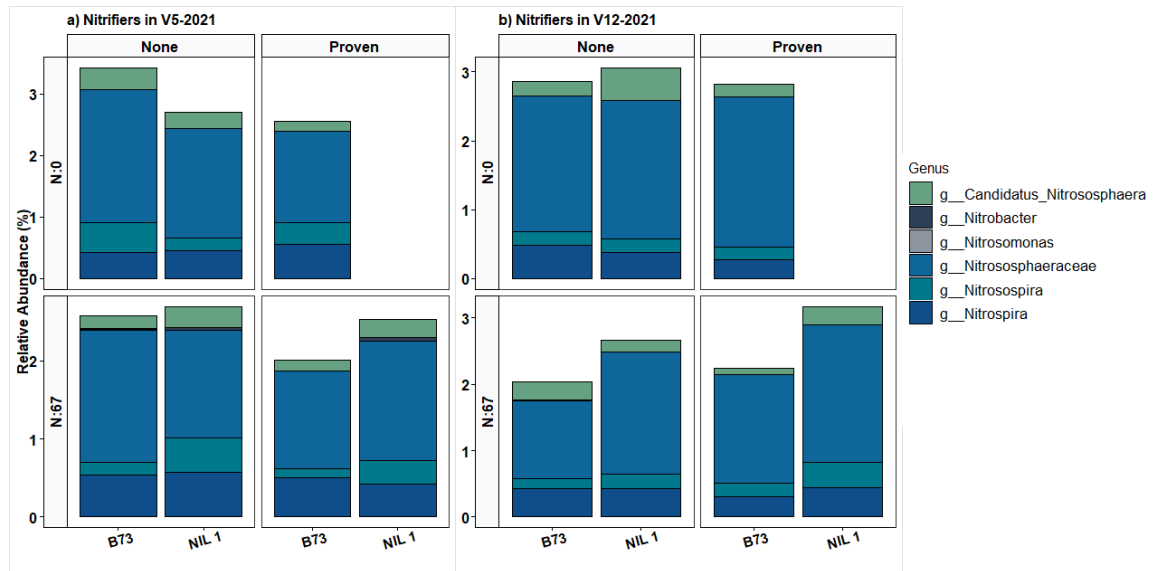
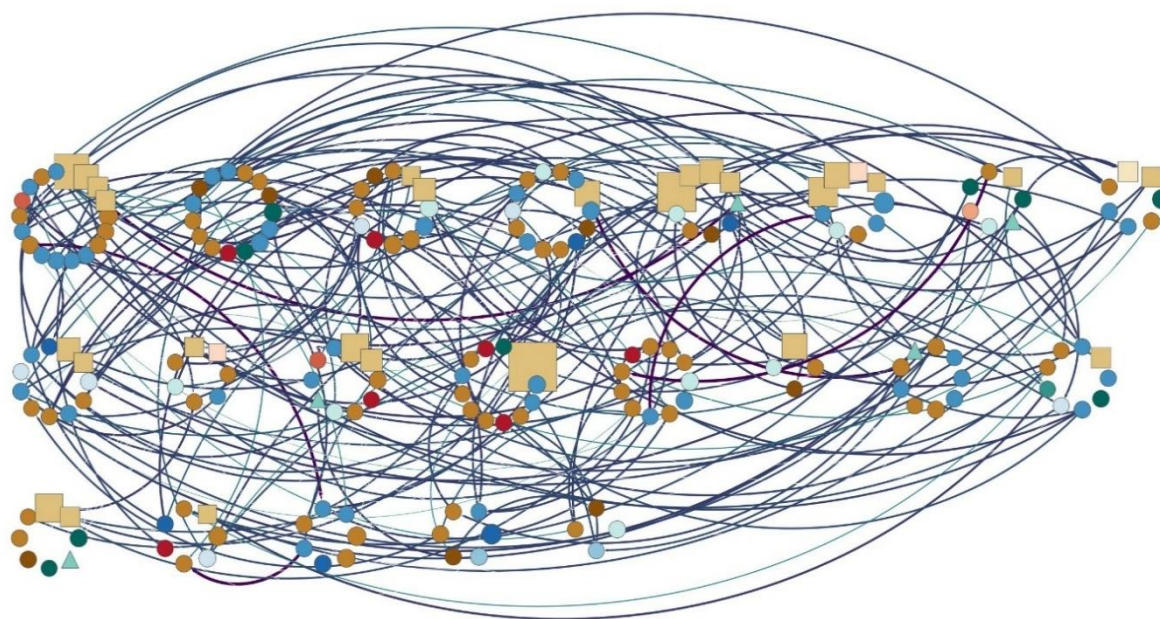
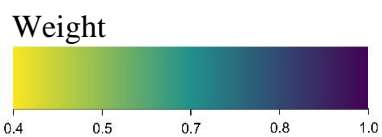
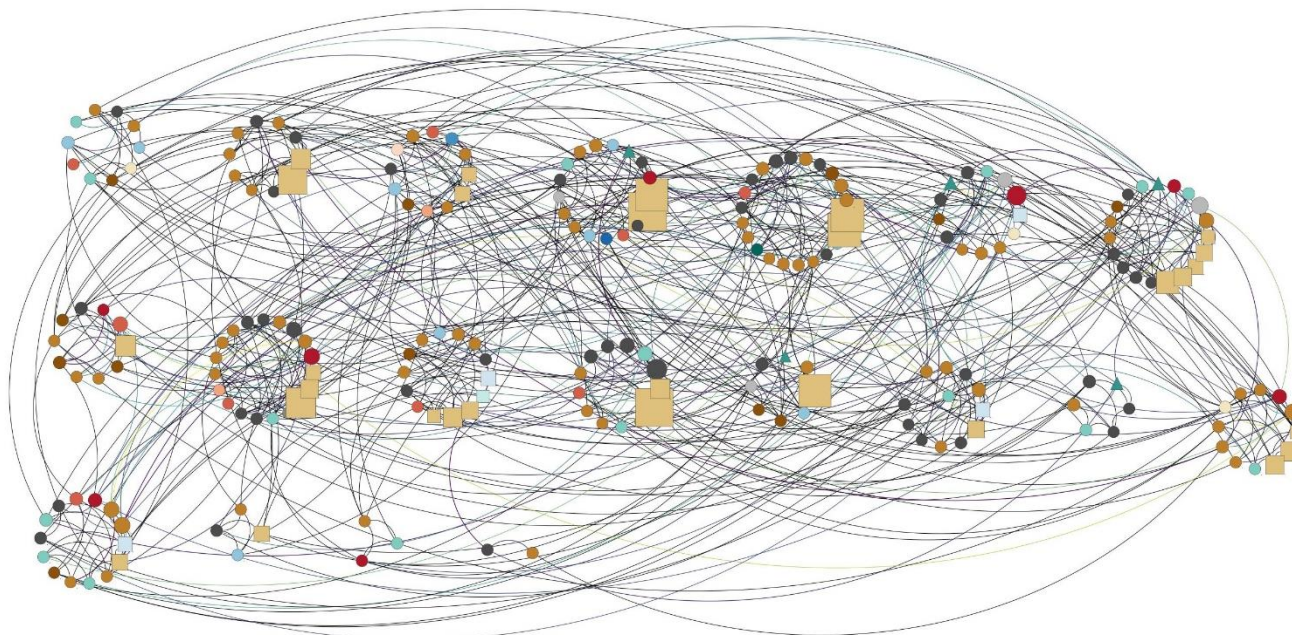
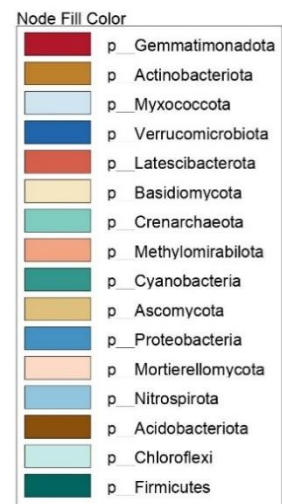


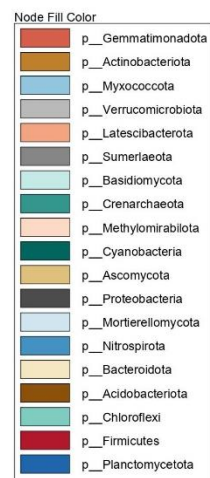
Figure S2a-b Comparing nitrifiers relative abundance across the genotypes and management with (a) and without (b) an N-fixing inoculant and N:67 kg ha⁻¹ amendment in 2021 at V5 and V12 growth stages of maize. Facets grouping the relative abundance of nitrifiers based on treatments: management: Inoculant (Proven, None), mineral N dosages amendment (N:0 kg ha⁻¹, N:67 kg ha⁻¹). NIL= near isogenic line, management (0 vs. 67 mineral N kg ha⁻¹ and none vs application of an N-fixing inoculant (Proven)).



