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Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields

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ABSTRACT

Biological nitrogen fixation contributes to the pool of plant-available N in both bulk soil and the rhizosphere. Here we investigated the co-association and assemblage process of diazotrophic community members in both rhizosphere and bulk soil of wheat fields. The diazotrophic community structure in the rhizosphere was significantly different and comprised a less competitive and more stable network structure when compared with that of the bulk soil. Deterministic versus stochastic community assemblage processes were quantified using betaNTI scores, demonstrating that deterministic processes decreased in importance with distance from plant roots. Soil pH was correlated with diazotrophic community structure and diversity, and community structure showed greater connectivity and stability in soils with neutral pH relative to those in acidic or alkaline soils. Stochastic processes dominated the assemblage of the diazotrophic community in soils with neutral pH, while deterministic processes dominated in acidic or alkaline soils. These results suggest that soil pH may play an essential role in the interaction and assemblage processes of the diazotrophic community in the rhizosphere and bulk soils, which could enhance our understanding of biological nitrogen fixation in agricultural soils.

1. Introduction

Biological nitrogen fixation (BNF) is one of the most significant steps of the nitrogen cycle in both ocean (Zehr et al., 1997, 2003) and terrestrial ecosystems (Orr et al., 2011; Silva et al., 2013; Zhou et al., 2016). In agricultural ecosystem, about 24% of nitrogen in crop biomass originated from non-symbiotic N_2 fixation (Ladha et al., 2016). Microorganisms capable of nitrogen fixation (diazotrophic organisms) that harbor the nifH gene have broad phylogenetic distribution (Zehr and McReynolds, 1989; Silva et al., 2013). Surveys of diazotrophic diversity have been conducted in a wide range of environments, including marine (Langlois et al., 2005; Turk et al., 2011), estuarine sediments (Affourtit et al., 2001), terrestrial geothermal springs (Hall et al., 2008; Hamilton et al., 2011), and terrestrial soils (Hamelin et al., 2002; Wang et al., 2017). Soil pH (Levy-Booth et al., 2014; Tu et al., 2016), soil organic matter (Wakelin et al., 2010; Gupta et al., 2014), soil moisture (Penton et al., 2016), and soil carbon: nitrogen ratio (Wang et al., 2017) have been shown to be dominant drivers of soil diazotrophic community structure. Collectively, these studies have revealed patterns in the distribution and diversity of diazotrophs in both natural ecosystems and agricultural bulk soils. However, diazotrophs often form close associations with the plant rhizosphere, which acts as a biological hotspot whose physicochemical properties differ substantially from the surrounding bulk soil (Philippot et al., 2013). While there have been studies of diazotrophic communities in the rhizosphere (Gupta et al., 2014), neither the physical nor chemical parameters that constrain the structure of diazotrophic communities within the rhizosphere of typical agricultural ecosystems have been examined.

In rhizosphere, plants exude organic compounds that support microbial activity near the roots, which, in turn, provide beneficial services to the plant (Dennis et al., 2010; Turner et al., 2013). A multistep model for root microbiome assembly from soil has been proposed and supported by research in rice (Edwards et al., 2015) and grapevines (Zarraonaindia et al., 2015). These studies have also shown that the composition of bacterial (Donn et al., 2015; Fan et al., 2017) and fungal (Zhang et al., 2017) communities varied significantly between rhizosphere and bulk soil, with bacterial diversity decreasing from the bulk soil towards roots. Co-occurrence networks provide evidence of the

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correlative changes in relative abundance between biomarkers, providing additional metrics to examine the relationships within the microbial community (Butland et al., 2005). Mendes et al. (2014) found a less complex network topology in rhizosphere when compared with bulk soil in a short-term plantation system. Similarly, Fan et al. (2017) detected a less complex, more hub based bacterial co-occurrence topology in the rhizosphere of the North China Plain. This reduced complexity supports the concept that the rhizosphere is a highly selective environment, which promotes specific taxonomic co-occurrences that could be indicative of reduced metabolic flexibility. One key metabolic process occurring within the rhizosphere is nitrogen fixation, and rhizospheric diazotrophs have wide ecological distribution and adaptability, While nitrogen fixation is of particular interest for improving productivity of sugarcane (Fischer et al., 2012) and commercial crops such as corn, rice, and wheat (Okon et al., 1977; Revers et al., 2000; Venieraki et al., 2011), the composition and co-occurrence of diazotrophic membership in different root-associated compartments of agricultural soils remains largely unknown.

