Geoderma 386 (2021) 114923

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

The geographical scale dependence of diazotroph assembly and activity: Effect of a decade fertilization

Xiangtian Meng [a,b,c], Hongkai Liao [d], Haoxin Fan [e], Xuechen Zhang [f], Yaying Li [a,b], Huaiying Yao [a,b,e,*], Bahar S. Razavi [g]

[a] *Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China*
[b] *Zhejiang Key Laboratory of Urban Environmental Processes and Pollution Control, Ningbo Urban Environment Observation and Research Station-NUEORS, Institute of Urban Environment, Chinese Academy of Sciences, Ningbo 315800, People's Republic of China*
[c] *University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China*
[d] *Guizhou Provincial Key Laboratory of Mountain Environment, Guizhou Normal University, Guiyang 550001, People's Republic of China*
[e] *Research Center for Environmental Ecology and Engineering, School of Environmental Ecology and Biological Engineering, Wuhan Institute of Technology, Wuhan 430073, People's Republic of China*
[f] *Department of Biogeochemistry of Agroecosystems, University of Göttingen, Göttingen, Germany*
[g] *Department of Soil and Plant Microbiome, Institute of Phytopathology, Christian-Albrecht-University of Kiel, Kiel, Germany*

ARTICLE INFO

Handling Editor: Naoise Nunan

Keywords:
Long-term fertilization
Soil diazotroph
Community assembly
Nitrogen fixation rate
Spatial scale dependence

ABSTRACT

Biological nitrogen (N) fixation is one of the most significant parts of the N cycle in terrestrial ecosystems, and this process is carried out by soil diazotrophs. However, knowledge of diazotroph assembly processes and activity in response to diverse fertilization strategies in agroecosystems across a large geographical scale is still lacking. Here, we selected nine agro-ecological experimental sites that covered a wide geographical distance (~3500 km) at a continental scale, and investigated the diazotrophic communities, assembly processes, distance-decay patterns and N_2 fixation activity in response to geographical factors and long-term fertilization strategies. The results showed that the dominant genera were *Bradyrhizobium* (~30.5%) and *Azospirillum* (~26.8%) in all samples, and RDA analysis showed that the relative abundance of *Bradyrhizobium* was positive correlated with MAP while specific negatively correlated with soil pH and the relative abundance of *Azospirillum.* Geographical factors (location and climate) and fertilization collectively drive diazotroph assembly processes and determine diazotroph activity. Diazotroph assembly processes were influenced by both stochastic (~36.2%) and deterministic (~63.8%) processes simultaneously at large geographical scales and under various fertilization strategies. Moreover, fertilization increased the proportion of deterministic processes under various fertilization strategies. The N fixation rate was determined by local soil properties. Fertilization changes but does not always suppress nitrogen fixation activity. Both geographical factors and fertilization through the shift of diazotroph community composition and the changes in soil properties, indirectly affected the assembly process and N fixation rate. Among soil properties, pH was the dominant factor and linearly related to diazotrophs assembly process, while N fixation rate reached peak at near-neutral pH. These results elucidate the mechanism of soil diazotroph assembly process and activity shaped by both geographical factors and fertilization; thus, expand the current understanding of the diazotroph community affected by fertilization strategies across a large geographical scale.

1. Introduction

Biological nitrogen (N) fixation refers to the process by which nitrogen-fixing microorganisms reduce atmospheric N to available ammonium (Galloway et al., 2008). Biological N fixation is one of the essential parts of the nitrogen cycle in terrestrial ecosystems (Kuypers et al., 2018), and it contributes 40 – 100 Tg N to the terrestrial ecosystem per year (Vitousek et al., 2013), which accounts for 75% of the N demand for plant growth worldwide. Only microorganisms in the domains bacteria and archaea are known to be capable of fixing nitrogen (Gaby

---

* Corresponding author at: Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China.

E-mail address: hyyao@iue.ac.cn (H. Yao).

https://doi.org/10.1016/j.geoderma.2020.114923
Received 25 September 2020; Received in revised form 22 December 2020; Accepted 26 December 2020
Available online 8 January 2021

and Buckley, 2012; Kuypers et al., 2018), termed diazotrophs. Diazotrophs perform nitrogen fixation using nitrogenases (Dixon and Kahn, 2004; Gaby et al., 2018) - an enzyme complex - encoded by the *nifH*, *nifD*, and *nifK* genes (Han et al., 2019). Of the three, *nifH*, the most highly conserved, has become the principal marker gene and is widely used for investigating the phylogeny, diversity and composition of diazotroph communities (Gaby et al., 2018; Zehr et al., 2003).

