Supplementary Materials for

Different resilience patterns of microbial community composition and network complexity within arbuscular mycorrhizal fungal hyphosphere

Chenchao Xu[1,†], Wanying Zhu[1,2†], Jing Xiao[1,*], Yongge Yuan[3], Lei Cheng[1]

[1] State Key Laboratory for Vegetation Structure, Function and Construction (VegLab), MOE Key Laboratory of Biosystems Homeostasis & Protection, College of Life Sciences, Zhejiang University, Hangzhou 310058, China

[2] Shanghai Majorbio Bio-Pharm Technology Co., Ltd, Shanghai 201321, China

[3] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

[†] These authors contributed equally to this work

[*] Corresponding author: jxiao@zju.edu.cn

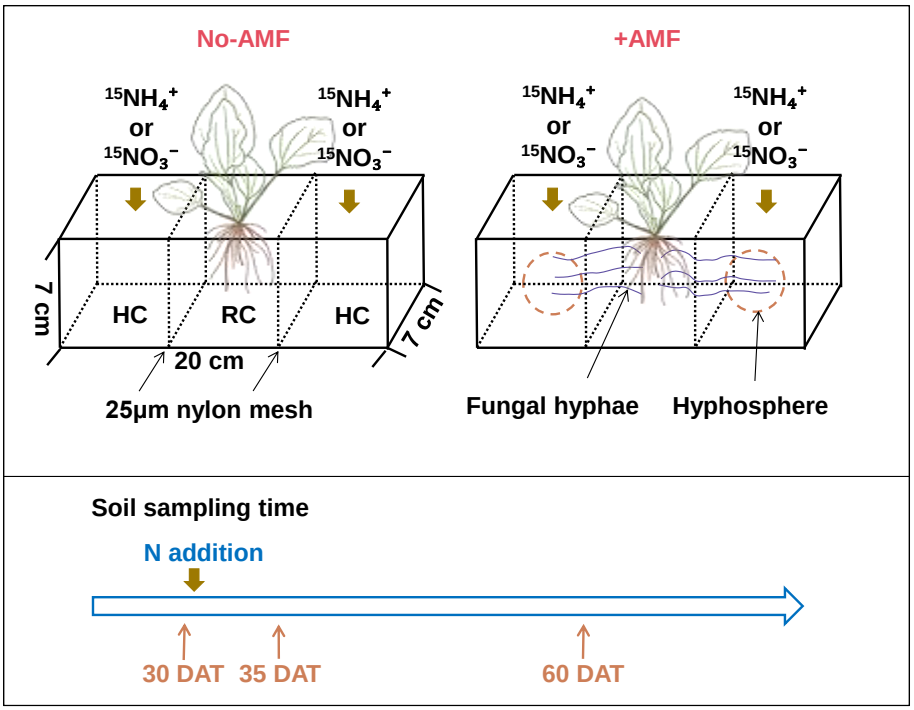

Fig. S1 Diagram of the experiment design. The experiment was carried out using a model mycorrhizal-plant system consisting of AMF species *Funneliformis geosporum* growing on roots of *Plantago asiatica* L. (*P. asiatica*). Each container was separated into three compartments, with a root compartment (RC) in the middle and two hyphae compartments (HC) on the two sides, by 25 μm nylon mesh which allowed fungal hyphae but not plant root to penetrate through. *P. asiatica* were germinated and pre-cultured in the greenhouse until seedlings were around 4 cm tall. *P. asiatica* were then transplanted in RC with 100g AMF-sand mixture in +AMF treatment and with 100 g autoclaved AMF-sand inoculum in No-AMF control. After 30 days after transplanting (DAT), the hyphosphere soils were first sampled to be the control. Just following the first sampling, ($(^{15}NH_4)_2SO_4$ or $Na^{15}NO_3$) was added in HC following a randomized complete block design. The hyphosphere soil samples in two HCs of each treatment were then taken at DAT 35 and 60 by destructive sampling.