Microbial community structures are shaped by a combination of deterministic and stochastic processes (Ofiţeru et al., 2010). Deterministic community assembly results from the predictable filtering of species by both environmental and biotic conditions (Leibold and McPeek, 2006), while stochastic community assembly occurs through the essentially random processes of dispersal, birth, death, and drift (Bell, 2001). As habitat heterogeneity declines at smaller scales, especially towards a strong selective force (e.g. rhizosphere), a more apparent contribution of stochastic processes over deterministic factors will be presented at these scales (Legendre et al., 2009; Chase, 2014). However, the scale at which this apparent shift becomes relevant for microbial community composition is still unclear (O'Brien et al., 2016). Previous studies have explored the relative importance of stochastic versus deterministic microbial community assemblage processes between rhizosphere and bulk soil. For example, a soybean cultivation study demonstrated that microbial community selection in the rhizosphere occurred via deterministic niche filtering, while the bulk soil microbial community seemed to be controlled by stochastic processes (Mendes et al., 2014). Despite the ecological and economic impact of diazotrophs (Werner and Newton, 2005; Brink, 2016), no attempt has yet been made to determine whether these microbes follow similar rules of community assembly in agricultural systems.

The North China Plain is an important agricultural area in China, with a traditional long-term (about 40 years) wheat-maize rotation system (Chen et al., 2004). Wheat (*Triticumaestivum* L.) is one of the main grain crops globally, but productivity increases per year have slowed to 0.9% (Fischer and Edmeades, 2010) in response to issues with field management, diseases, and poor nitrogen nutrition. It is possible that targeted manipulation of nitrogen fixing microbial community could lead to a more environmentally and economically sustainable production systems. In our study, we collected soils across the North China Plain from three compartments: namely bulk soil, loosely bound soil, and tightly bound soil, providing a rough gradient of root proximity (Donn et al., 2015). Two hypotheses were proposed in the current study. First, the diversity and co-occurrence topology of the rhizospheric diazotrophic community will be simpler when compared to the bulk soil. Second, the rhizospheric diazotrophic communities will demonstrate deterministic assemblage processes when compared to the bulk soil.

2. Materials and methods

2.1. Sample collection

Nine sampling sites were chosen from the typical wheat planting fields by GIS map across a broad area (~800,000 km^2) (32° N~38° N; 110° E~118° E) on the North China Plain (Fig. S1; Table S1) with wheat-maize rotation. The soil type in most sampling sites were Epiaquepts, Haplustalfs, Humaquepts and Calciustepts according to soil taxonomy of the USA (Table S2). Samples were collected during the wheat filling stage (22nd −27th of May 2015). At each sampling site, five replicate locations were measured within a ~100 km^2 square plot. A group of ten to twelve wheat plants were extracted in every place by digging around the group to keep the root systems as intact as possible. Loosely bound soil samples were collected by gently shaking off the soil which lightly adhered to the root, and the tightly bound soil samples were collected by brushing the soil which tightly adhered to roots. The topsoil (0–15 cm), beside each group and ~50 cm away from plants, was collected using an auger corer as bulk soil (Fan et al., 2017). All samples were packed into polyethylene bags and shipped on ice packs (4 °C) to the laboratory. The soils were sieved through 2 mm meshes, handpicked to remove fine roots, residues, and stones. Each sample was then divided into two parts: one was stored at −40 °C for DNA extraction within two weeks, and the other one was stored at 4 °C for soil chemical analyses.

2.2. Soil physical and chemical analysis

Soil moisture was measured gravimetrically by drying 5 g fresh soil until the soil reached a constant weight. Soil texture was tested by using Laser Particle Sizer (LS13320) with air dried soil (Table S2). Soil for total carbon (TC), total nitrogen (TN), total phosphorus (TP) and total potassium (TK) analyses was air dried, sieved (1 mm mesh), determined by combustion (CNS-2000; LECO, St. Joseph, MI, USA). Soil pH was determined by pH monitor (Thermo Orion-868) with a fresh soil to water ratio of 1:5 (Table S3).

2.3. High throughput sequencing and bioinformatics analysis

A half gram of fresh soil was used for DNA extraction using the Power Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Primers nifHF (5′-TGYGAYCCNAARGCNGA-3′) and nifHRb (5′-ADNGCCATCATYTCNCC-3′) (Gaby and Buckley, 2012) were used for amplification of the nifH gene. These amplified PCR products were sequenced on the Illumina MiSeq PE 300 platform. Sequences obtained from this research were submitted in the NCBI Sequence Read Archive (SRA) with accession number SRP 113262.

After sequencing, nifH nucleotide sequences were analyzed using the QIIME pipeline (http://qiime.sourceforge.net/) (Caporaso et al., 2010). The low-quality sequences that had a quality score < 20, contained ambiguous nucleotides, or did not match the primer and barcode, were removed. The remaining sequences were further converted to amino acid sequences using the FunGene Pipeline of the Ribosomal Database Project (Wang et al., 2013). Sequences whose translated proteins did not match the nifH protein sequence or that contained termination codons were discarded. The remaining sequences were aligned against the nifH gene database (Gaby and Buckley, 2014), and both failed and chimeric sequences were also removed. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) with UCLUST (Edgar, 2010) running in de novo mode at 95% amino acid similarity, and all singleton OTUs were deleted. As the nifH gene provides sufficient phylogenetic resolution (Pinto et al., 1995), it has been used frequently in ecological studies. A phylogenetic tree was estimated based on aligned representative sequences by using FastTree (Price et al., 2010).