A central aim of current microbial ecology research is to investigate the mechanisms by which environmental changes affect the assembly of soil microbial communities (Hanson et al., 2012; Nemergut et al., 2013; Zhang et al., 2016). Historically, biological community assembly has been dominated by niche-based theory and neutral theory (Jia et al., 2018). The niche theory raises ecological selection as a unique environmental process that influences the identities and abundance of species and emphasizes the importance of determinism and fits well within two postulates: 'Everything is everywhere, but the environment selects' (Baas Becking, 1934) and 'the niche that is occupied by one organismal type is very unlikely to also be occupied by another one' (Gause, 1934). The neutral theory stresses that neutral and often stochastic processes can largely mediate the assembly and dynamic changes in community structures (Hubbell, 2001). This theory assumes that random demographic aspects, probabilistic dispersal, speciation, and unpredictable disturbance are the mechanisms generating divergence across community types. Currently thinking of biological community assembly, biological community assembly is simultaneously affected by deterministic (including environmental filtering, species sorting, and biotic interactions) and stochastic (including random birth, death, dispersal, and colonization) processes rather than previous dichotomy polarization (Dini-Andreote et al., 2015; Jia et al., 2018; Stegen et al., 2013). Increasing evidence has emerged that both deterministic processes and stochastic processes may dominate the soil microbial community assembly process alone or together. Recent studies have suggested that diazotroph assemblies processed in a certain area are derived by either deterministic (Wang et al., 2017a, 2017b) or stochastic (Feng et al., 2018) processes. However, knowledge of the contribution of deterministic and stochastic processes to the diazotroph assembly process at a large geographical scale is lacking. In addition, the mechanism of the assembly process is still not clear.

Shifts in the assembly process may be related to changes in environmental conditions, such as spatial and temporal changes (Dini-Andreote et al., 2015). Microbial communities are expected to be dominated by stochasticity (turnover in community composition shows little deviation from the null expectation) initially, and then changes in environmental conditions progressively increase the strength of selection. If the environmental factors that change through time or space do not impose selection, stochastic factors are expected to remain dominant such that the system will remain ecologically neutral; the environmental factors that increase spatial heterogeneity cause turnover in community composition to be higher than the null expectation; in contrast, spatial environmental homogeneity causes turnover in community composition to be lower than the null expectation. Collectively, spatial and anthropogenic activities (especially fertilization) may affect diazotroph assembly by modulating environmental conditions that impose a selective pressure. Much less is known, however, about the long-term consequences (~decade) of soil fertilization (e.g., chemical and organic fertilizers) on diazotroph assembly processes at a large geographical scale.

Fertilization has major consequences for soil properties and diazotroph communities (Gruber and Galloway, 2008; Li et al., 2018; Reganold et al., 2001). Some studies have found that fertilization, especially its long-term application, strongly suppressed N fixation activity and changed the key diazotroph community structure (Fan et al., 2019; Liu et al., 2020). N fixation is an energy-expensive process that consumes 16 molecules of ATP per unit of N fixed (Bothe et al., 2010). Fertilization provides a large amount of available N in the soil in the short or even long-term, which is obviously cheaper for the soil ecosystem. Over time, nitrogen-fixing activity will be weakened, and microorganisms will be diminished. In contrast, other studies found that fertilization also promotes N fixation activities. This scenario mainly refers to nonnitrogen fertilizers, organic amendments, and other trace element fertilizers. These fertilizers provide diazotrophs with various elements (e.g., P and Mo) (Dong, 2017; Ma et al., 2019; Wang et al., 2018), nutrients and energy resources (Liao et al., 2018) necessary for life. There are also studies suggesting that fertilization changes the diazotrophic community structure through changes in the soil physical and chemical properties, of which the most important factor is soil pH. Soil pH plays an essential role in regulating diazotrophic community assemblage processes and shifting soil dominant taxa (Fan et al., 2018; Han et al., 2019; Wang et al., 2017a, 2017b).

Current studies researching the effect of fertilization on diazotrophs are mostly concentrated in a certain place or area (e.g., similar environmental factors or soil types), which may not be widely applicable to all soils due to their own unique characteristics. As the geographical distance increases, the similarity of nitrogen-fixing communities decreases (Gao et al., 2019; Hanson et al., 2012). For example, *Geobacter* spp, the keystone of N fixers in lime concretion black soil (Fan et al., 2019), were rarely detected or not dominant in other soil types (e.g., red soil, fluvo-aquic soil and black soil (Han et al., 2019; Wang et al., 2017a, 2017b)). In addition, the application of organic amendments (chicken manure) has been found to significantly improve N fixation activity in paddy soil (Liao et al., 2018). Moreover, even the assembly process of the diazotrophic community varies depending on soil type. For instance, stochastic processes were found to be dominant in Calcic Kastanozems (the FAO soil classification system), (Feng et al., 2018), while deterministic processes dominated in Kastanozems and Cambisols, (Wang et al., 2017a, 2017b). Abundant contradictory examples valid in some systems but not others suggest the probability of a major knowledge gap.

