Type of contribution: Regular papers

Date of preparation: 25th July, 2018

Number of text pages: 31, **number of tables:** 0, **number of figures:** 6

Number of Supplementary tables: 12, **Number of Supplementary figures:** 3

Manuscript title: Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil

Author Names: Kunkun Fan[1,2], Pamela Weisenhorn[3], Jack A. Gilbert[3,4,5], Haiyan Chu[1,*]

Affiliation:

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, 71 East Beijing Road, Nanjing 210008, China.

[2]University of Chinese Academy of Sciences, Beijing 100049, China.

[3]Argonne National Laboratory, Biosciences Division, Argonne, IL 60439, USA.

[4]Department of Surgery, University of Chicago, Chicago, IL 60637, USA.

[5]Marine Biological Laboratory, Woods Hole, MA 02543, USA.

***Corresponding author**: Haiyan Chu

Corresponding address: State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, China

Tel.: +862586881356, **Fax:** +8602586881000, **E-mail:** hychu@issas.ac.cn

Abstract

The rhizosphere harbors complex microbial communities, whose dynamic associations are considered critical for plant growth and health but remain poorly understood. We constructed co-occurrence networks for archaeal, bacterial and fungal communities associated with the rhizosphere and bulk soil of wheat fields on the North China Plain. Rhizosphere co-occurrence networks had fewer nodes, edges, modules and lower density, but maintained more robust structure compared with bulk soil, suggesting that a less complex topology and more stable co-occurrence pattern is a feature for wheat rhizosphere. Bacterial and fungal communities followed a power-law distribution, while the archaeal community did not. Soil pH and microbial diversity were significantly correlated with network size and connectivity in both rhizosphere and bulk soils. Keystone species that played essential roles in network structure were predicted to maintain a flexible generalist metabolism, and had fewer significant correlations with environmental variables, especially in the rhizosphere. These results indicate that distinct microbial co-occurrence patterns exist in wheat rhizosphere, which could be associated with variable agricultural ecosystem properties.

Key words: co-occurrence pattern; rhizosphere microbes; network structure; keystone species

1. Introduction

Agricultural ecosystems have lower plant diversity and greater spatial homogeneity when compared to natural environments, as a result of directed and persistent human intervention (Kennedy and Smith, 1995). The rhizosphere is a complex ecological and biological zone where root exudation can alter biogeochemistry and sustain microbial activity (Turner et al., 2013). Edwards *et al.* (2015) proposed a multistep model for root microbiome assembly from soil, with each root-associated compartment harboring a distinct microbiome during pure cultivation or in greenhouse. In real agricultural systems, bacterial (Fan et al., 2017) and fungal (Zhang et al., 2017) community composition have been found to differ significantly between root-associated soils and bulk soil, with a decrease in microbial diversity closer to the root (Donn et al., 2015; Fan et al., 2017). However, most studies have focused on the bacterial or fungal community in isolation, so that the interaction between archaeal, bacterial, and fungal populations in the rhizosphere and bulk soil of agricultural crops remains unclear.

Microbial communities consist of species which compete for space and resources (Hibbing et al., 2010) or engage in symbiotic interactions (Faust and Raes, 2012). Keystone species are defined as those which other species rely on such an extent that if they were removed the ecology of an ecosystem would be dramatically altered (Ze et al., 2013). Keystone species have been identified in many environments (Zaura et al., 2009) by defining the degree of node-specific interaction for taxa within co-association networks (Fisher and Mehta, 2014). Species occupying key positions in

these networks, namely as hubs or connectors, have the potential to act as keystone species, as the removal of these nodes can have outsized impact on overall network structure. Keystone species often have more defined ecological roles, such as bacteria that suppress fungal root pathogens in the rhizosphere (Mendes et al., 2011). Shi *et al.* (2016) identified keystone species in soils associated with wild oats and found that some of them had low relative abundance. However, few studies have focused on microbial keystone species among multiple kingdoms in agricultural ecosystems, let alone attempt to determine the environmental parameters that shape their distribution and co-associations.

Network analyses have been used to explore the ecological interaction patterns among microbial species in many different environments including human gut (Chow et al., 2014; Sung et al., 2017) , oceans (Fuhrman and Steele, 2008) and soils (Ma et al., 2016; Jiang et al., 2017). Co-occurrence patterns can help decipher the structure and assembly of complex microbial communities (Barberán et al., 2012), and predict potential interactions (Kara et al., 2013). Because co-occurrence patterns are based solely on simultaneous changes in pairwise taxa abundance, it is not possible to differentiate between environmental filtering (species with similar niches changing in response to the same environmental gradients) and direct interspecific interactions. However, species occupying similar niches are likely to compete under many, though not all circumstances (Tilman, 1982), so differentiation between direct interactions and environmental filtering may only be necessary when specific interactions are critical to understanding community behavior. Mendes *et al.* (2014) used co-occurrence networks

to demonstrate that the rhizosphere community was a subset of the bulk soil community, and the rhizosphere bacterial community had a less complex network compared to that of bulk soil in a short-term plantation system (Mendes et al., 2014). Ma *et al.* (2016) investigated the microbial community co-occurrence patterns of forest soil across five climate regions, demonstrating a random distribution of interactions within the archaeal community and a non-random pattern for bacterial and fungal communities. Jiang *et al.* (2017) found the alkaline phosphomonoesterase (ALP) producing *Mesorhizobium* by analyzing the network correlations between bacterivores and ALP-producing bacteria in maize rhizosphere. However, there is little information about the topological shifts of archaeal, bacterial and fungal co-occurrence interactions in rhizosphere compared with bulk soil.

The North China Plain has a long agricultural history with a wheat-maize rotation system (Zhao et al., 2006; Liu et al., 2010). Wheat (*Triticumaestivum L.*) is one of the most important crops globally, however, the increase of wheat productivity has slowed down to 0.9% per year (Fischer and Edmeades, 2010). One potential way to increase wheat productivity is by manipulating microbial community interactions that support plant health, especially those in the rhizosphere. In this study, we investigated archaeal, bacterial, and fungal communities in wheat rhizosphere and bulk soil on the North China Plain. We proposed two hypotheses: *1) Microbial co-occurrence patterns in wheat rhizosphere are distinct from those in bulk soil, which is affected by both abiotic and biotic factors; 2) The keystone microbial species are usually metabolic generalists that demonstrate fewer correlations with environmental variables.*

2. Materials and Methods

2.1. Sample collection and soil physiochemical analysis

Samples were collected from nine sampling sites across the typical wheat planting fields (32° N~38° N; 110° E~118° E) on the North China Plain during the wheat filling stage (22^{th} -27^{th} of the May, 2015). The soil type in most sampling sites were Fluvic Calcaric Eutric Cambisols, Haplic Luvisols, Cambic Calcisols, Calcaric Eutric Cambisols and Endocalcaric Luvisols according to the soil taxonomy of FAO. At each sampling site (~100 km^2 plot), five replicate locations were sampled. In each location, ten to twelve wheat plants were extracted. After shaking off the loosely bound soil, we brushed off the tightly adhered soil, which serve as rhizosphere soil (RS). Beside each wheat group, the topsoil (0-15 cm) without plants were collected by soil auger, which serve as bulk soil (BS). Soil pH was determined by pH meter (Thermo Orion-868) with a 1:5 fresh soil to water ratio. Soil texture was determined by using Laser Particle Sizer (LS13320). Soil moisture was determined gravimetrically by drying 5 g fresh soil to the constant weight under 105 ℃ for 12 hours. Total carbon (TC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were determined by $K_2Cr_2O_7$-H_2SO_4 oxidation method, semi-micro Kjeldahl method, Mo-Sb colorimetry method and flame spectrophotometry method, respectively.

2.2. High throughput sequencing

DNA was extracted from 0.5 g fresh soil using the Power Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The archaeal and bacterial 16S rRNA genes were amplified by primer pairs 524F-10-ext

(5’-TGYCAGCCGCCGCGGTAA-3’)/Arch958-modR(5’-YCCGGCGTTGAVTCCAATT-3’) (Baker et al., 2003) and 515F (5'-GTGCCAGCMGCCGCGGTAA-3’) / 907R (5'-CCGTCAATTCCTTTGAGTTT-3’) (Biddle et al., 2008), respectively; the fungal ITS2 region was amplified by primer pair ITS3 (5'-GCATCGATGAAGAACGCAGC-3') /ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gade et al., 2013). The sequences have been submitted to the NCBI Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra/SRP117302) with accession number SRP 117302.