2.4. Statistical analysis

SPSS20.0 was used to perform ANOVA, pairwise *t*-test and covariance analysis to calculate significant differences in the dominant microbial taxon composition, alpha diversity and soil variables. NMDS were performed for the diazotrophic community data by calculating the Bray-Curtis dissimilarity. The NMDS, SIMPER analysis and Mantel test

were conducted using the 'vegan' R package (http://cran.stat.sfu.ca/). The co-occurrence network was constructed with the 'WGCNA' package (Langfelder and Horvath, 2012) by using the Spearman correlation, and the network and sub-network properties were calculated in the 'igraph' R package. We deleted the rare OTUs with relative abundances less than 0.01% of the total number of nifH sequences, and adjusted all P-values by using the Benjamini and Hochberg false discovery rate (FDR) for multiple testing (Benjamini et al., 2006) which was implemented in the 'multtest' R package. We set the adjusted P-values cutoff as 0.001. The network images was generated with Cytoscape (http://www.cytoscape.org/) and Gephi (http://gephi.github.io/). We defined the 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) referring to their roles in network structure (Poudel et al., 2016). The algorithms were based on the methods of metabolic networks (Guimera and Amaral, 2005).

To test the assembly processes of the diazotrophic community, the nearest taxon index (NTI) was calculated in each sampling plot, and beta nearest taxon index (betaNTI) was calculated in paired joined plots. NTI measures the mean nearest taxon distance (MNTD) among individuals and it therefore estimates the 'terminal' phylogenetic dispersion of the community (Webb, 2000). Positive (negative) NTI values indicate shorter (longer) nearest taxon distances within a community than expected by chance. NTI was calculated in the R 'picante' package (Purcell et al., 2007). Positive (negative) betaNTI values indicate greater (less) than expected phylogenetic turnover, and betaNTI was calculated in phylocom 4.2 (Hardy, 2008). For both metrics, values between −2 and +2 values indicates the expectation under neutral community assembly while the individual values below −2 or above +2 are statistically significant (Stegen et al., 2012; Diniandreote et al., 2015).

3. Results

3.1. Diazotrophic community structure in wheat rhizosphere and bulk soil

In total 5753 OTUs (95% nucleotide identity) were clustered from 4,567,615 high-quality nifH reads across 135 soil samples. *Polaromonas, Burkholderia, Bradyrhizobium, Opitutaceae* and *Rhizobium* (Fig. 1) dominated all sample types across wheat fields of the North China Plain. ANOVA (Table S4) identified *Polaromonas* (18.01%), *Burkholderia* (12.95%), *Hyphomicrobium* (6.06%), *Leptothrix* (4.93%), *Verrucomicrobiae* (3.33%), *Mesorhizobium* (2.54%) and *Gluconacetobacter* (2.51%) as being significantly more abundant in tightly bound soil compared to loosely bound soil (P < 0.05) and bulk soil (P < 0.05); while

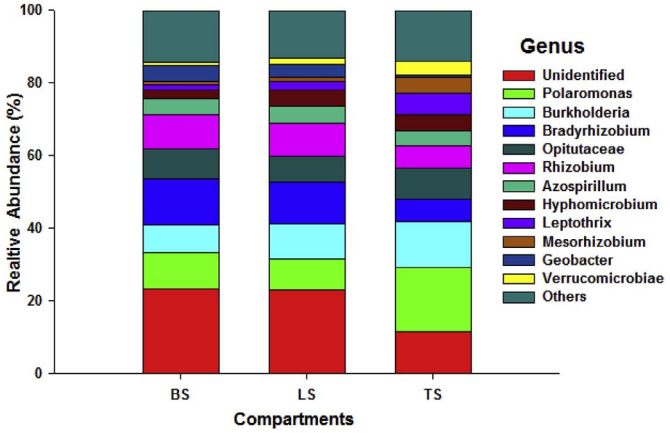

Fig. 1. The relative abundance of the dominant diazotrophic genus in bulk soil, loosely bound soil and tightly bound soil. BS: bulk soil; LS: loosely bound soil; TS: tightly bound soil.

Bradyrhizobium (6.76%), *Geobacter* (0.58%) and *Dechlorosoma* (0.73%) were significantly less abundant in tightly bound soil compared to the other compartments (P < 0.05). SIMPER analysis (Fig. S2) suggested that the variance between the bulk soil and loosely bound soil could mostly be described by changes in the abundance of *Burkholderia* and *Geobacter*, while *Bradyrhizobium* and *Rhizobium* described variance between bulk soil and tightly bound soil, and *Azospirillum* and *Polaromonas* had the greatest variance between loosely bound soil and tightly bound soil. There were no significant differences in nifH alpha diversity across sample types (Table S5). ANOSIM and Adonis tests (Table S6) suggested that diazotrophic community structure in tightly bound soil significantly differed from that of loosely bound soil and bulk soil.