In the current study, we aimed to investigate the impact of long-term fertilization on diazotroph assembly processes and activity across a broad spatial scale. We raised 3 main questions: i) How do geographical factors and fertilization cooccurrence cooperatively affect diazotroph assembly processes and activity? ii) What are the dominant influential factors that regulate the diazotroph assembly process and activity? iii) What are the biological mechanisms of geographical factors and fertilization mediating diazotrophs assembly processes and activity? Accordingly, we hypothesized that 1) at a large geographical scale, deterministic processes dominate diazotroph assembly processes and account for a greater proportion of diazotrophs due to fertilization. 2) Geographical factors determine diazotroph activity, and long-term fertilization (N-based chemical fertilization and chemical plus organic amendment fertilization) always suppresses the agricultural soil N fixation rate, which may be related to changes in diazotroph community composition and soil properties. 3) Soil pH plays a primality role in diazotroph assembly processes and activity. Hence, to test these hypotheses, we selected nine agro-ecological experimental sites that covered a wide geographical area (~3500 km), and each site was set for more than a decade and contained experimental treatments of three fertilization strategies: control (without fertilizer), NPK (inorganic N, P, and K fertilizers) and OM (inorganic N, P, K fertilizers plus organic amendment). The diazotrophic communities were analyzed by sequencing *nifH* gene amplicons with Illumina MiSeq technology (San Diego, CA, USA), and the N fixation rate was measured by the $^{15}N_2$-labeling method.

2. Materials and methods

2.1. Experimental site and sampling

A total of 81 soil samples were collected from 9 long-term fertilization experimental stations across China (Table 1), which extends from 28° N to 45°N, and 86° E to 123° E, with the Mean annual precipitation (MAP) ranges from 186 mm to 1476 mm, and mean annual temperature (MAT) ranges from 6.6 °C to 18.0 °C. Nine long-term experimental sites

Table 1
Description of field experimental sites.

Sampling sites	Shaoxing	Jinhua	Ningxiang	Shenyang	Wuhan	Chengdu	Quzhou	Fengqiu	Fukang
Soil Geographical background									
Province	Zhejiang	Zhejiang	Hunan	Liaoning	Hubei	Sichuan	Hebei	Henan	Xinjiang
Longitude	120°41′E	119°39′E	112°18′E	123°36′E	114°20′E	103°37′E	115°1′E	114°24′E	86°15′E
Latitude	29°45′N	29°15′N	28°7′N	41°48′N	30°29′N	30°31′N	36°52′N	35°00′N	44°18′N
MAP (mm)	1461	1476	1358	721.9	1300	918	566.7	615	186
MAT (°C)	18.0	17.6	16.8	7.4	16.7	16.2	13.2	14.0	6.6
Crop rotation	Rice-Wheat	Rice-Wheat	Rice-Wheat	Corn	Rice-Wheat	Rice-Wheat	Wheat-Corn	Wheat-Corn	Wheat
Soil chemical properties at the Control treatment									
pH	5.66	5.57	6.47	6.74	7.24	7.82	8.58	8.66	9.21
TN (g kg^{-1})	1.54	1.18	1.90	1.04	0.74	1.10	0.78	0.47	0.59
NH_4^+ (mg kg^{-1})	6.75	8.73	30.8	14.16	14.20	2.33	10.47	4.80	4.86
NO_3^- (mg kg^{-1})	24.23	24.02	28.6	12.54	13.31	1.28	17.11	6.30	9.89
SOM (g kg^{-1})	2.67	1.89	3.55	1.68	0.88	1.75	1.08	0.57	0.96
AP (mg kg^{-1})	62.82	32.36	2.42	1.82	3.35	2.23	2.04	0.78	4.37
Soil texture	Loam	Loam	Silt loam	Silt loam	Silt loam	Loam	Silt loam	Silt loam	Silt loam
Clay (%)	20	18	13	22	27	9	12	11	10
Silt (%)	38	39	68	52	64	43	69	72	50
Sand (%)	42	43	19	26	9	48	19	17	40

planted crops including rice, wheat, corn, etc., and have been fertilizing for more than 10 years. In the current study, three treatments were selected from each site, Control (without fertilization), NPK (mineral NPK fertilizers) and OM (mineral NPK fertilizers plus organic amendments), and each treatment have three replicates. Details of experimental sites are fully described in Tables 1 and S1.