2.3. Sequence analysis

The Quantitative Insight into Microbial Ecology (QIIME) pipeline (http://qiime.sourceforge.net/) was used to analyze the sequence data (Caporaso et al., 2010). 1,545,509 high quality sequences of archaea; 3,595,706 high quality sequences of bacteria; 2,383,721 high quality sequences of fungi were acquired after removing < 200 bp long and average quality score < 25 reads. OTUs were generated based on a 97% similarity level through UCLUST (Edgar, 2010). The greengenes database (http://greengenes.lbl.gov/) was used to assign the taxonomic identity of each phylotype of archaea and bacteria; fungal taxonomic identity was determined using the UNITE database (Kõljalg et al., 2005).

2.4. Statistical analysis

NMDS (based on Bray-Curtis distance), Mantel test, Envfit, ANOSIM, MRPP and ADONIS analyses were conducted using the ‘vegan’ R package (Oksanen et al., 2013) in R×32 (3.2.2) (https://CRAN.R-project.org/package=vegan). And the

physicochemical parameters were fitted on the NMDS map based on non-permutation regression. The rank abundance distribution, which was calculated by the frequency of sequences (OTU Table), was used to test whether stochastic or deterministic processes best explain the community assembly of archaeal, bacterial, and fungal communities. TeTame (Jabot et al., 2008) was used to test whether a rank abundance was consistent with zero-sum multinomial (ZSM) distribution, predicting the dominance of stochastic processes (Hubbell et al., 2001). Tests for dominance of deterministic processes, i.e. rank abundance distributions fitting the Broken stick model, Pre-emption model, Log-normal model, or Zipf-Mandlebrot model were performed using 'radfit' in the 'vegan' R package (Oksanen, 2010).

The co-occurrence network was constructed with the 'WGCNA' R package based on the Spearman correlation matrix (Langfelder and Horvath, 2012). We removed OTUs occurring in less than 30% of all samples, kept OTUs with relative abundances greater than 0.01% for archaeal, bacterial, and fungal communities (Ma et al., 2016). The nodes and the edges in the network represent OTUs and the correlations between pairs of OTUs, respectively. P-values were adjusted by Benjamini and Hochberg false discovery rate (FDR) test (Benjamini et al., 2006), and the adjusted P-values had a 0.001 cutoff. We calculated the network properties with the 'igraph' R package (http://igraph.org), and generated network images with Gephi (https://gephi.org/). The natural connectivity provides sensitive discrimination of network structural robustness, we estimated network stability by removing nodes in the static network to assess how quickly robustness degraded and we assessed network robustness by natural

connectivity (Peng and Wu, 2016).

In addition, the z-score and c-score cut-offs were based on the methods of metabolic networks (Guimera and Amaral, 2005). Here we define nodes as network hubs (z-score > 2.5; c-score > 0.6), module hubs (z-score > 2.5; c-score < 0.6), connectors (z-score < 2.5; c-score > 0.6) and peripherals (z-score < 2.5; c-score < 0.6), based on their within-module degree (z-score) and participation coefficient (c-score) threshold value (Poudel et al., 2016), which could determine how the node is positioned within a specific module or how it interacts with other modules (Rives and Galitski, 2003; Han et al., 2004). The network hubs were highly connected, both in general and within a module, the module hubs were highly connected within a module, the connectors provided links among multiple modules, and the peripherals had few links to other species (Poudel et al., 2016). Network hubs, module hubs, and connectors were termed keystone network topological features; these are considered to play important roles in the stability and resistance of microbial communities (Tylianakis and Morris, 2017); thus, we define the OTUs associated with these nodes as keystone species.

3. Results

3.1. Microbial community and co-occurrence patterns in wheat rhizosphere and bulk soil

For the microbial community, the archaeal community composition did not differ significantly between wheat rhizosphere and bulk soil, while the relative abundance of most dominant bacteria (*Proteobacteria*, *Actinobacteria*, *Acidobacteria, Bacteroidetes*) and fungi (*Sordariomycetes*, *Dothideomycetes*) in wheat rhizosphere

were significantly different from those of bulk soil (Table S1). Further, the archaeal community had no clear clustering by soil compartments (rhizosphere vs bulk soil), while bacterial and fungal community structure showed significant clustering by soil compartments (ANOSIM; bacteria: $R = 0.51$, $P = 0.001$; fungi: $R = 0.50$, $P = 0.001$; Table S3). In addition, most of archaeal (P-value < 0.0001), bacterial (P-value < 0.0001) and fungal (P-value < 0.0001) diversity indices were significantly lower in the rhizosphere when compared to the bulk soil (Table S2).

When constructing the co-occurrence network, the empirical network structures were verified as reliable and nonrandom comparing with random network analysis (Table S4). Multiple network topological metrics consistently showed that microbial co-occurrence pattern in the rhizosphere differed profoundly from the bulk-soil network. Rhizosphere formed smaller networks with less nodes (2209) than the bulk-soil network (2310); meanwhile the rhizosphere networks contained less connections (edges) between nodes which decreased the density of connections and kept only one cluster and fewer modules which created less intricate rhizosphere network patterns when compared with bulk-soil network (Fig 1A). In addition, the rhizosphere network had more positive correlations (63.2%) especially intraspecific correlations within archaeal, bacterial, and fungal communities (Table S5) when compared with the bulk-soil network (55.7%) (Fig 1A). The natural connectivity in the rhizosphere network was greater than that of bulk soil (Fig 1B), indicating greater network robustness in rhizosphere. There were larger number of correlations within each kingdom than between kingdoms, and the network degree for the fungal community was lower than

217 that of the archaeal or bacterial communities, in both bulk soil and the rhizosphere
218 (Table S6). At the taxonomic level of order, the correlations within the same order were
219 also greater than those between orders (Table S7).

220 Network degree for paired bacterial and fungal nodes obeyed a power-law
221 distribution (BS: $P < 0.001$; RS: $P < 0.001$), indicating a non-random distribution
222 pattern, while the degree for paired archaeal nodes did not follow a typical power-law
223 distribution pattern (BS: $P = 0.03$; RS: $P = 0.02$) with little fluctuation for the degree of
224 each node (Fig 2; Fig S1). In addition, the network degree did not increase with the
225 relative abundance of species: the OTUs with high degree often had medium or low
226 relative abundance while the OTUs with high relative abundance sometimes had low
227 degrees (Fig S2). When combining the different models with AIC values, a zero-sum
228 multinomial model gave the best fit for the rank abundance distribution of archaeal taxa
229 in most samples of bulk soil (64.4%) and rhizosphere (71.1%), suggesting stochastic
230 processes for archaeal community assembly. The rank abundance distribution of
231 bacterial taxa in most bulk soil (71.1%) and rhizosphere (95.6%) samples had a good
232 fit with the Zipf-Mandelbrot model, suggesting niche-based community assembly; the
233 rank abundance distribution of fungal taxa in most samples of bulk soil (68.9%) and
234 rhizosphere (62.3%) also had a good fit with the Zipf-Mandelbrot model, but also with
235 the Log-normal model and Zipf model, which also suggested niche-based community
236 assembly (Table S8).

237 ***3.2. Abiotic and biotic factors influencing the microbial co-occurrence patterns in***
238 ***wheat rhizosphere and bulk soil***

We used a mantel test (Table S9) which demonstrated that soil pH described the greatest variance in archaeal, bacterial, and fungal community structures in both bulk and rhizosphere soil, which is consistent with the results of the envfit analysis (Fig 3). We also calculated how co-occurrence network complexity correlated with environmental variables. In bulk soil, network size (number of nodes) and network connectivity (number of edges) were significantly correlated with soil pH, moisture and total phosphorus. While in the rhizosphere, network size was uncorrelated with any environmental factors, and network connectivity was correlated with soil pH and moisture (Table S10). Finally, microbial diversity has been widely used to determine the influence of biotic factors on the microbial co-occurrence patterns (Shi et al., 2016), and we found that increased network size and connectivity in the rhizosphere was correlated with an increase in archaeal, bacterial, and fungal Shannon diversity (Fig 4).