3.2. Interaction relationships and network topological features of diazotrophic community in three compartments

A co-occurrence network was built for each sample type (bulk soil, loosely bound soil, and tightly bound soil) with the relative abundance of nifH OTUs as nodes, and correlation between relative abundances based on Spearman rank with p-values adjusted with FDR (Benjamini et al., 2006) (Table S7). Random network analysis confirmed network reliability and accuracy (Table S8). There were a greater number of negatively correlated edges between OTUs and between genera in bulk soil (13.3%), compared with loosely bound soil (11.1%), and tightly bound soil (9.4%) (Fig. 2A; Table S8). The negative links of total network and the negative correlation rates of sub-network in bulk soil were significantly more than those in either loosely bound soil or tightly bound soil, which could be interpreted as a reduction in competitive relationships within the rhizosphere (Table S9; S10). OTUs associated with the same genera had stronger interactions and clustered together in the network map (Fig. S3). The measure of an OTU's within-module degree and its participation coefficient, which define how the node is positioned within its own module and with respect to other modules, can provide insight on the role of the species represented by that node (Rives and Galitski, 2003; Han et al., 2004). Network hubs are defined as OTUs that are highly connected both in general and within a module, module hubs are OTUs that are highly connected only within a module; OTUs that link modules are known as connectors, while peripherals have few links to other species (Poudel et al., 2016). The proportion of both network hubs and module hubs decreased with proximity to roots, with tightly bound soil having few to no hubs, while the proportion of connector nodes was greatest closest to roots (Fig. 2B). Together, this suggests that the diazotrophic community in wheat rhizosphere is more interconnected than that in bulk soil.

We estimated network stability by removing nodes in the static network and assessing how quickly robustness degrades. This network attack method pre-specifies node order based on hub characteristics (e.g. degree and betweenness centrality) and assesses stability by natural connectivity (Peng and Wu, 2016). When comparing the network stability of these sample types (Fig. S4), the network structure in the tightly bound soil was more stable than those of loosely bound soil and bulk soil. Topological features of degree distribution patterns showed that the diazotrophic community in the bulk soil ($R^2 = 0.662$, $P < 0.001$) and loosely bound soil ($R^2 = 0.633$, $P < 0.001$) presented typical power-law distribution patterns, which changed with the distance to roots, and presented a random distribution pattern in the tightly bound soil ($R^2 = 0.308$, $P = 0.005$) (Fig. S5).

3.3. The physicochemical factors affecting diazotrophic community in rhizosphere and bulk soil

Mantel tests identified soil pH as the dominant factor correlating with diazotrophic community structure (Table S11) in all three sample types (Fig. S6). The spearman correlations between soil physicochemical properties and diazotrophic diversity showed that soil pH, total