Soil samples were collected in June 2017. Each soil sample was combined with 5 cores taken at a depth of 0–20 cm using a 20-mm diameter gouge auger. Five cores were mixed, roots and stones were removed and then put in a dry-icebox immediately and transported to the laboratory. Each sample was divided into three aliquots. One aliquot was air-dried, sieved and used for the analysis of soil pH, soil organic matter (SOM), total nitrogen (TN) and soil Olsen P (AP); one aliquot was sieved and stored at 4 °C for the soil inorganic N (NH_4^+ and NO_3^-) and N fixation rate, and the other aliquot was freeze-dried and stored at −80 °C for DNA extraction.

2.2. Soil properties analysis

Soil pH was measured from a soil/distilled water ratio of 1:2.5 with a pH meter. SOM was determined by the wet oxidation redox titration method. TN was determined by a Vario MAX CNS elemental analyzer (ELEMENTAR, Germany). AP was determined using the Olsen method (Olsen et al., 1954). The soil NO_3^- and NH_4^+ contents determined by a continuous flow injection analyzer (FLA star 5000 Analyzer, Foss, Denmark) extracted by 1 mol L^{-1} KCl (soil/KCl, 1:10).

N fixation rates were measured by $^{15}N_2$-labeling method which was common and widely applied for studying diazotrophs activity (Fan et al., 2019; Hsu and Buckley, 2009). Briefly, 5 g soil was placed into 120 ml bottles, and the headspace was replaced with synthetic air containing 20% O_2 and 80% $^{15}N_2$ (99.9 atom % ^{15}N, Isotec, Miamisburg, OH, USA). The controls were filled with unlabeled N_2 gas and processed in parallel. The bottles were incubated horizontally in the dark at 25 °C for 20 days. The atom % ^{15}N of soil samples was determined using a stable isotope ratio mass spectrometer (Flash 2000 HT/Conflo IV/Delta V, Thermo Fisher Scientific, Germany). Then, we calculated the net potential N fixation rate by comparing the difference of total ^{15}N in soils receiving $^{15}N_2$ relative to control.

2.3. DNA extraction, PCR amplification, high throughput sequencing and bioinformatics analysis

Soil DNA was extracted from 0.5 g soil by using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. After that, the extracted DNA was evaluated by a NanoDrop 2000 (NanoDrop Technologies, Wilmington, USA). The *nifH* gene were amplified with primer pairs *nifH*-F/*nifH*-R (5′-AAAGGYGGWATCGGYAARTCCACCAC-3′/5′- TTGTTSGCSGCRTACATSGCCATCAT-3′) (Rösch et al., 2002) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The 20 μL PCR mixture contained 2 μL of template DNA, 22.5 μL of 2 × PCR Master Mix, 1.5 μL of each primer, and 0.2 μL of bovine serum albumin (BSA) made up to 50 μL with sterile water (ddH_2O). The PCR amplification of *nifH* gene was performed as follows: 2 min of denaturation, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 45 s, and single extension at 72 °C for 7 min, and end at 4 °C. The PCR products were run on a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP128014).

After sequencing, the quality score less than 30 or shorter than 40 bp sequences were removed by cutadapt 1.9.1 and the rest Illumina MiSeq fastq reads were analyzed using the QIIME2 platform (version 2019.4) and were processed by the DADA2 program. The actual biological sequences present in data were called amplicon sequence variants (ASVs), and can be compared between different studies. In QIIME 2, these ASVs have a higher resolution than the QIIME 1 default of 97% OTUs, and their quality is higher since these quality control steps are better than those implemented in QIIME 1. This should result in more accurate estimates of the diversity and taxonomic composition of samples than achieved with QIIME 1. The taxonomy was assigned based on both nucleotide and translated amino acid sequences (Gaby et al., 2018; Gaby and Buckley, 2014). The FastTree method was used to build a phylogenetic tree based on aligned representative sequences and demonstrated in MEGA 6.0. Sequences reads are archived in the NCBI Sequence Read Archive, accession PRJNA661676.

2.4. Statistical analyses

The richness and Shannon indexes were calculated using 'Mutidiversity' and 'picante' in the 'vegan' package of R (R 3.6.1) (Dixon, 2003; R Core Team, 2017) to represent taxonomic diversity based on the ASV-table. The mean nearest taxon distance (MNTD) was measured to describe phylogenetic diversity. The smaller the value of MNTD, the closer the relationship between species. The nearest taxon index (NTI) was calculated to quantify the degree of phylogenetic relatedness among species within each plot. MNTD and NTI was calculated using 'mntd' and 'ses.mntd' in the 'picante' package of R (R 3.6.1) (Kembel et al., 2010). MNTD measures mean nearest taxon distance between all pairs of OTUs within one sample and NTI measures the deviation of observed MNTD from MNTD in a null model, in which the tip labels of the phylogeny are randomized (null.model = 'taxa.labels' in 'ses.mntd') (Webb et al., 2002). If NTI > 2 or mean NTI > 0 in a community, coexisting taxa are significantly more closely related, indicating phylogenetic clustering. In contrast, if NTI < -2 or mean NTI < 0, then coexisting taxa are significantly more distantly related (phylogenetic overdispersion) (Stegen et al., 2012).