3.3. Keystone species in wheat rhizosphere and bulk soil

The proportion of network hubs (BS:0.5%; RS:0.05%) and module hubs (BS:0.7%; RS:0) decreased from bulk soil to rhizosphere while the proportion of connectors increased from bulk soil (17.8%) to the rhizosphere (28.8%; Fig S3), indicating a less hub-based and more connected network structure in the rhizosphere. Although the proportion of connectors for bacteria increased from the bulk soil to the rhizosphere, the proportion decreased for archaea and did not significantly change for fungi (Fig 5A). For archaea, there were eight network hub OTUs, which are most closely related to *Thermococcus waiotapuensis* (2 OTUs), *Methanomassiviicoccus luminyensis* (5 OTUs), and *Nitrososphaera viennensis* (1 OTU), and nine module hub

OTUs belonging to *Methanomassiviicoccus luminyensis* (4 OTUs) and *Nitrososphaera viennensis* (5 OTUs) (Fig 5A; Table S11). For bacteria, there were two module hub OTUs in bulk soil belonging to *Gemmatimonas phototrophica* and *Edaphobacter aggregans*, and one single network hub in rhizosphere belonging to *Pseudonocardia seranimata*. For fungi, there were three network hubs belonging to *Chaetomium piluliferum* (1 OTUs) and *Aspergillus piperis* (2 OTUs), and two module hubs belonging to *Pleurophama sp* and *Cercophara caudate* (Fig 5A; Table S11). Although the number of connector archaeal OTUs decreased in the rhizosphere, the relative abundance of the *E2* and *Nitrososphaerales* orders increased. The relative abundance of connector bacterial OTUs belonging to *Gammaproteobacteria*, *Acidobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, and *Verrucomicrobia* all increased in the rhizosphere compared to the bulk soil. The relative abundance of connector fungal OTUs belonging to *Agaricomycetes*, *Dothideomycetes,* and *Leotiomycetes* increased in the rhizosphere, while *Eurotiomycetes* and *Sordariomycetes* decreased (Fig 5B).

3.4. The environmental factors influencing the keystone species

We constructed new networks by correlating the relative abundance of keystone species with soil physicochemical properties (Fig 6; Table S12). The physicochemical properties had no significant correlation with the majority of the keystone species in both bulk soil (67.3%) and rhizosphere (75.7%), and, when significant correlations were present, the strength of correlations decreased in the rhizosphere (Fig 6A). When considering the correlated links, the proportion of negative correlations between

keystone species and soil properties was greater than the number of positive correlations, and the negative correlations were numerically greater in the rhizosphere (60.2%) compared to bulk soil (51.9%) (Fig 6B). Soil pH, total phosphorus and moisture content were correlated with more keystone species than other soil properties in both bulk soil and rhizosphere soil, while the total potassium was more significantly associated with the keystone species in the rhizosphere than that in the bulk soil (Fig 6B; TableS12).

4. Discussion

4.1. A less complex but more stable microbial co-occurrence pattern in wheat rhizosphere

In our study, the microbial co-occurrence network in wheat rhizosphere showed less negative correlations, which could be expected as the rhizosphere is a resource-enriched soil compartment, with approximately 17% of carbon fixed by photosynthesis transferred to the root-associated soil through root exudation (Nehls et al., 2016). These additional resources reduce competition; thus they may allow more species to maintain free-living populations (Hubbell, 2005; Costello et al., 2012). The network structure of the microbial community in the rhizosphere was more stable than in bulk soil, which to some extent reflects the less dynamic structure (Costa et al., 2006) and greater ecological stability of the rhizosphere (Thébault and Fontaine, 2010). In addition, microbial interactions were consistently stronger within a kingdom than between kingdoms, even at the order or genus level. This pattern of OTUs being more likely to co-occur with other OTUs of the same kingdom may be a reflection of similar

environmental filtering processes (Baker et al., 2017).

Both abiotic and biotic factors showed significant correlation with the variance in microbial co-occurrence patterns in wheat rhizosphere and bulk soil. While pH, moisture and phosphorus were significantly correlated with network properties in bulk soil, fewer environmental factors correlated with rhizosphere network properties. For the biotic properties, the increased network size and connectivity in the rhizosphere was accompanied by increasing archaeal, bacterial and fungal Shannon diversity. Also, consistent with the multistep model (Edwards et al., 2015) of root filtering, archaeal, bacterial, and fungal alpha diversity decreased from bulk soil to the rhizosphere, which was also associated with a decreased network complexity in wheat rhizosphere.

4.2. Varied degree distribution patterns for different microbial communities

We found that the network degree of OTUs within the fungal community were lower than those within the archaeal or bacterial communities, potentially due to the differences in the predicted metabolism for these organisms. The majority of archaea and bacteria respond much more rapidly to changes in environmental conditions and the abundance of other taxa compared to eukaryotes such as fungi (Paul, 2014). Fungi can degrade complex organic matter outside the cell enzymatically, therefore reducing their obligate dependency on bacterial and archaeal taxa (Pivato et al., 2008); this could explain why fungi had fewer correlative interactions.

Our study also showed that the distribution of interactions among bacterial and fungal OTUs followed a power-law distribution, whereas the distribution of archaeal interactions did not. A power-law network structure is more widespread than the

binomial distribution pattern, and many real-world (internet, social and biological) networks follow the power-law distribution (Adamic and Huberman, 2000; Bergman and Siegal, 2003). The non-power-law distribution for archaeal degree indicated that each interaction between paired archaeal OTUs was equally likely (Newman, 2003; Ma et al., 2016), suggesting increased distributional homogenization for archaeal species across the different communities analyzed. We speculated that the variable degree distribution patterns for different microbial communities were probably associated with their assembly processes as seen for forest soils (Ma et al., 2016). Further supporting this, our analysis of the rank abundance distributions for different taxa showed that the archaeal community was mainly influenced by stochastic processes, while bacterial and fungal communities were dominated by deterministic factors in both bulk soil and rhizosphere. Zheng *et al.*（2013) also found that stochastic processes mainly influenced soil archaeal diversity patterns. Decreased variation between compartments and the dominance of stochastic assembly processes might be associated with a decreased habitat dependence for the archaeal community (Galand et al., 2008).

4.3. Network structure of keystone species and ecological functions

Topologically, network hubs and connectors represent the regulators, mediators or adaptors. Module hubs can be regarded as integral elements within distinct modules, which may mediate important functions but tend to function at a lower level within the overall community (Han et al., 2004). Generally, connectors are more conserved than hubs (Guimera and Amaral, 2005). We defined the hubs and connectors as keystone species, by which we mean if these taxa were removed, the modules and networks may

also break apart; thus, they play essential roles in network structure and could be identified as targets for microbial modulation to improve crop productivity (Olesen et al., 2007).

These keystone species have also vital ecological functions in the microbial community. The archaeal network hubs, *Thermococcus waiotapuensis* and *Methanomassiliicoccus luminyensis*, have been shown to grow under a wide range of environmental conditions and as such have flexible metabolic and physiological requirements (González et al., 1999; Dridi et al., 2012). Meanwhile, the archaeal module hub *Nitrososphaera viennensis* (Kerou et al., 2016) has the capacity for biofilm formation, cell surface modification, carbohydrate conversions and detoxification. The bacterial network hub, *Pseudonocardia seranimata*, has diverse carbon metabolisms and cooperates with fungal species by stimulating the fungal production of branched substrate mycelium and aerial hyphae (Zhang et al., 2014). The bacterial module hub *Gemmatimonas phototrophica* has been found to contain purple bacterial photosynthetic reaction centers which probably control carbon synthesis within specific modules (Zeng et al., 2016) while the module hub *Edaphobacter aggregans* can adapt to low carbon concentrations and slightly acidic conditions, and is often associated with methane-oxidizing proteobacterial species (Koch et al., 2008). The bacterial connector species belonging to the phyla *Bacteroidetes* and Verrucomicrobia have been reported as important contributors to nutrient turnover (Werner and Newton, 2005), especially in the rhizosphere (Yousuf et al., 2012; Chaparro et al., 2014). The bacterial connectors belonging to the phylum *Proteobacteria* are considered fast growers with the ability to

371 utilize a majority of root-derived carbon substrates (Lauber et al., 2009; Philippot et al.,
372 2013). For fungal network hubs, *Chaetomium piluliferum* (Garg and Modi, 1999) and
373 *Aspergillus piperis* (Nielsen et al., 2009) are known lignin degraders and play essential
374 roles in carbon and nitrogen cycling through cooperation with other organisms. The
375 fungal connector species belonging to *Agaricomycetes* (Drechsler-Santos et al., 2009),
376 *Dothideomycetes* (Schoch et al., 2009), and *Leotiomycetes* (Wang et al., 2006) have the
377 potential to improve nutrient acquisition and combat pathogenic taxa, and maintain
378 cooperative metabolic associations with other species. In general, network hubs,
379 module hubs and connectors had diverse metabolisms.

380 In our study, although overall network topological features were correlated with
381 abiotic factors, most of the keystone species were not. The keystone species in wheat
382 rhizosphere were less correlated to the changes in environmental conditions, and as
383 such any disturbances may be expected to spread more slowly through those networks
384 with more keystone species in the rhizosphere, but once disrupted, the ecosystem will
385 have difficulty in recovering (Olesen et al., 2007).