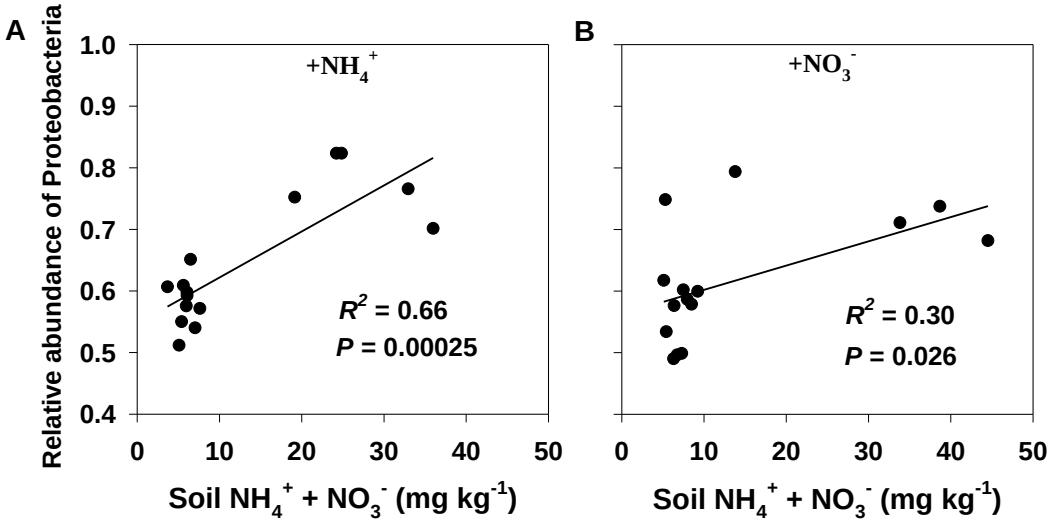

Fig. S2 The relationship between the contents of inorganic-N and relative abundance of Proteobacteria within the AMF hyphosphere following the addition of $^{15}NH_4^+$ (A) or $^{15}NO_3^-$ (B). *P* and R^2 values for the linear regression are shown in each of the figure panels.

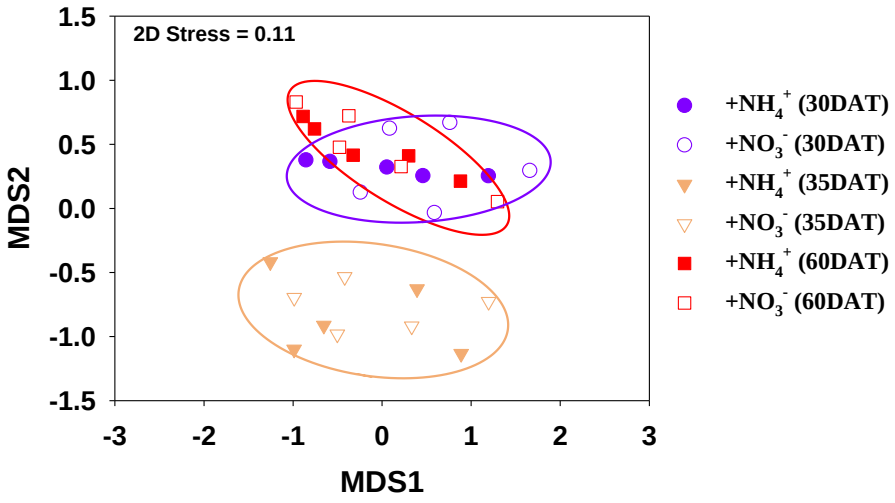

Fig. S3 Effects of AMF and inorganic-N addition on changes of microbial community composition in the hyphospheric soil. Genomic DNA was extracted from soils taken at DAT 30, 35 and 60 with the addition of either $^{15}NH_4^+$ or $^{15}NO_3^-$. Nonmetric multidimensional analysis (NMDS) of community dissimilarities based on 16S rRNA gene profiling.

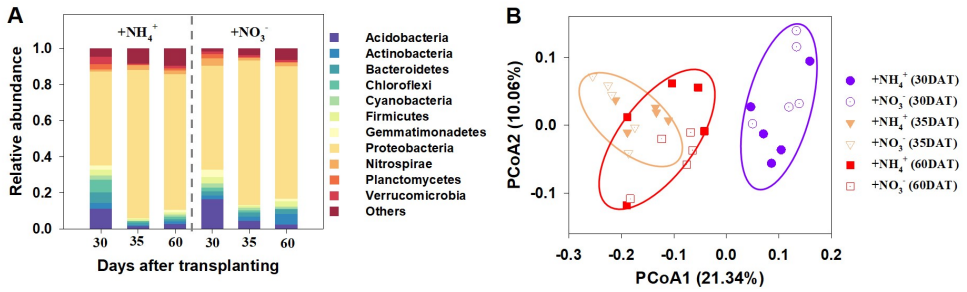

Fig. S4 Effects of inorganic-N addition on changes of microbial community composition. Genomic DNA was extracted from soils taken at DAT 30, 35 and 60 with the addition of either $^{15}NH_4^+$ or $^{15}NO_3^-$ under No-AMF treatment. (A) The relative abundance of dominant phyla (> 1%) before and after inorganic-N addition. (B) Principal co-ordinates analysis (PCoA) of community dissimilarities based on 16S rRNA gene profiling.