Phylogenetic turnover (phylogenetic β-diversity) was calculated by the unweighted-UniFrac distance, β mean nearest taxon distance (βMNTD) and βNTI; βMNTD is the mean phylogenetic distance to the closest relative between pairs of communities, and βNTI is the between-assemblage analog of NTI. βMNTD measures MNTD between all pairs of OTUs distributed in two different samples and was calculated using 'comdistnt' in the R package 'picante' (R 3.6.1) and βNTI measures the deviation of observed βMNTD from mean βMNTD in the null model in which taxa are randomized across the tips of phylogenetic trees. βNTI was calculated using in the R package 'picante' (R 3.6.1). According to the framework of Stegen et al. (2012), $|\beta\text{NTI}| < 2$ indicates stochastic community assembly because of immigration and weak selection; on the contrary, $|\beta\text{NTI}| > 2$ indicates a deterministic process, which is subdivided into $\beta\text{NTI} > 2$, which indicates a heterogeneous selective environment leads to variable selection, and $\beta\text{NTI} < -2$, which indicates a homogeneous selective environment leads to homogeneous selection. Unweighted UniFrac and βMNTD were demonstrated by principal coordinates analysis (PCoA) by the 'vegan' package in R (R 3.6.1).

The significance of differences in environmental variables (i.e., MAT, MAP and soil properties), diazotrophic diversity (i.e., Richness, Shannon and MNTD) and N fixation rate were analyzed by two-way ANOVA test (i.e., sites and fertilization strategies were the two variables, and their interactions were also tested) using the software SPSS 22 at $p < 0.05$. And then the significance of differences of environmental variables, diazotrophic diversity and N fixation rate were tested by the Tukey honestly significant difference (HSD) test using the software SPSS 22, at $p < 0.05$. Phylogenetic dissimilarity of the diazotrophic community among the sites and fertilization strategies was analyzed by PERMANOVA ('vegan' package in R 3.6.1). The relationships between diazotrophic diversity, N fixation rate, and soil properties were examined with linear regressions, and the relationships between environmental variables and diazotrophic community structure were assessed using a partial Mantel test with Pearson correlation ('vegan' package in R 3.6.1). Redundancy analysis (RDA) was performed to determine the relationships between dominant soil diazotrophic genera and environmental variables (permutations = 999) ('vegan' package in R 3.6.1). Aggregated boosted tree analysis (ABT) was carried out to quantitatively evaluate the relative influence of environmental variables on the N fixation rate. ABT analysis was performed using 'gbmplus' package (R 2.7.1) (De'ath, 2007). The rate of distance decay (i.e., slope of the distance decay curves) of the diazotrophic communities was calculated based on the following equation:

$$\lg(Similarity) = \lg(a) + b\lg(Distance)$$

where *Similarity* is diazotrophic phylogenetic community similarity; *Distance* is geographical distance or chemical distance. Geographical distance was calculated using the latitudinal and longitudinal coordinates of each sampling position. The chemical distance was calculated using 5 properties (pH, SOM, NH_4^+, NO_3^- and AP) of each soil sample, we first normalize the data and calculate it according to Euclidean distance ('vegan' package in R 3.6.1). a is an intercept parameter and b is the slope coefficient of the distance-decay curve (Chen et al., 2017a, 2017b). Then, permutation tests were used to test the significance of slopes: control vs. NPK, control vs. OM and NPK vs. OM. Partial least squares path modeling (PLS-PM) was used to explore the direct, indirect, and interactive relationships between various factors for the diazotrophic assembly process (β diversity based on βMNTD) and the N fixation rate. The model included the following variables: site (longitude and latitude), fertilization, climate (MAT and MAP), soil properties (soil pH, TN, SOM, NH_4^+, NO_3^- and AP) and diazotrophic communities (diazotrophic taxa in genera). Indirect effects were defined as multiple path coefficients between predictor and response variables, including all possible paths, excluding the direct effect. PLS-PM analysis was performed using 'plspm' package in R 3.6.1 (Sanchez, 2013).