386 In conclusion, the network topological features of microbial communities
387 showed dramatic dissimilarity between wheat rhizosphere and bulk soils. The microbial
388 co-occurrence pattern in rhizosphere was less complex but more stable than that in bulk
389 soil, and the microbial diversity was positively correlated with network size and
390 connectivity. The keystone species were identified by their network position (i.e.,
391 network hubs, module hubs, and connectors) and were predicted to have greater
392 metabolic and phenotypic flexibility than other taxa. The keystone species were less

likely to correlate with changing soil physicochemical parameters, especially in the rhizosphere. Our results suggest a more stable and flexible microbial network structure within wheat rhizosphere, which might be associated with ecosystem functions in the agricultural soils.

Acknowledgements

We thank Yu Shi, Yuntao Li, Ruibo Sun, Congcong Shen, Xingjia Xiang, Dan He, Kaoping Zhang, Yingying Ni and Teng Yang for their assistance in soil sampling and lab analyses. This work was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15010101) and the National Program on Key Basic Research Project (2014CB954002). The authors declare no conflict of interest.

References

Adamic, L.A., Huberman, B.A., 2000. Power-law distribution of the world wide web. Science 287, 2115-2115.

Baker, G., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. Journal of Microbiological Methods 55, 541-555.

Baker, J.L., Bor, B., Agnello, M., Shi, W., He, X., 2017. Ecology of the Oral Microbiome: Beyond Bacteria. Trends in Microbiology.

Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6, 343-351.

Benjamini, Y., Krieger, A.M., Yekutieli, D., 2006. Adaptive linear step-up procedures that control the false discovery rate. Biometrika, 491-507.

Bergman, A., Siegal, M.L., 2003. Evolutionary capacitance as a general feature of complex gene networks. Nature 424, 549-552.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E., House, C.H., 2008. Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. Proceedings of the National Academy of Sciences of the United States of America 105, 10583-10588.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335.

Chaparro, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. The ISME Journal 8, 790-803.

Chow, C.-E.T., Kim, D.Y., Sachdeva, R., Caron, D.A., Fuhrman, J.A., 2014. Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. The ISME Journal 8, 816-829.

Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., Smalla, K., 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiology Ecology 56, 236-249.

Costello, E.K., Stagaman, K., Dethlefsen, L., Bohannan, B.J., Relman, D.A., 2012. The application of ecological theory toward an understanding of the human microbiome. Science 336, 1255-1262.

Donn, S., Kirkegaard, J.A., Perera, G., Richardson, A.E., Watt, M., 2015. Evolution of bacterial communities in the wheat crop rhizosphere. Environmental Microbiology 17,

610-621.

Drechsler-Santos, E.R., Gibertoni, T.B., Góes-Neto, A., Cavalcanti, M.A.Q., 2009. A re-evaluation of the lignocellulolytic Agaricomycetes from the Brazilian semi-arid region. Mycotaxon 108, 241-244.

Dridi, B., Fardeau, M.-L., Ollivier, B., Raoult, D., Drancourt, M., 2012. Methanomassiliicoccus luminyensis gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. International Journal of Systematic and Evolutionary Microbiology 62, 1902-1907.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-2461.

Edwards, J., Johnson, C., Santos-Medellin, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A., Sundaresan, V., 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proceedings of the National Academy of Sciences of the United States of America 112, E911-920.

Fan, K., Cardona, C., Li, Y., Shi, Y., Xiang, X., Shen, C., Wang, H., Gilbert, J.A., Chu, H., 2017. Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. Soil Biology and Biochemistry 113, 275-284.

Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nature Reviews Microbiology 10, 538-550.

Fischer, R.A., Edmeades, G.O., 2010. Breeding and Cereal Yield Progress. Crop Science 50, S-85-S-98.

459 Fisher, C.K., Mehta, P., 2014. Identifying keystone species in the human gut
460 microbiome from metagenomic timeseries using sparse linear regression. PloS One 9,
461 e102451.
462 Fuhrman, J.A., Steele, J.A., 2008. Community structure of marine bacterioplankton:
463 Patterns, networks, and relationships to function. Aquatic Microbial Ecology 53, 69-81.
464 Gade, L., Scheel, C.M., Pham, C.D., Lindsley, M.D., Iqbal, N., Cleveland, A.A.,
465 Whitney, A.M., Lockhart, S.R., Brandt, M.E., Litvintseva, A.P., 2013. Detection of
466 fungal DNA in human body fluids and tissues during a multistate outbreak of fungal
467 meningitis and other infections. Eukaryotic Cell 12, 677-683.
468 Galand, P.E., Lovejoy, C., Pouliot, J., Vincent, W.F., 2008. Heterogeneous archaeal
469 communities in the particle-rich environment of an arctic shelf ecosystem. Journal of
470 Marine Systems 74, 774-782.
471 Garg, S.K., Modi, D.R., 1999. Decolorization of pulp-paper mill effluents by white-rot
472 fungi. Critical Reviews in Biotechnology 19, 85-112.
473 González, J.M., Sheckells, D., Viebahn, M., Krupatkina, D., Borges, K.M., Robb, F.T.,
474 1999. Thermococcus waiotapuensis sp. nov., an extremely thermophilic archaeon
475 isolated from a freshwater hot spring. Archives of Microbiology 172, 95-101.
476 Guimera, R., Amaral, L.A.N., 2005. Functional cartography of complex metabolic
477 networks. Nature 433, 895.
478 Han, J.-D.J., Bertin, N., Tong, H., Goldberg, D.S., 2004. Evidence for dynamically
479 organized modularity in the yeast protein-protein interaction network. Nature 430, 88.
480 Hibbing, M.E., Fuqua, C., Parsek, M.R., Peterson, S.B., 2010. Bacterial competition:

481 surviving and thriving in the microbial jungle. Nature Reviews Microbiology 8, 15-25.
482 Hubbell, S.P., 2005. Neutral theory in community ecology and the hypothesis of
483 functional equivalence. Functional Ecology 19, 166-172.
484 Hubbell, S.P., Ahumada, J.A., Condit, R., Foster, R.B., 2001. Local neighborhood
485 effects on long‐term survival of individual trees in a Neotropical forest. Ecological
486 Research 16, 859-875.
487 Jabot, F., Etienne, R.S., Chave, J., 2008. Reconciling neutral community models and
488 environmental filtering: theory and an empirical test. Oikos 117, 1308-1320.
489 Jiang, Y., Liu, M., Zhang, J., Chen, Y., Chen, X., Chen, L., Li, H., Zhang, X.-X., Sun,
490 B., 2017. Nematode grazing promotes bacterial community dynamics in soil at the
491 aggregate level. The ISME Journal 11, 2705.
492 Kõljalg, U., Larsson, K.-H., Abarenkov, K., Nilsson, R.H., Alexander, I.J., Eberhardt,
493 U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S.,
494 Tedersoo, L., Vrålstad, T., 2005. UNITE: a database providing web-based methods for
495 the molecular identification of ectomycorrhizal fungi. New Phytologist 166, 1063-1068.
496 Kara, E.L., Hanson, P.C., Hu, Y.H., Winslow, L., McMahon, K.D., 2013. A decade of
497 seasonal dynamics and co-occurrences within freshwater bacterioplankton
498 communities from eutrophic Lake Mendota, WI, USA. The ISME Journal 7, 680-684.
499 Kennedy, A., Smith, K., 1995. Soil microbial diversity and the sustainability of
500 agricultural soils. Plant and Soil 170, 75-86.
501 Kerou, M., Offre, P., Valledor, L., Abby, S.S., Melcher, M., Nagler, M., Weckwerth, W.,
502 Schleper, C., 2016. Proteomics and comparative genomics of Nitrososphaera

503 viennensis reveal the core genome and adaptations of archaeal ammonia oxidizers.
504 Proceedings of the National Academy of Sciences of the United States of America 113,
505 7937.
506 Koch, I.H., Gich, F., Dunfield, P.F., Overmann, J., 2008. Edaphobacter modestus gen.
507 nov., sp. nov., and Edaphobacter aggregans sp. nov., acidobacteria isolated from alpine
508 and forest soils. International Journal of Systematic and Evolutionary Microbiology 58,
509 1114-1122.
510 Langfelder, P., Horvath, S., 2012. Fast R functions for robust correlations and
511 hierarchical clustering. Journal of Statistical Software 46.
512 Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based
513 assessment of soil pH as a predictor of soil bacterial community structure at the
514 continental scale. Applied Environmental Microbiology 75, 5111-5120.
515 Liu, Y., Wang, E., Yang, X., Wang, J., 2010. Contributions of climatic and crop varietal
516 changes to crop production in the North China Plain, since 1980s. Global Change
517 Biology 16, 2287-2299.
518 Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z., Brookes, P.C., Xu, J., Gilbert,
519 J.A., 2016. Geographic patterns of co-occurrence network topological features for soil
520 microbiota at continental scale in eastern China. The ISME Journal 10, 1891-1901.
521 Mendes, L.W., Kuramae, E.E., Navarrete, A.A., Van Veen, J.A., Tsai, S.M., 2014.
522 Taxonomical and functional microbial community selection in soybean rhizosphere.
523 The ISME Journal 8, 1577.
524 Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.,