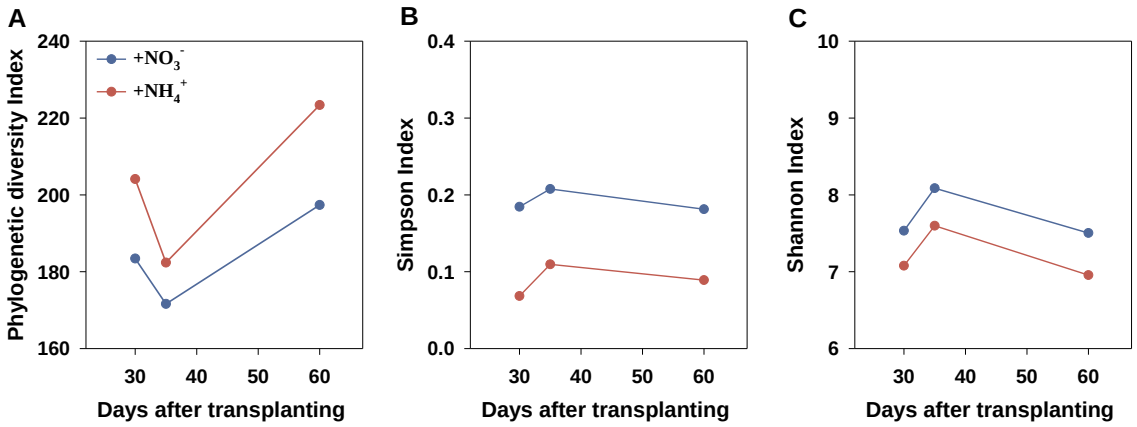

Fig. S5 The alpha diversity of microbial communities within the AMF hyphosphere. The Faith's Phylogenetic diversity index (A), Simpson index (B) and Shannon index (C) were calculated to represent the alpha diversity of microbial communities before (DAT 30) and after the addition of $^{15}NH_4^+$ or $^{15}NO_3^-$ (DAT 35 and 60) .

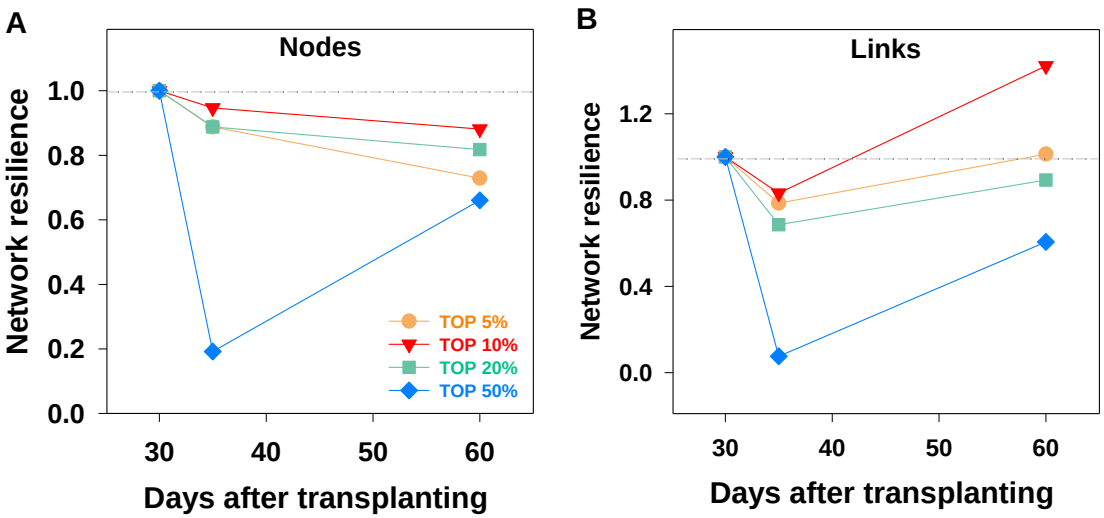

Fig. S6 Changes in the network resilience of the AMF hyphospheric microbiomes in response to inorganic-N additions. Variation in network nodes (A) and network links (B) of top 5%, 10%, 20%, 50% most abundant taxa (based on abundance ranking at DAT 30) before and after inorganic-N additions. Values are averages across both inorganic-N addition treatments ($^{15}NH_4^+$ or $^{15}NO_3^-$).

Table S1. Significance test of inorganic-N form or sampling time effects on the taxonomic of microbial communities using three different non-parametric multivariate analyses.