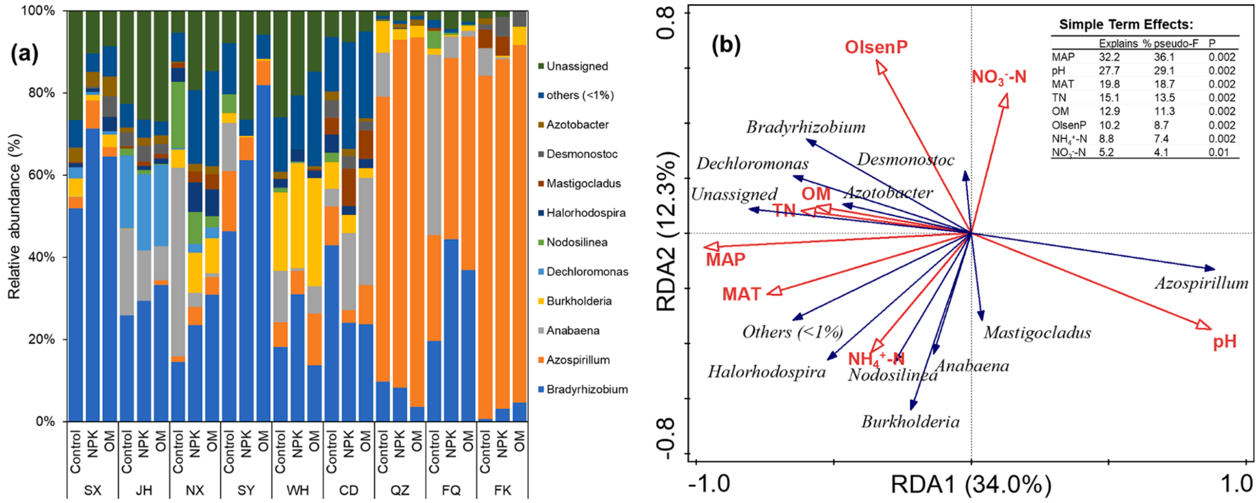
3. Result

3.1. Diazotrophic community composition and diversity

A total of 120,570 high-quality reads were clustered as 3502 ASVs at the single-nucleotide resolution across 78 soil samples, discarding 3 samples with extremely low diazotrophic ASVs richness. According to results from high-throughput sequencing, we found 11.0% of ASVs were unassigned, and all assigned ASVs be categorized into 5 phyla and 59 genera. At the phylum level, the dominant phyla (relative abundance > 1%) were Proteobacteria and Cyanobacteria in all samples (Fig. S1), and they contribute to 87.6% of all ASVs when added up. The dominant genera (relative abundance > 2%) were *Bradyrhizobium* (~30.5%), *Azospirillum* (~26.8%), *Anabaena* (~8.9%), *Burkholderia* (~5.2%) and *Dechloromonas* (~2.4%) (Fig. 1a). Two-way ANOVA showed that the relative abundance of dominant genera was strongly affected by both sites and fertilization strategies ($p < 0.05$). When we focus on each individual site, we found that the NPK treatment and OM treatment significantly increased the relative abundance of *Bradyrhizobium* compared with the control treatment in all sites except Chengdu and Quzhou, and significantly decreased relative abundance of *Anabaena* and *Nodosiline* (both are Cyanobacteria) compared with the control except Chengdu.

RDA diagrams (Fig. 1b) showed that environmental variables explained 52.6% of the total diazotrophic community composition, and the two factors with the highest simple term effects were MAP and soil pH, which explained 36.1% and 27.7% of the variation, respectively. In addition, soil pH was positively correlated with the relative abundance of *Azospirillum* and was negatively correlated with MAP and *Bradyrhizobium*.

The richness, Shannon and MNTD indexes were used to estimate diazotrophic taxonomic and phylogenetic diversities among different geographical sites and fertilization strategies (Table 2). The richness and diversity indexes of soil diazotrophs ranged from 33.5 ± 4.9 to 85.7 ± 7.6, 3.74 ± 0.11 to 6.00 ± 0.11 and 0.021 ± 0.004 to 0.159 ± 0.032, respectively. Two-way ANOVA showed that geographical sites strongly affected richness and diversity ($p < 0.05$) and explained more than half of the variation ($\eta^2 > 0.5$). However, fertilization only explained less than 1% of the variation; it also significantly affected the Shannon index and MNTD (site × fertilization factor, $p < 0.05$). Correlation analysis (Table 2) showed that richness, Shannon index and MNTD were positively correlated with MAT, MAP, TN and SOM. However, soil pH solely showed a robust positive correlation with MNTD rather than taxonomic diversities, suggesting that soil pH was a key factor in phylogenetic diazotrophic diversities. The mean nearest taxon index (NTI) was used to determine whether the species were more phylogenetically related to one another than to other species found in that soil (Table 2 and 3); for all samples, the NTI was significantly greater than 0, and ~74% of the samples had a value higher than +2, showing that the diazotroph community was more phylogenetically clustered. NTI was strongly affected by both site and fertilization strategies ($p < 0.05$), and fertilization increased the range of phylogenetic clustering (Fig. S2). Linear regression analysis (Table 3) showed that NTI was significantly negatively correlated with MAT, MAP, TN, NH_4^+, SOM and AP and positively