525 Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A., 2011. Deciphering the
526 rhizosphere microbiome for disease-suppressive bacteria. Science 332, 1097-1100.
527 Nehls, U., Das, A., Neb, D., 2016. Carbohydrate metabolism in ectomycorrhizal
528 symbiosis. Molecular Mycorrhizal Symbiosis 10, 161-178.
529 Newman, M.E., 2003. The structure and function of complex networks. SIAM Review
530 45, 167-256.
531 Nielsen, K.F., Mogensen, J.M., Johansen, M., Larsen, T.O., Frisvad, J.C., 2009. Review
532 of secondary metabolites and mycotoxins from the Aspergillus niger group. Analytical
533 and Bioanalytical Chemistry 395, 1225-1242.
534 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson,
535 G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan'. Community
536 Ecology Package, Version 2.
537 Oksanen, P., 2010. Vegan 1.17-0 in R version 2.10. 1 (2009-12-14). URL http. cc. oulu.
538 fi~ jarioksa/softhelp. vegan. html.
539 Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of
540 pollination networks. Proceedings of the National Academy of Sciences of the United
541 States of America 104, 19891-19896.
542 Paul, E.A., 2014. Soil microbiology, ecology and biochemistry. Academic Press.
543 Peng, G.-S., Wu, J., 2016. Optimal network topology for structural robustness based on
544 natural connectivity. Physica A: Statistical Mechanics and its Applications 443, 212-
545 220.
546 Philippot, L., Raaijmakers, J.M., Lemanceau, P., Van Der Putten, W.H., 2013. Going

547 back to the roots: the microbial ecology of the rhizosphere. Nature Reviews
548 Microbiology 11, 789.
549 Pivato, B., Gamalero, E., Lemanceau, P., Berta, G., 2008. Colonization of adventitious
550 roots of Medicago truncatula by Pseudomonas fluorescens C7R12 as affected by
551 arbuscular mycorrhiza. FEMS Microbiology Letters 289, 173-180.
552 Poudel, R., Jumpponen, A., Schlatter, D., Paulitz, T., Gardener, B.M., Kinkel, L.,
553 Garrett, K., 2016. Microbiome networks: A systems framework for identifying
554 candidate microbial assemblages for disease management. Phytopathology 106, 1083-
555 1096.
556 Rives, A.W., Galitski, T., 2003. Modular organization of cellular networks. Proceedings
557 of the National Academy of Sciences of the United States of America 100, 1128-1133.
558 Schoch, C.L., Crous, P.W., Groenewald, J.Z., Boehm, E.W.A., Burgess, T.I., de Gruyter,
559 J., de Hoog, G.S., Dixon, L.J., Grube, M., Gueidan, C., Harada, Y., Hatakeyama, S.,
560 Hirayama, K., Hosoya, T., Huhndorf, S.M., Hyde, K.D., Jones, E.B.G., Kohlmeyer, J.,
561 Kruys, Å., Li, Y.M., Lücking, R., Lumbsch, H.T., Marvanová, L., Mbatchou, J.S.,
562 McVay, A.H., Miller, A.N., Mugambi, G.K., Muggia, L., Nelsen, M.P., Nelson, P.,
563 Owensby, C.A., Phillips, A.J.L., Phongpaichit, S., Pointing, S.B., Pujade-Renaud, V.,
564 Raja, H.A., Plata, E.R., Robbertse, B., Ruibal, C., Sakayaroj, J., Sano, T., Selbmann, L.,
565 Shearer, C.A., Shirouzu, T., Slippers, B., Suetrong, S., Tanaka, K., Volkmann-
566 Kohlmeyer, B., Wingfield, M.J., Wood, A.R., Woudenberg, J.H.C., Yonezawa, H.,
567 Zhang, Y., Spatafora, J.W., 2009. A class-wide phylogenetic assessment of
568 Dothideomycetes. Studies in Mycology 64, 1-15-S10.

569 Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The
570 interconnected rhizosphere: high network complexity dominates rhizosphere
571 assemblages. Ecology Letters 19, 926-936.
572 Sung, J., Kim, S., Cabatbat, J.J.T., Jang, S., Jin, Y.S., Jung, G.Y., Chia, N., Kim, P.J.,
573 2017. Global metabolic interaction network of the human gut microbiota for context-
574 specific community-scale analysis. Nature Communications 8, 15393.
575 Thébault, E., Fontaine, C., 2010. Stability of ecological communities and the
576 architecture of mutualistic and trophic networks. Science 329, 853-856.
577 Tilman, D., 1982. Resource competition and community structure. Princeton University
578 Press.
579 Turner, T.R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D.,
580 Osbourn, A., Grant, A., Poole, P.S., 2013. Comparative metatranscriptomics reveals
581 kingdom level changes in the rhizosphere microbiome of plants. The ISME Journal 7,
582 2248-2258.
583 Tylianakis, J.M., Morris, R.J., 2017. Ecological networks across environmental
584 gradients. Annual Review of Ecology, Evolution, and Systematics 48, 25-48.
585 Wang, Z., Johnston, P.R., Takamatsu, S., Spatafora, J.W., Hibbett, D.S., 2006. Toward
586 a Phylogenetic Classification of the Leotiomycetes Based on rDNA Data. Mycologia
587 98, 1065-1075.
588 Werner, D., Newton, W.E., 2005. Nitrogen fixation in agriculture, forestry, ecology, and
589 the environment. Springer Science & Business Media.
590 Yousuf, B., Keshri, J., Mishra, A., Jha, B., 2012. Application of targeted metagenomics

591 to explore abundance and diversity of CO 2-fixing bacterial community using cbbL
592 gene from the rhizosphere of Arachis hypogaea. Gene 506, 18-24.
593 Zaura, E., Keijser, B.J., Huse, S.M., Crielaard, W., 2009. Defining the healthy" core
594 microbiome" of oral microbial communities. BMC Microbiology 9, 259.
595 Ze, X., Le Mougen, F., Duncan, S.H., Louis, P., Flint, H.J., 2013. Some are more equal
596 than others: the role of “keystone” species in the degradation of recalcitrant substrates.
597 Gut Microbes 4, 236-240.
598 Zeng, Y., Baumbach, J., Barbosa, E.G.V., Azevedo, V., Zhang, C., Koblížek, M., 2016.
599 Metagenomic evidence for the presence of phototrophic Gemmatimonadetes bacteria
600 in diverse environments. Environmental Microbiology Reports 8, 139-149.
601 Zhang, D.-F., Jiang, Z., Li, L., Liu, B.-B., Zhang, X.-M., Tian, X.-P., Zhang, S., Li, W.-
602 J., 2014. Pseudonocardia sediminis sp. nov., isolated from marine sediment.
603 International Journal of Systematic and Evolutionary Microbiology 64, 745-750.
604 Zhang, K., Adams, J.M., Shi, Y., Yang, T., Sun, R., He, D., Ni, Y., Chu, H., 2017.
605 Environment and geographic distance differ in relative importance for determining
606 fungal community of rhizosphere and bulk soil. Environmental Microbiology 19, 3649-
607 3659.
608 Zhao, R.-F., Chen, X.-P., Zhang, F.-S., Zhang, H., Schroder, J., Römheld, V., 2006.
609 Fertilization and nitrogen balance in a wheat–maize rotation system in North China.
610 Agronomy Journal 98, 938-945.
611 Zheng, Y.-M., Cao, P., Fu, B., Hughes, J.M., He, J.-Z., 2013. Ecological drivers of
612 biogeographic patterns of soil archaeal community. PloS One 8, e63375.

613

614 **Figure legends**

615 **Fig 1** The co-occurrence network interactions of soil archaea, bacteria, and fungi (A);
616 and the robustness of microbial network for bulk soil and rhizosphere (B). The edges
617 refer to significant (P-value < 0.001) correlations; the nodes represent unique OTUs in
618 the data sets. The size of each node is proportional to the relative abundance. BS:bulk
619 soil; RS:Rhizosphere soil.

620 **Fig 2** The network degree distribution patterns of archaea, bacteria, and fungi in bulk
621 soil and rhizosphere. BS:bulk soil; RS:Rhizosphere soil.

622 **Fig 3** Archaeal, bacterial, and fungal community structures (based on Bray-Curtis
623 distance) in the soils as indicated by non-metric multidimensional scaling plots, and the
624 environmental factors are fitted on the plot by doing envfit analysis. BS:bulk soil;
625 RS:Rhizosphere soil. TC: total carbon; TN: total nitrogen; TP: total phosphorus; TK:
626 total potassium.