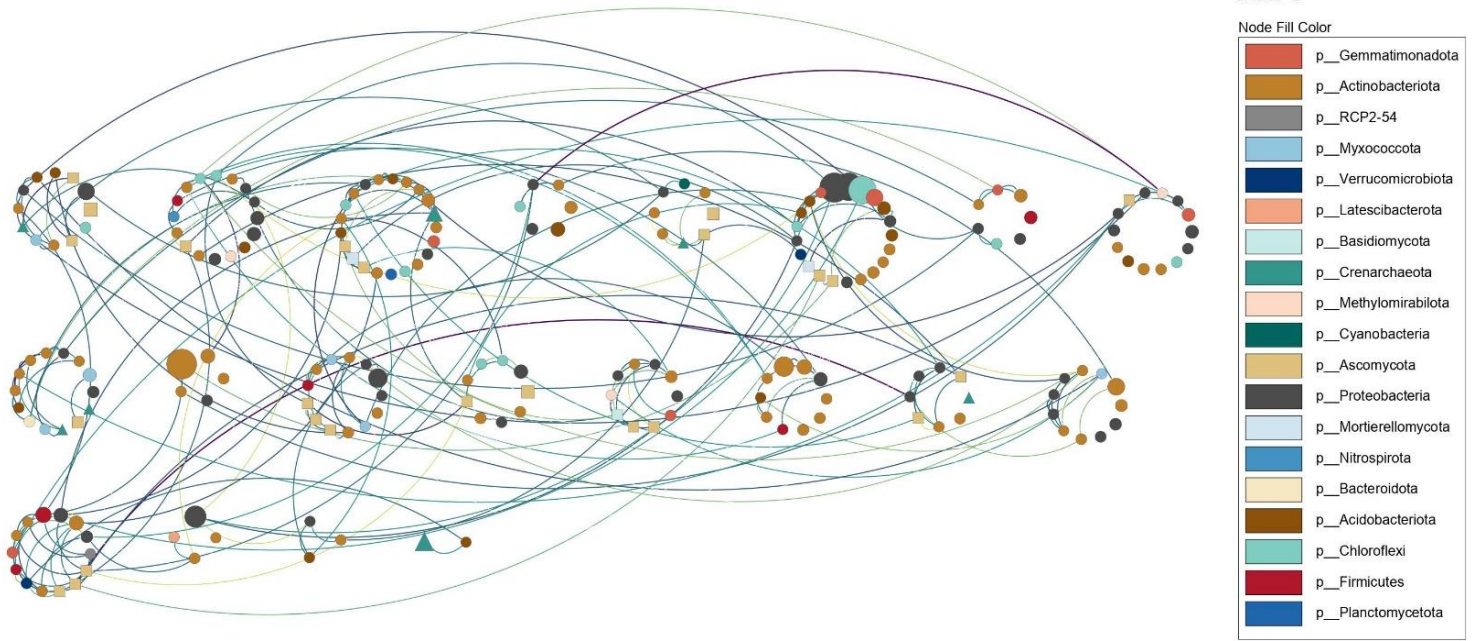
B73



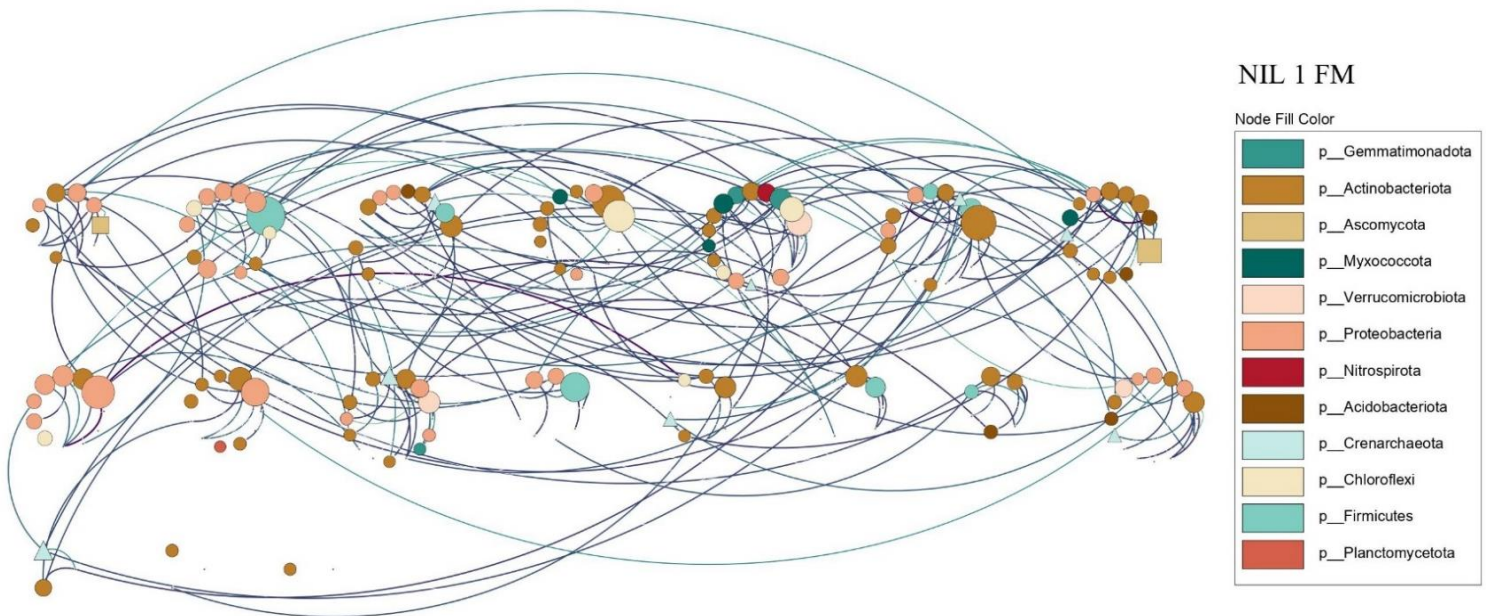
B73 FM



NIL 1



NIL 1 FM



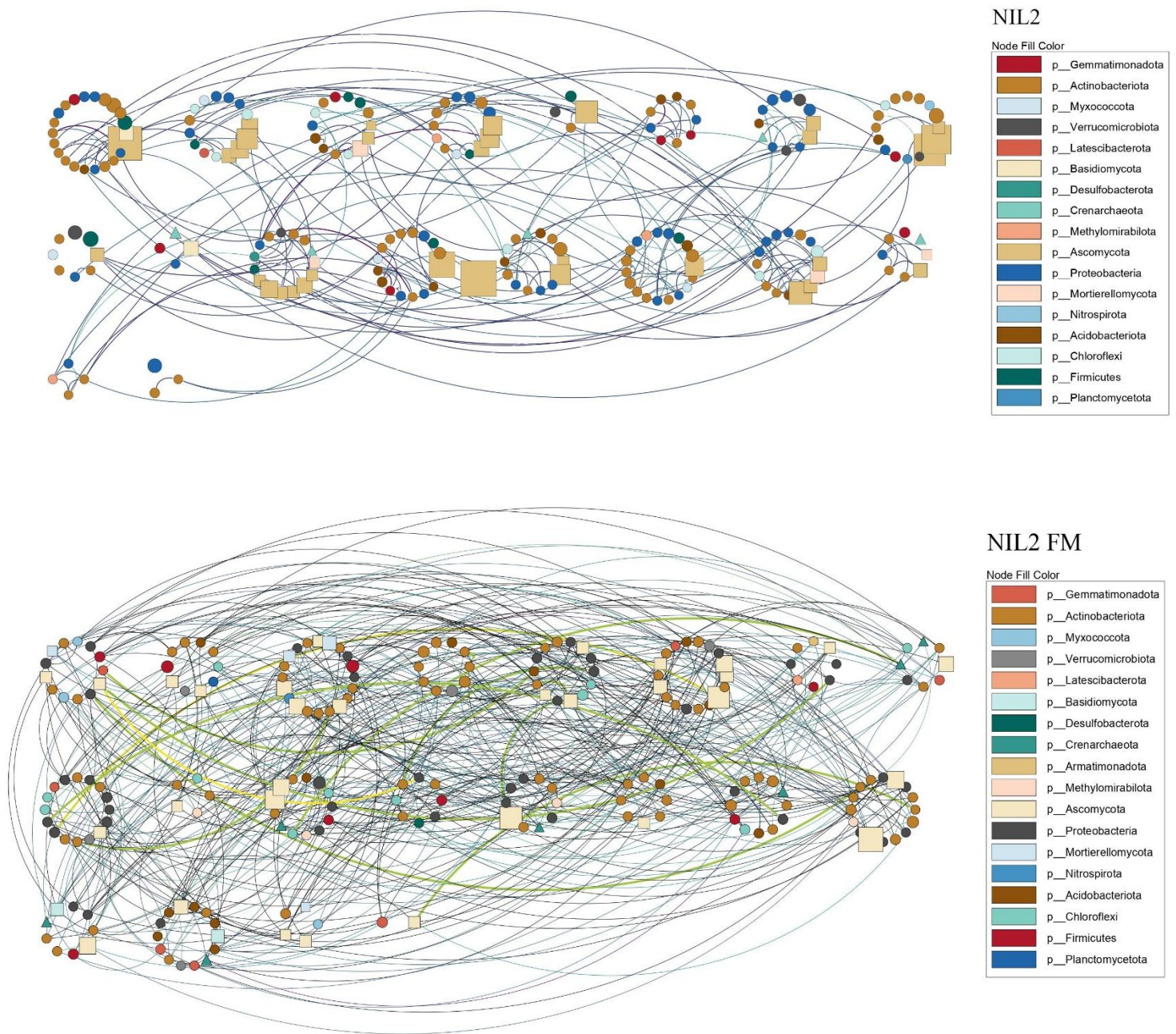


Figure S3a-f Modules and among modules connectivity under genotype (B73, NIL 1, and NIL 2) (a-c) and genotype: management (management = FM with an N-fixing inoculant (Proven) and mineral N amendment (N:67 kg ha⁻¹) (high N availability) interactions (d-f) are shown. The presence and size of edges represent among-modules-connectivity. The nodes are color-coded to indicate ASVs relevant to phyla present in the co-occurrence network. Node sizes are proportional to phyla relative abundance, and weights have been used for edge thickness. Networks' properties and details of their ecological relevance are defined in Table S11. Bacteria are shown by a circle, Archaea are shown by a triangle and Fungi are shown by a square.