	Explains %	pseudo-F	P
MAP	32.2	36.1	0.002
pH	27.7	29.1	0.002
MAT	19.8	18.7	0.002
TN	15.1	13.5	0.002
OM	12.9	11.3	0.002
OlsenP	10.2	8.7	0.002
NH_4^+-N	8.8	7.4	0.002
NO_3^--N	5.2	4.1	0.01

Fig. 1. Distributions of the dominant diazotroph genera (>1%) responses to sites and fertilization strategies (a) and Redundancy analysis (RDA) diagrams (b) based on Relative abundance of diazotrophic communities in genera level as affected by sites and fertilization. Data are means of 3 replicates. Control: without fertilizers; NPK: mineral NPK fertilizers; OM: mineral NPK fertilizers plus organic amendments.

correlated with pH ($p < 0.05$).

3.2. Diazotroph phylogenetic community structure and assembly process

principal coordinates analysis (PCoA) using both unweighted UniFrac and βMNTD showed that diazotrophic communities were more phylogenetically clustered by geographical site than by fertilization (Fig. 2). Permutational ANOVA showed that fertilization had a significant effect on the diazotrophic community structure through an interaction with sites. The partial Mantel test (Table 3) showed that soil pH and NO_3^- were correlated with the diazotrophic communities.

βNTI scores described diazotroph phylogenetic structure turnover between sites in the same fertilization strategy (Figs. 3 and S3). Only 31.9% ~ 44.4% of the βNTI scores for the diazotrophic communities in our study were in the range of −2 to +2, suggesting that deterministic processes dominated diazotrophic community dynamics. Compared with the control treatment, the two fertilization treatments strongly increased variable selection, which originated from stochastic processes, indicating that fertilization shifts the assemblage process from stochastic to deterministic processes. In addition, ~15.7% of the βNTI scores were lower than −2 and were not affected by fertilization, mainly in the conversion between Fukang, Fengqiu and Quzhou, and in all site replicates.

Distance-decay relationship (DDR) curves showed that diazotrophic phylogenetic community similarity (Unweight-UniFrac) significantly decreased with increasing geographical distance ($p < 0.05$) (Fig. 4a), suggesting that geographical distance has a vast influence on diazotrophic community distribution. The distance-decay slopes in the control, NPK and OM treatments were −0.125, −0.128 and −0.136, respectively. Negative correlations were again observed for chemical distance (Fig. 4b), with slopes of −0.428, −0.453 and −0.441, respectively, in the three fertilization strategies. Fertilization had a tendency to reduce the slope of both geographical and chemical DDR curves, but the difference was not significant (Fig. 4c).

3.3. The N fixation rate

The N fixation rate ranged from 30.0 ± 6.9 to 177.2 ± 13.3 μg kg^{-1} d^{-1} and was significantly affected by geographical site, fertilization and their interaction ($p < 0.05$). In particular, geographical factors explained 78.6% of the total variation (Fig. 5a). The highest N fixation rate was found in Wuhan' control treatment, 177.2 μg kg^{-1} d^{-1}, which was significantly higher than that of other sites and treatments. The lowest nitrogen fixation rate was found at the FK site, 31.9 μg kg^{-1} d^{-1} on average, which was lower than that at the other sites ($p < 0.01$). In Shaoxing, Jinhua, Ningxiang, and Wuhan, NPK application reduced the N fixation rate compared with the control ($p < 0.01$). In contrast, other sites caused no changes or increased the N fixation rate. At the same time, the OM treatment maintained an N fixation rate similar to that of the control treatment in most regions.

3.4. Relative contributions of geographical factors and fertilization to diazotroph assembly processes and activity

PLS-PM was constructed to deeply explore how the diazotroph assembly processes and activity were affected by geographical factors and fertilization (Fig. 6). Geographical location directly determined the local climate characteristics; the lower the latitude and longitude, the higher the MAT and MAP. We collectively refer to latitude, longitude and climate factors as geographical factors. Geographical factors were the dominant factors that directly and indirectly impacted the diazotroph assembly processes. In contrast, fertilization only indirectly influenced diazotroph assembly processes. Both diazotroph community composition and soil properties played a key role in geographical factors and fertilization, indirectly affecting the assembly processes.