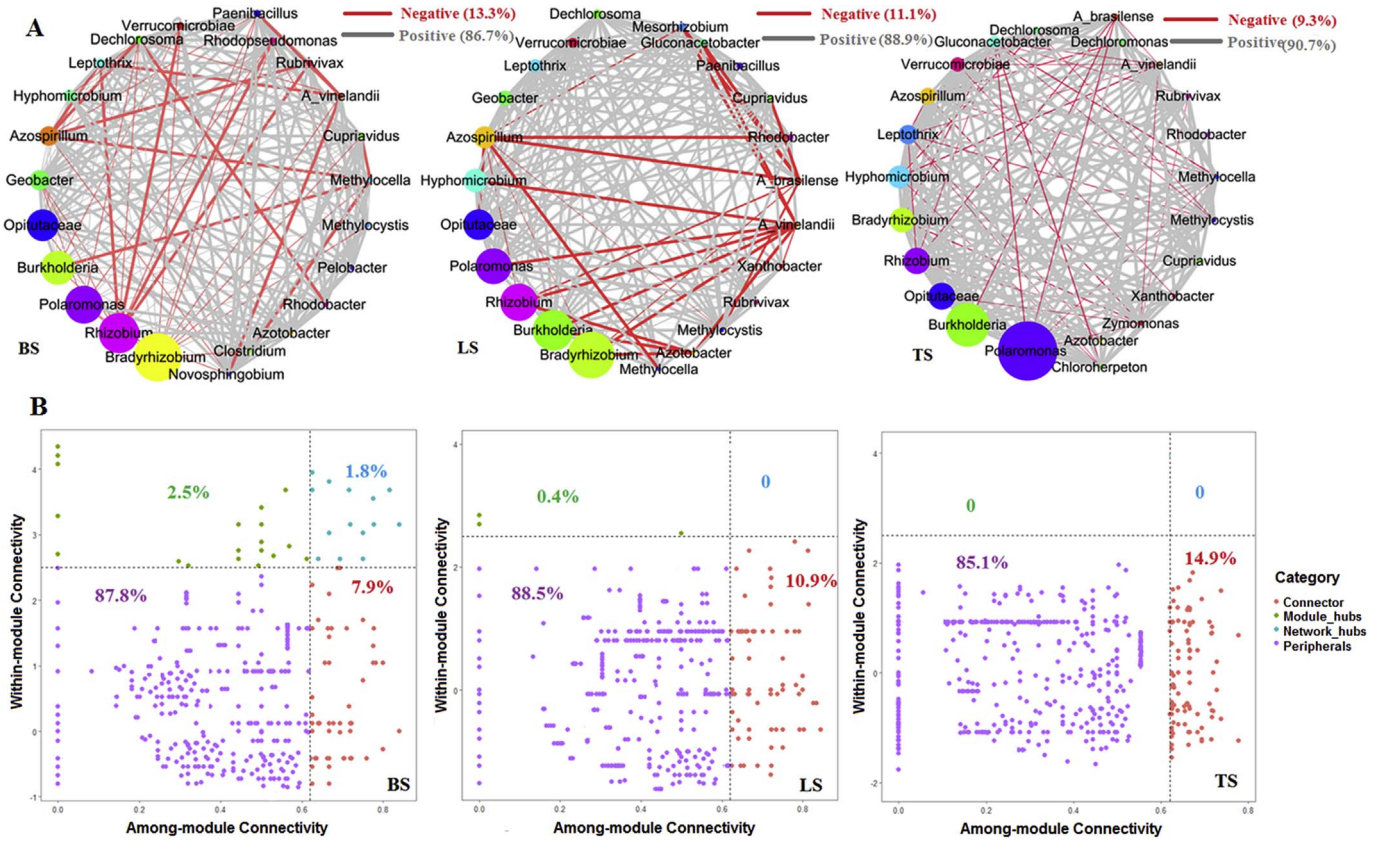

Fig. 2. The co-occurrence network structure of diazotrophic community at genus level (A); and network roles of analysing module feature at OTU level (B). BS: bulk soil; LS: loosely bound soil; TS: tightly bound soil.

phosphorus and soil texture were positively correlated with alpha diversity (Table S12). Additionally, Spearman correlations between network topological features and soil physiochemical properties in the different soils showed that soil pH, total phosphorus, soil moisture and soil texture were significantly correlated with all network topological features, while total potassium was less correlated with network degree, betweenness, closeness, or transitivity (Table S13).

3.4. Assemblage processes of the diazotrophic community in rhizosphere and bulk soil

Both deterministic and stochastic processes are responsible for structuring the microbial communities (Chave, 2004). The majority of the betaNTI scores for the nifH OTU derived communities in our study were below −2 (75.7% in bulk soil; 60.5% in loosely bound soil; and 54.1% in tightly bound soil) (Fig. 3), which indicated that deterministic processes dominated diazotrophic community dynamics in all sample types. However, betaNTI scores in the range of −2 to +2 (24.3% in bulk soil; 39.5% in loosely bound soil; and 45.9% in tightly bound soil), which are indicative of neutral processes to be greater in the tightly bound, rhizospheric soil (Fig. 3).

3.5. Network structure and assemblage processes of diazotrophic community along soil pH gradients

As the diazotrophic community structure and network topological features correlated with soil pH, we binned all samples into four groups according to soil pH (4.5–5.5, 5.5–6.5, 6.5–7.5, 7.5–8.5), and analyzed the network relationship and assemblage processes in detail. The relative abundance of *Bradyrhizobium* and *Geobacter* decreased

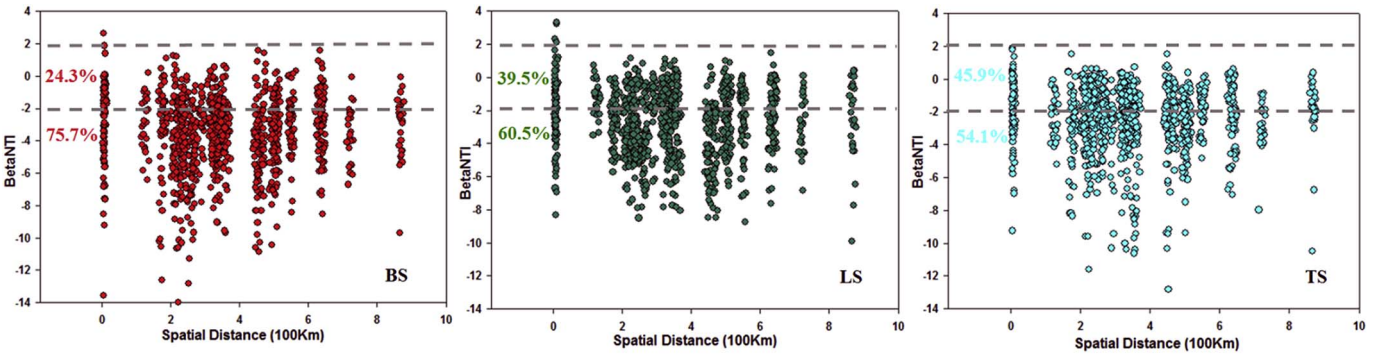

Fig. 3. Distribution of beta Nearest Taxon Index (betaNTI) according to the environmental distance which indicating assamblage processes of diazotrophic communities. The horizontal dotted blue line (above +2 or below −2 are statistically significant) shows the 95% confidence intervals around the expectation under neutral community assembly. BS: bulk soil; LS: loosely bound soil; TS: tightly bound soil.