Microbial communities		Adonis†		ANOSIM‡		MRPP¶	
		R^2	*P*	*R*	*P*	δ	*P*
N form effect ($+NH_4^+$ vs. $+NO_3^-$)	30 DAT	0.810	0.546	-0.172	0.920	17580	0.402
	35 DAT	0.913	0.601	-0.172	0.920	50150	0.917
	60 DAT	0.844	0.198	-0.116	0.920	25990	0.446
Time effect (35 DAT vs. 30 DAT)	$+NH_4^+$	2.436	**0.050**	0.444	**0.048**	36930	**0.018**
	$+NO_3^-$	2.786	**0.050**	0.568	**0.048**	30800	**0.027**
Time effect (60 DAT vs. 35 DAT)	$+NH_4^+$	2.874	**0.050**	0.496	**0.048**	42580	**0.018**
	$+NO_3^-$	2.587	0.065	0.452	0.052	33570	**0.018**
Time effect (60 DAT vs. 30 DAT)	$+NH_4^+$	1.347	0.308	0.072	0.402	24400	0.094
	$+NO_3^-$	1.553	0.268	0.152	0.277	19170	0.109

†Adonis, per mutational multivariate analysis of variance using distance matrices.

‡ANOSIM, analysis of similarities.

¶MRPP, multi response permutation procedure.

All the *P*-values have undergone False Discovery Rate (FDR) correction. $P < 0.05$ are considered statistically significant.

Table S2. Topological properties of the empirical networks of hyphospheric microbial communities.

Sampling time (DAT)		30	35	60
Commonly present OTU NO.		6652	6486	7762
Empirical Network	Total nodes	2608	2428	1818
	Total links	4412	3096	2556
	Centralization of degree (CD)	0.014	0.021	0.021
	Average clustering coefficient (avgCC)	0.125	0.119	0.119
	Harmonic geodesic distance (HD)	6.697	6.726	6.361
	Similarity threshold	0.960	0.960	0.980
	Connectedness	0.268	0.288	0.261
	Modularity	0.847	0.858	0.851

Datasets S1.

Treatments		Days after transplanting	Replicates	Soil NH_4^++NO_3^- (mg kg^{-1} soil)
AMF	NH_4^+	30	1	7.62
			2	6.48
			3	5.96
			4	5.39
			5	5.08
		35	1	44.09
			2	40.81
			3	32.86
			4	20.31
			5	26.82
		60	1	6.06
			2	6.06
			3	5.60
			4	3.68
			5	7.03
	NO_3^-	30	1	6.37
			2	6.76
			3	5.12
			4	6.31
			5	5.41
		35	1	9.43
			2	18.70
			3	38.68
			4	13.79
			5	44.52
		60	1	5.84
			2	7.91
			3	7.65
			4	4.22
			5	4.72
No-AMF	NH_4^+	30	1	7.72
			2	6.15
			3	5.12
			4	4.14
			5	3.27
		35	1	58.25
			2	45.13
			3	35.25
			4	57.12
			5	58.13
		60	1	23.14
			2	26.45
			3	45.49
			4	43.43
			5	37.14
	NO_3^-	30	1	8.13
			2	6.36
			3	6.46
			4	4.13
			5	3.46
		35	1	40.15
			2	35.52
			3	38.25
			4	47.45
			5	58.76
		60	1	13.58
			2	36.43
			3	30.88
			4	13.43
			5	37.38

Datasets S2.

Treatments		Days after transplanting	Replicates	Plant biomass (g)	AMT%	d $^{15}N/^{14}N$	Plant ^{15}N(mg)
AMF	NH_4^+	60	1	3.47	0.75	1961.38	0.28
			2	3.94	0.75	1918.87	0.32
			3	3.22	0.97	1654.08	0.31
			4	4.72	0.95	2761.31	0.62
			5	3.61	0.92	1746.74	0.34
	NO_3^-	60	1	4.14	0.95	2592.25	0.52
			2	3.77	1.00	2114.49	0.43
			3	3.25	0.87	2315.53	0.34
			4	4.93	0.90	1783.20	0.45
			5	4.54	0.99	1716.38	0.45
No-AMF	NH_4^+	60	1	2.13	0.44	971.18	0.07
			2	1.57	0.63	918.47	0.07
			3	1.67	0.25	1274.08	0.04
			4	0.48	0.58	1761.12	0.03
			5	1.75	0.74	756.85	0.08
	NO_3^-	60	1	1.57	0.25	1585.14	0.04
			2	1.58	0.86	1211.37	0.11
			3	1.55	0.41	1105.11	0.05
			4	2.49	0.32	1983.00	0.09
			5	1.79	0.43	1826.57	0.08

R script for PCoA analysis：

```
install.packages("vegan")
library(vegan)
dist=vegdist(data, method = "bray")
pcoa=cmdscale(dist)
pcoa
scores=scores(pcoa,choices=c(1,2))
scores
```

R script for NMDS analysis:

```
install.packages("vegan")
library(vegan)
dist=vegdist(data,method="bray")
nmds=monoMDS(dist)
nmds
scores=scores(nmds,choices=c(1,2))
scores
```