627 **Fig 4** Alpha diversity metrics of archaeal, bacterial, and fungal Shannon Index are
628 positively correlated with increasing network complexity in terms of network size
629 (nodes) and network connectivity (links) in bulk soil and rhizosphere soil. *r*-values
630 represent Pearson correlation coefficients. BS:bulk soil; RS:Rhizosphere soil.

631 **Fig 5** Network roles of analysing module feature at OTU level for archaea, bacteria and
632 fungi with the composition of network hubs and module hubs (A); the composition of
633 connectors for archaea, bacteria and fungi in bulk soil and rhizosphere soil. BS:bulk
634 soil; RS:Rhizosphere soil.

635 **Fig 6** The contribution of environmental factors correlated with network hubs, module

hubs and connectors (A); the network correlations between environmental factors and network hubs, module hubs and connectors (B). BS:bulk soil; RS:Rhizosphere soil.

Fig 1

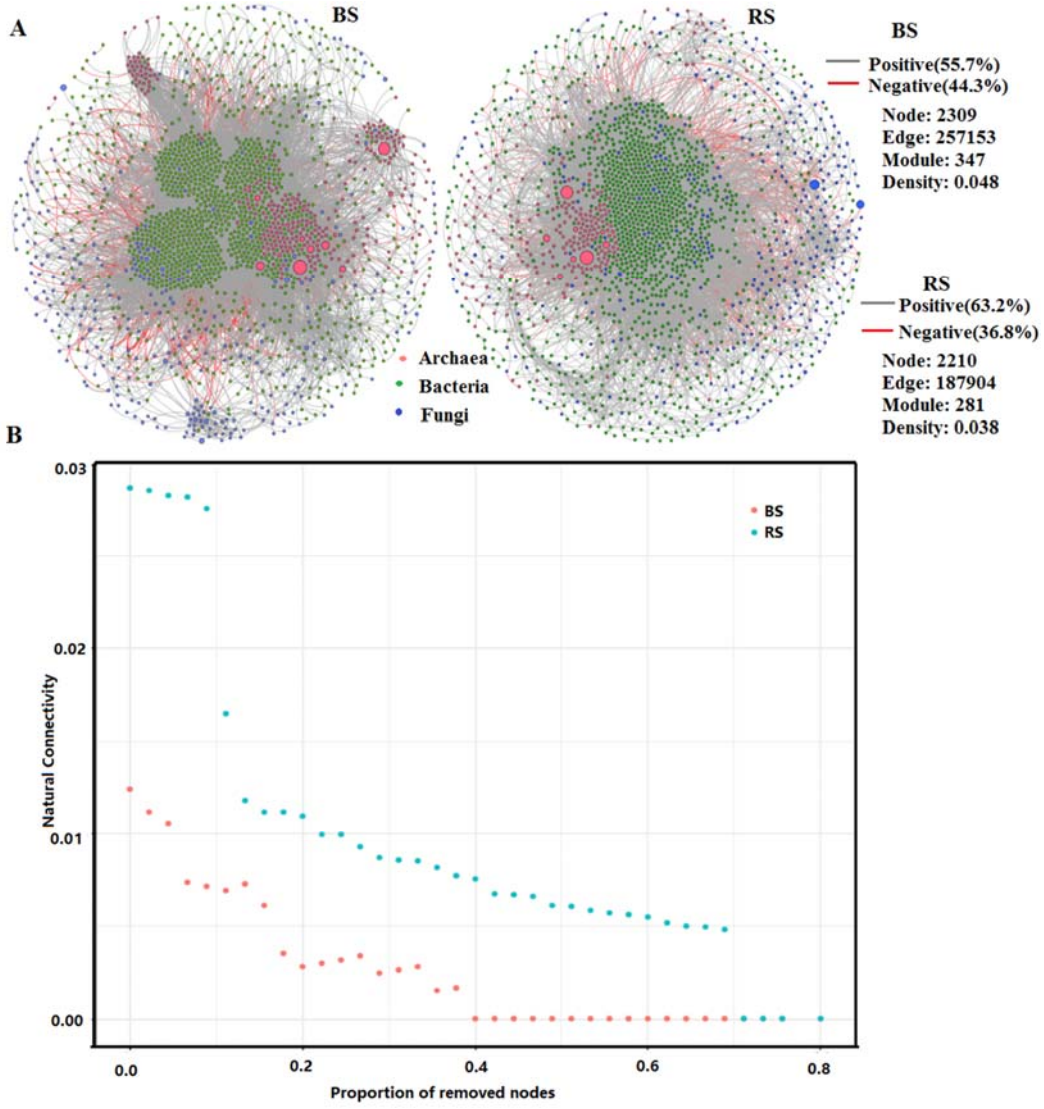

A
BS
RS
BS
Positive(55.7%)
Negative(44.3%)
Node: 2309
Edge: 257153
Module: 347
Density: 0.048
RS
Positive(63.2%)
Negative(36.8%)
Node: 2210
Edge: 187904
Module: 281
Density: 0.038
Archaea
Bacteria
Fungi
B
0.03
0.02
0.01
0.00
Natural Connectivity
0.0
0.2
0.4
0.6
0.8
Proportion of removed nodes
BS
RS

Fig 2

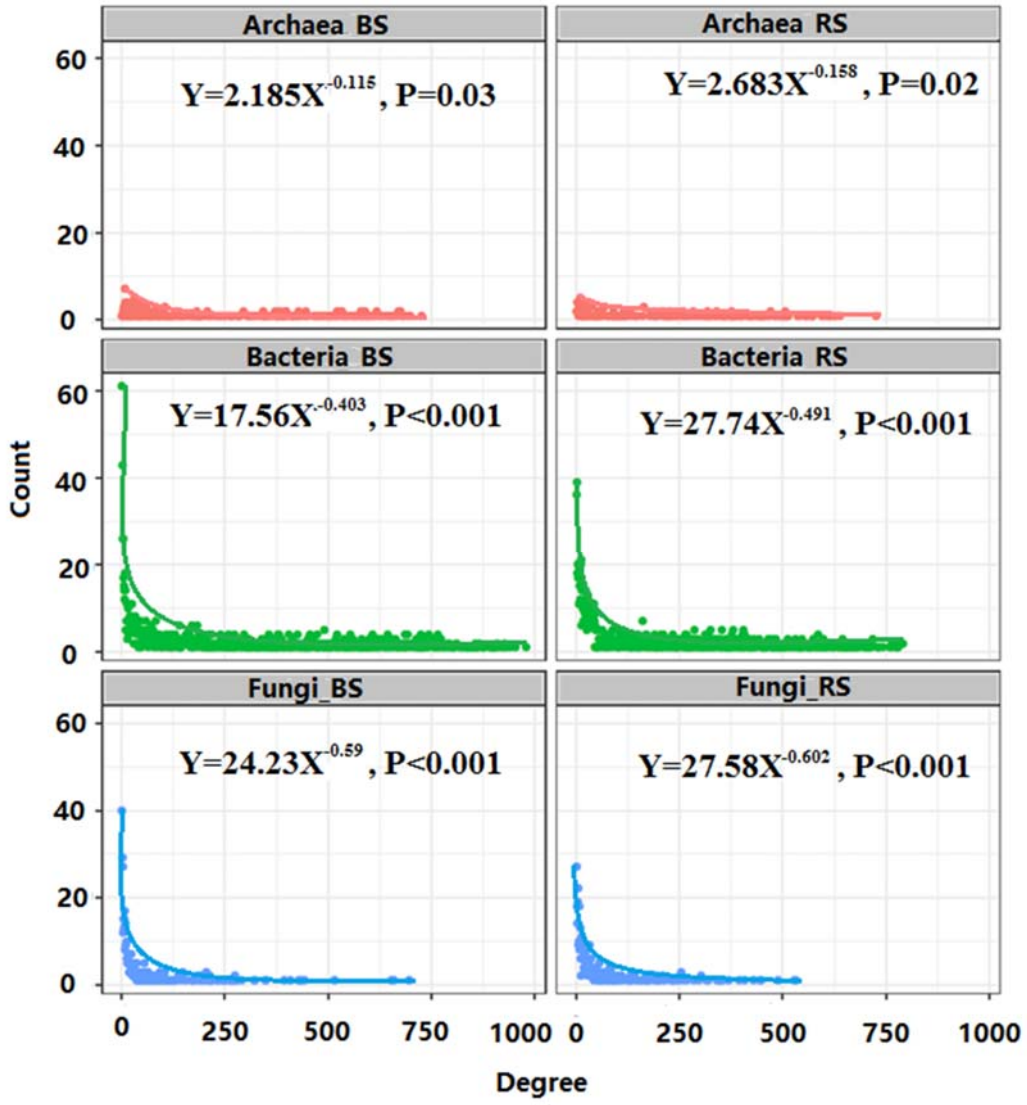

Archaea_BS
Y=2.185X^-0.115, P=0.03
Archaea_RS
Y=2.683X^-0.158, P=0.02
Bacteria_BS
Y=17.56X^-0.403, P<0.001
Bacteria_RS
Y=27.74X^-0.491, P<0.001
Fungi_BS
Y=24.23X^-0.59, P<0.001
Fungi_RS
Y=27.58X^-0.602, P<0.001
Count
Degree
0
20
40
60
0
250
500
750
1000