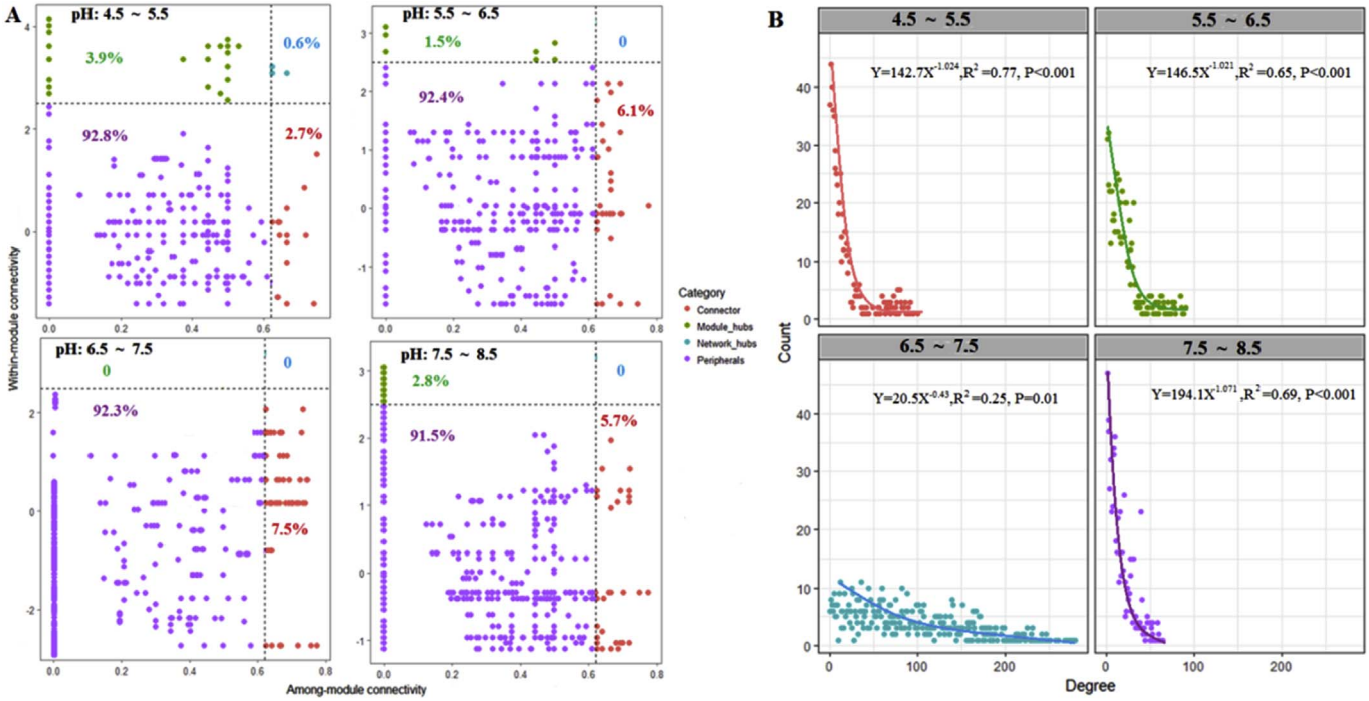

Fig. 4. Co-occurrence network module analysis (A); and degree distribution pattern (B) for the diazotrophic communities in different soil pH gradients.

significantly, while *Opitutaceae, Azospirillum, Leptothrix* and *Rhizobium* increased significantly with increasing pH (ANOVA $p < 0.05$; Table S14). *Polaromonas, Burkholderia* and *Hyphomicrobium* had the greatest relative abundance in the pH range of 6.5–7.5. Alpha diversity also increased with increasing pH (Table S15).

Co-occurrence network topology differed significantly with soil pH. Networks had a greater number of negative correlations with nodes at a pH range of 7.5–8.5 when compared with the other pH ranges (Table S16). We also observed a greater number of negative correlations among different genera in the 7.5–8.5 pH range (Fig. S7). There were no module hubs or network hubs from samples with a soil pH range of 6.5–7.5 (Fig. 4A), while the proportion of network connectors increased, suggesting a less hub-based and more connected structure at a neutral pH range. Topological patterns of degree distribution demonstrated that nifH OTUs at neutral pH did not have a typical power-law distribution pattern (Fig. 4B). When comparing the network stability among the four pH groups, less fluctuation from natural connectivity patterns was observed in response to the proportion of removed nodes in the soil pH range 6.5–7.5 (Fig. S8), which indicated a more stable network topological structure.