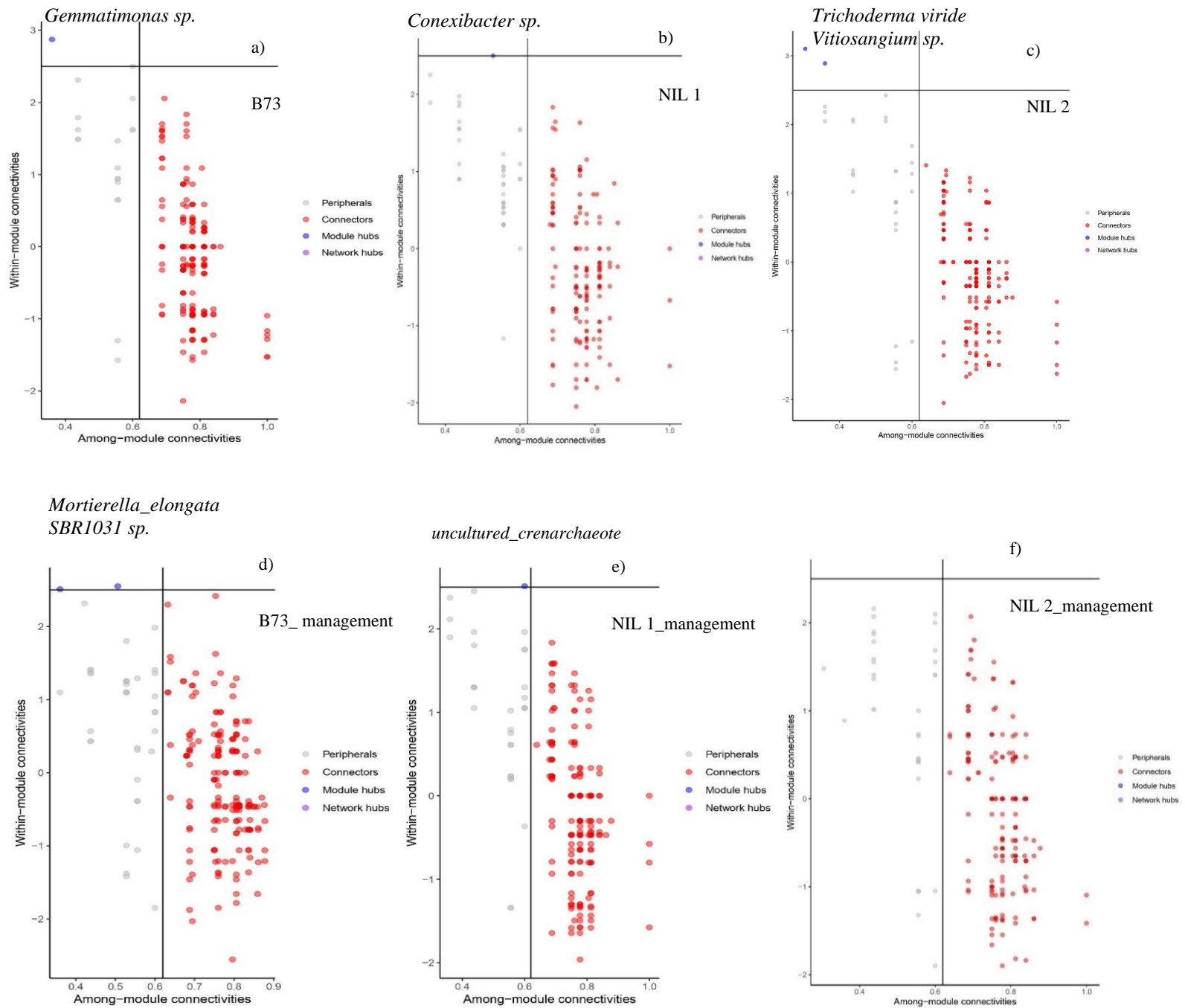


Figure S4a-F Classification of nodes to identify putative keystone species among the factors. The plots illustrate the modularity of networks constructed from predominant prokaryotic and fungal ASVs at the same depth, (2022 trial). The nodes are classified based on connectivity patterns: module hubs (blue dots display highly connected nodes within modules), network hubs (purple dots show highly connected nodes in the entire network), connectors (red dots indicate nodes connecting modules), and peripherals (gray nodes display interconnected nodes in modules with few outside connections). Figures a-c exhibit the genotype effect and figures d-f show the interactive effects of genotype and management on the connectivity pattern of cross-domain network of microbes. Genotypes (B73, NIL 1, and NIL 2) and a combination of genotype: management (Inoculant (Proven, None), mineral N dosages amendment (N:0 kg ha⁻¹, N:67 kg ha⁻¹)). Network properties are defined in Table S11.