Fig 3

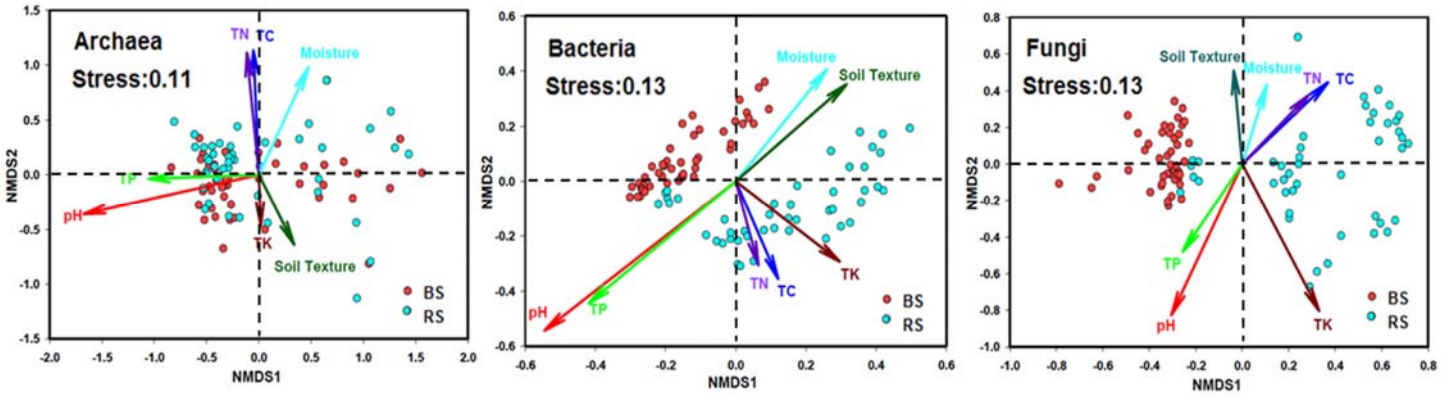

Archaea
Stress:0.11
TN
TC
Moisture
TP
pH
TK
Soil Texture
BS
RS
NMDS1
NMDS2
Bacteria
Stress:0.13
Moisture
Soil Texture
pH
TP
TN
TC
TK
BS
RS
NMDS1
NMDS2
Fungi
Stress:0.13
Soil Texture
Moisture
TN
TC
TP
pH
TK
BS
RS
NMDS1
NMDS2

Fig 4

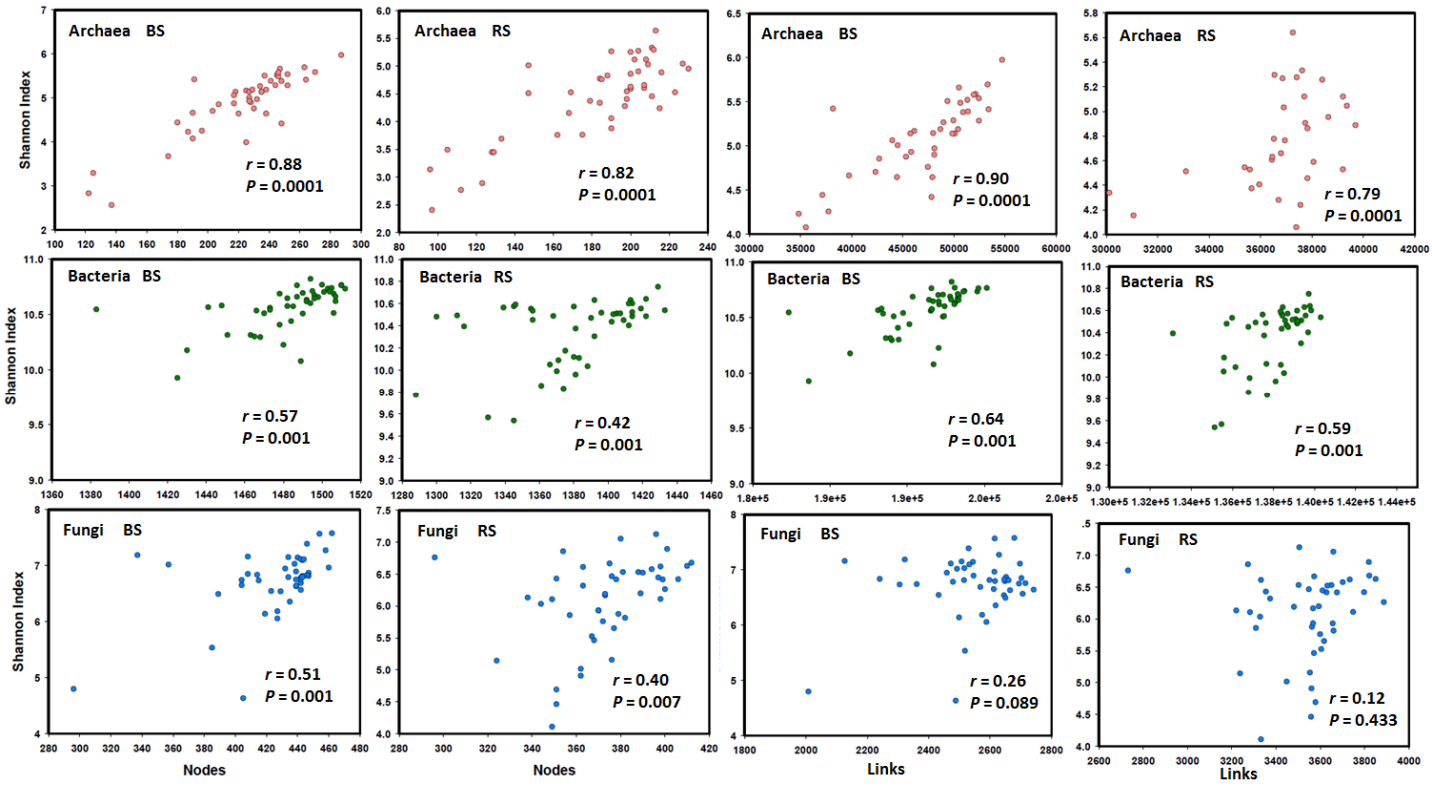

Archaea BS
r = 0.88
P = 0.0001
Archaea RS
r = 0.82
P = 0.0001
Archaea BS
r = 0.90
P = 0.0001
Archaea RS
r = 0.79
P = 0.0001
Bacteria BS
r = 0.57
P = 0.001
Bacteria RS
r = 0.42
P = 0.001
Bacteria BS
r = 0.64
P = 0.001
Bacteria RS
r = 0.59
P = 0.001
Fungi BS
r = 0.51
P = 0.001
Fungi RS
r = 0.40
P = 0.007
Fungi BS
r = 0.26
P = 0.089
Fungi RS
r = 0.12
P = 0.433
Shannon Index
Nodes
Links