To test whether common assemblage mechanisms explain the assembly of the diazotrophic community at different pH ranges, we calculated the NTI and betaNTI for paired samples (Fig. 5). NTI scores positively correlated with soil pH, with samples in a pH range of 4.5–5.5 and 5.5–6.5 mostly having scores below −2, and samples with a soil pH range of 6.5–7.5 mostly having scores between −2 and +2, while a pH range of 7.5–8.5 mostly resulted in scores above 2 (Fig. 5A). The relative frequency of betaNTI from soils in the 6.5–7.5 pH range suggested that stochastic processes dominated the diazotrophic communities in the neutral pH range (Fig. 5B). The betaNTI scores in the other pH ranges (4.5–5.5, 5.5–6.5, and 7.5–8.5) are suggestive of a dominance of deterministic processes at an acid or alkaline pH (Fig. 5B).

4. Discussion

The diazotrophs that were more abundant in the tightly bound soil, such as *Polaromonas, Burkholderia, Hyphomicrobium, Leptothrix, Verrucomicrobiae*, and *Gluconacetobacter*, are related to the non-symbiotic nitrogen-fixing bacteria (Kennedy et al., 2004). They are frequent colonizers of the rhizosphere, rhizoplane or the interior of the roots of grasses and graminaceous crop plants, such as maize, wheat, sugarcane, and rice (Baldani et al., 1997). *Mesorhizobium*, which was enriched in the tightly bound soil, is known to fix atmospheric nitrogen (Chen et al., 2015). *Mesorhizobium* was recently shown to be a key species in rhizosphere biological networks, where it played an important role in phosphorus cycling and bacterial biomass production (Jiang et al., 2017). Similar to the bacterial (Fan et al., 2017) and fungal communities (Zhang et al., 2017) of wheat fields on the North China Plain, soil pH is the most important environmental driver for diazotrophic community structure across the samples from the rhizosphere to bulk soil. *Bradyrhizobium* was more abundant at acidic pH (4.5–5.5), while *Azospirillum* and *Rhizobium* increased in abundance with increasing pH. As *Bradyrhizobium* species have wide niches and are excellent survivors across diverse conditions (Bottomley et al., 1991), high abundance at low soil pH gradients will to be expected. The genera *Azospirillum* and *Rhizobium* have been found to form biofilms (Rinaudi and Giordano, 2010; Herath et al., 2015), thus increasing soil pH may not affect these communities. In our study, the alpha diversity of diazotrophic community was affected by soil pH. *Polaromonas, Burkholderia* and *Hyphomicrobium* had the greatest relative abundance at a pH range of 6.5–7.5, which is the optimal pH range for most soil microbes.

Co-occurrence networks had fewer negative correlations in the tightly bound soil when compared with that of either bulk soil or loosely bound soil, which can be interpreted as reduced inter-species competition in the tightly bound soil. The rhizosphere has a more enriched carbon resource pool than the bulk soil, and an estimated 17% of photosynthetically fixed carbon is transferred to the thin layer of soil surrounding the root through exudation (Martin, 2016). Greater resource availability is thought to reduce competition in microbial communities (Hubbell, 2005; Costello et al., 2012); thus root exudates in the rhizosphere likely contribute to reduced competition. As nitrogen fixation is an energy-expensive process (Orr et al., 2012), the increase in C availability within rhizosphere soil may have a more pronounced effect on competition of diazatrophic species in the bulk soil likely compete due to reduced carbon substrate availability, with the strength