Fig 5

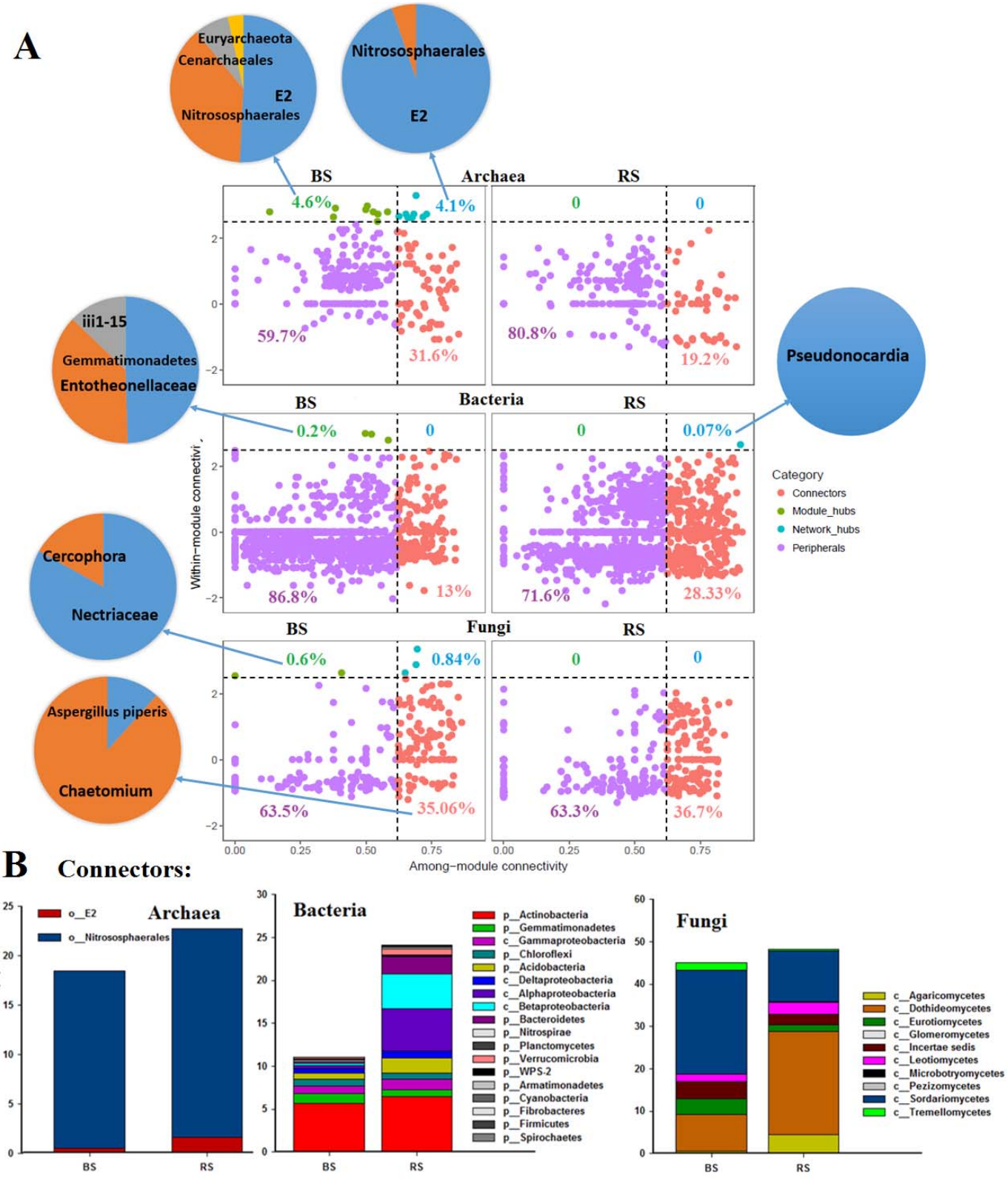
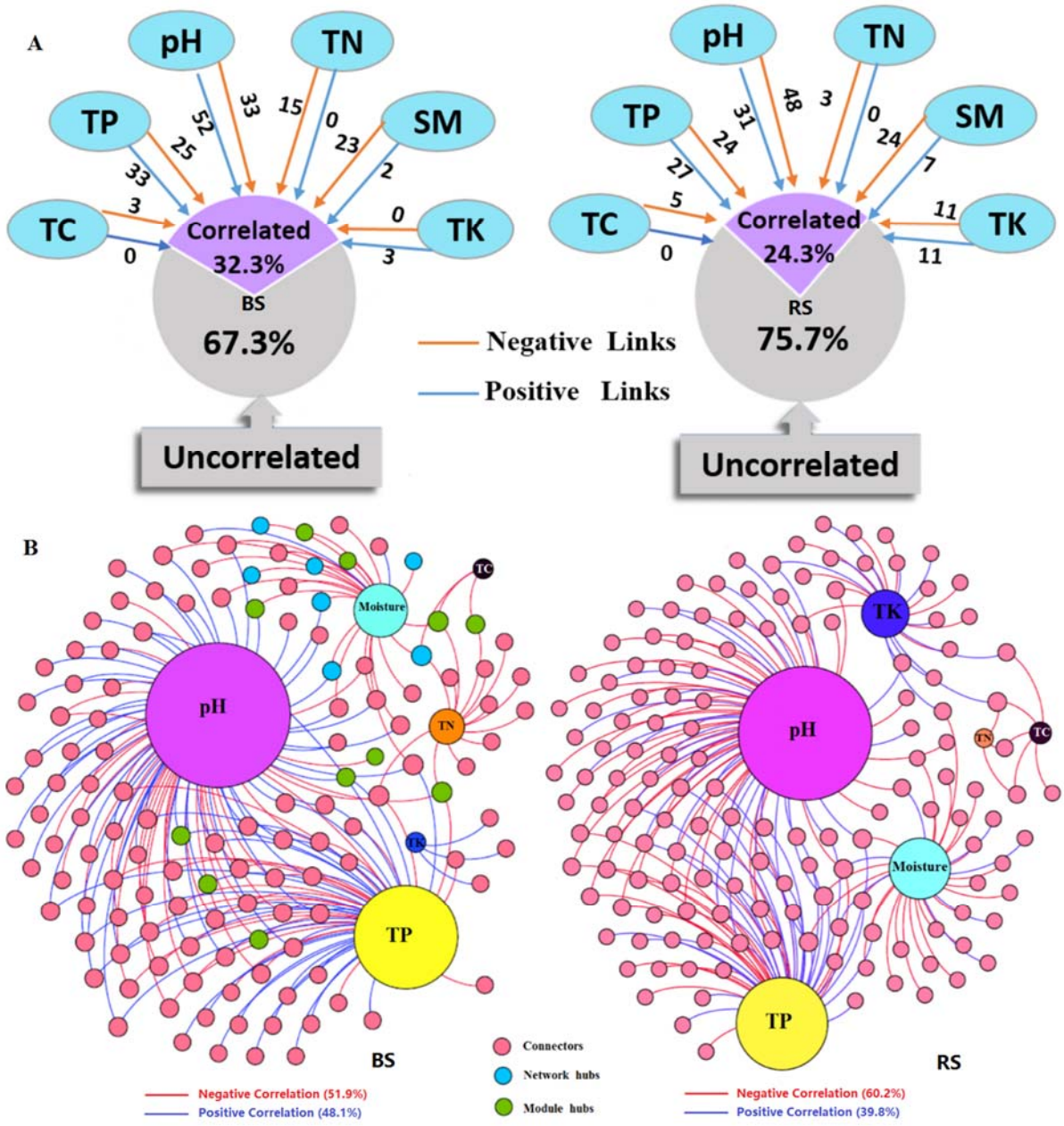

A
Euryarchaeota
Cenarchaeales
E2
Nitrososphaerales
Nitrososphaerales
E2
BS
Archaea
RS
4.6%
4.1%
0
0
59.7%
31.6%
80.8%
19.2%
iii1-15
Gemmatimonadetes
Entotheonellaceae
Pseudonocardia
BS
Bacteria
RS
0.2%
0
0
0.07%
86.8%
13%
71.6%
28.33%
Cercophora
Nectriaceae
BS
Fungi
RS
0.6%
0.84%
0
0
Aspergillus piperis
Chaetomium
63.5%
35.06%
63.3%
36.7%
Within−module connectivity
Among−module connectivity
Category
Connectors
Module_hubs
Network_hubs
Peripherals
B
Connectors:
Archaea
o__E2
o__Nitrososphaerales
Relative Abundance(%)
Bacteria
p__Actinobacteria
p__Gemmatimonadetes
c__Gammaproteobacteria
p__Chloroflexi
p__Acidobacteria
c__Deltaproteobacteria
c__Alphaproteobacteria
c__Betaproteobacteria
p__Bacteroidetes
p__Nitrospirae
p__Planctomycetes
p__Verrucomicrobia
p__WPS-2
p__Armatimonadetes
p__Cyanobacteria
p__Fibrobacteres
p__Firmicutes
p__Spirochaetes
Fungi
c__Agaricomycetes
c__Dothideomycetes
c__Eurotiomycetes
c__Glomeromycetes
c__Incertae sedis
c__Leotiomycetes
c__Microbotryomycetes
c__Pezizomycetes
c__Sordariomycetes
c__Tremellomycetes
BS
RS

Fig 6


A
pH
TN
TP
SM
TC
TK
Correlated
32.3%
BS
67.3%
Uncorrelated
Correlated
24.3%
RS
75.7%
Uncorrelated
Negative Links
Positive Links
B
Moisture
pH
TN
TC
TP
TK
BS
RS
Connectors
Network hubs
Module hubs
Negative Correlation (51.9%)
Positive Correlation (48.1%)
Negative Correlation (60.2%)
Positive Correlation (39.8%)