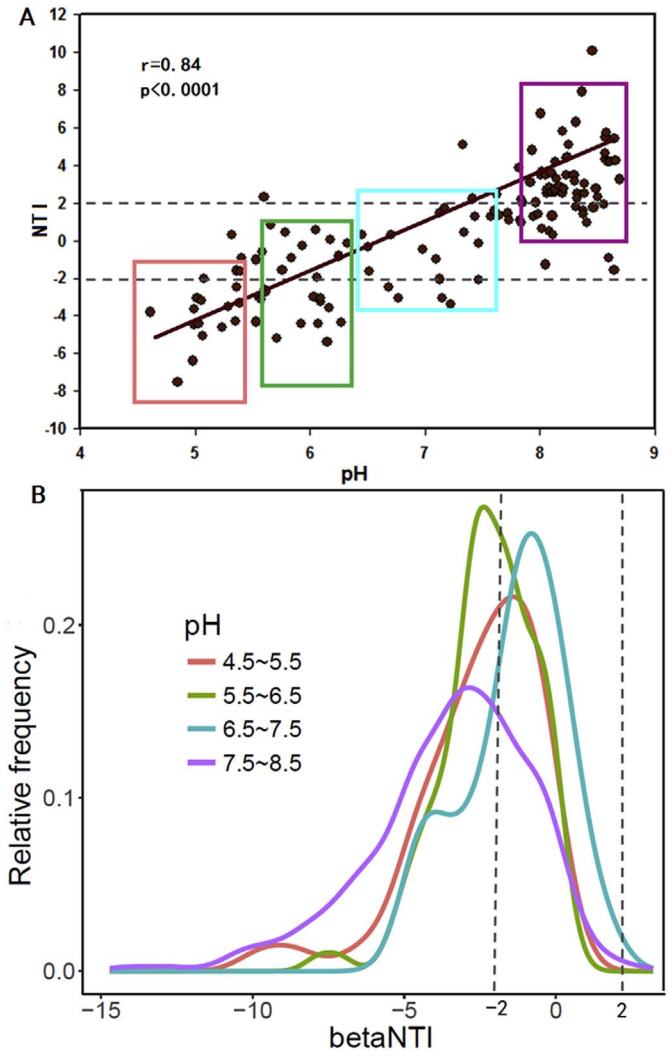

Fig. 5. Variation of nearest taxon index (NTI) along pH gradients (A); and the distributions of between community analog (betaNTI) in different pH gradients (B). Each observation is the number of null model standard deviations the observed value is from the mean of its associated null distribution. For both metrics, individual values below −2 or above +2 are statistically significant, values within indicated the expectation under neutral community assembly.

of competition increasing from the tightly bound soil to the loosely bound soil and then to the bulk soil (Engelmoer et al., 2014).

The proportion of both network hubs and module hubs decreased to zero closer to roots, while the proportion of connectors increased in the tightly bound soil, suggesting that the network structure within the rhizosphere was not hub-based. Rhizosphere network structure also followed a less typical power-law distribution pattern, meaning that all interactions between OTUs were equally likely (Ma et al., 2016). Tightly bound soil had a more robust network structure (Dunne et al., 2002) compared to bulk soil and loosely bound soil. This ecological stability is likely driven by the greater resource availability and niche habitability of the rhizosphere (Thébault and Fontaine, 2010).

The network structure across a gradient of pH demonstrated a more hub-based network pattern in either acid or alkaline conditions, while there were no module hubs or network hubs, but more connectors, at neutral pH, suggestive of a stable network, and hence microbial community and structure (Guimera and Amaral, 2005). The degree distribution pattern at neutral pH was random, which differed from the power-law distribution pattern in both acid and alkaline pH ranges. A power-law distribution pattern is often observed in biological networks (Bergman and Siegal, 2003); however, a random distribution pattern is rare in microbial communities, with one example being archaeal communities in flooded and non-flooded soils (Zheng et al., 2013). In our study, the random degree distribution pattern at neutral pH meant that all interactions between OTUs are equally likely with the links homogeneously distributed among diazotrophic OTUs, which would suggest neutral assembly (Chave, 2004). That stochastic processes may be more important than deterministic processes for diazotrophic communities at neutral pH, concurs with global microbial network analysis (Mendes et al., 2014).

NTI correlated with increasing pH, with the diazotrophic community tending to have greater phylogenetic evenness (Hamilton et al., 2011) at acidic pH, and greater phylogenetic clustering at alkaline pH. The neutral pH range is the optimal environment for the majority of microbes (Bååth, 1996), and a majority of diazotrophs are enriched at neutral pH, with greater relative nitrogenase activity and N-fixation rates (Hsu and Buckley, 2009). We hypothesize that the release from environmental stress and weakened environmental filtering at neutral pH, resulted in an increased dominance of stochastic processes in structuring the diazotrophic community at a pH range of 6.5–7.5 (Diniandreote et al., 2015). We observed that network topological patterns in the rhizosphere were similar to the patterns observed at neutral pH. Rhizosphere pH was more homogenous and closer to neutral, with 40% of samples in the tightly bound soil in the neutral pH range; more than that of loosely bound soil (6.7%) and bulk soil (4.4%) (Table S3). The decreasing dominance of deterministic assembly factors from the bulk soil to the rhizosphere may be related to more favorable conditions in the rhizosphere, because of the availability of root-derived products (Bürgmann et al., 2005; Mendes et al., 2014) and the neutral pH range.

In conclusion, the diazotrophic community, as determined by nifH sequencing, differed between the rhizosphere and bulk soils, and the network structure of the diazotrophic community in the rhizosphere soil was less competitive and more stable compared with the bulk soil. Deterministic factors played a larger role in diazotrophic community assembly in the bulk soil than in the rhizosphere soil. The network structure of the diazotrophic community in the soils with neutral pH was more stable, and stochastic processes dominated the community assembly under these conditions. These results suggest that soil pH controls the interactions and assemblage processes of soil diazotrophic communities in the wheat fields, and soils with neutral pH may have a more stable diazotrophic community.

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Appendix A. Supplementary data

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