In contrast, both geographical factors and fertilization affected the N fixation rate indirectly rather than directly. Soil properties played a prominent role in the N fixation rate. The aggregated boosted tree (ABT) model further illustrated the levels of the interaction effects of the environmental variables on the N fixation rate (Fig. 7a) and confirmed that soil pH was the major factor determining its rate, accounting for approximately 40.3% of the relative influence. Correlation analysis showed that the N fixation rate increased at pH nearly neutral (6.8) rather than a linear correlation (Fig. 7b).

4. Discussion

4.1. How do geographical factors and fertilization cooccurrence cooperatively affect diazotroph assembly processes?

Both stochastic and deterministic processes influenced diazotrophic community assembly simultaneously at large geographical scales and under various fertilization strategies (Fig. 3 and S3). Among them, deterministic processes were more imperative and accounted for more than 55% in the control treatment, in line with hypothesis 1, suggesting that environmental filtering, species sorting and biotic interactions play

Table 2
Taxonomic and phylogenetic diversities indices of diazotrophic communities as affected by geographical sites and fertilization strategies.

Site	Treatments	Taxonomic Diversity		Phylogenetic Diversity	
		Richness	Shannon	MNTD	NTI
Shaoxing	Control	45.0 ± 1.70	4.90 ± 0.45	0.140 ± 0.018	1.87 ± 1.04
	NPK	47.7 ± 8.70	5.09 ± 0.20	0.127 ± 0.012	2.18 ± 0.25
	OM	51.3 ± 5.0	5.28 ± 0.09	0.136 ± 0.014	2.06 ± 0.8
Jinhua	Control	54.3 ± 14.6	4.62 ± 0.06	0.138 ± 0.032	1.25 ± 0.43
	NPK	47.3 ± 3.1	4.71 ± 0.17	0.133 ± 0.004	1.69 ± 0.19
	OM	56.3 ± 11.2	4.88 ± 0.23	0.117 ± 0.015	1.93 ± 0.26
Ningxiang	Control	67.0 ± 21.5	4.45 ± 0.39	0.063 ± 0.018	2.49 ± 0.19
	NPK	80.0 ± 14.0	5.88 ± 0.22	0.127 ± 0.026	1.70 ± 0.90
	OM	85.7 ± 7.6	6.00 ± 0.11	0.115 ± 0.007	2.18 ± 0.40
Shenyang	Control	47.3 ± 9.1	4.31 ± 0.26	0.106 ± 0.028	1.88 ± 0.35
	NPK	32.5 ± 3.5	3.74 ± 0.11	0.037 ± 0.012	3.46 ± 0.18
	OM	40.3 ± 11.6	3.83 ± 0.36	0.059 ± 0.036	2.72 ± 0.60
Wuhan	Control	50.0 ± 14.7	4.98 ± 0.22	0.123 ± 0.041	2.26 ± 1.04
	NPK	44.7 ± 20	4.78 ± 0.80	0.159 ± 0.032	1.28 ± 0.98
	OM	46.3 ± 12.3	4.92 ± 0.55	0.085 ± 0.027	3.44 ± 0.64
Chengdu	Control	80.0 ± 7.0	5.71 ± 0.23	0.105 ± 0.007	2.40 ± 0.34
	NPK	78.3 ± 31.6	5.42 ± 0.67	0.078 ± 0.013	3.16 ± 0.79
	OM	80.0 ± 9.5	5.41 ± 0.08	0.076 ± 0.014	2.80 ± 0.27
Quzhou	Control	48.7 ± 5.0	4.84 ± 0.08	0.052 ± 0.015	3.87 ± 0.14
	NPK	45 ± 2.6	4.84 ± 0.07	0.052 ± 0.025	4.49 ± 0.95
	OM	44.5 ± 7.5	4.84 ± 0.17	0.031 ± 0.008	5.03 ± 0.19
Fengqiu	Control	54.3 ± 7.5	5.04 ± 0.04	0.073 ± 0.008	3.80 ± 0.45
	NPK	42.0 ± 10.6	4.54 ± 0.28	0.047 ± 0.011	4.01 ± 0.16
	OM	35.3 ± 10.6	4.42 ± 0.34	0.039 ± 0.012	4.40 ± 0.37
Fukang	Control	42.0 ± 11.5	4.83 ± 0.46	0.021 ± 0.004	5.49 ± 0.75
	NPK	39.3 ± 7.0	4.95 ± 0.21	0.039 ± 0.029	5.62 ± 1.02
	OM	33.5 ± 4.9	4.75 ± 0.09	0.068 ± 0.032	4.75 ± 1.28
Source of variance					
Site	*p*	**0.001**	**0.001**	**0.001**	**0.001**
	η2	0.637	0.561	0.615	0.719
Fertilization	*p*	0.565	0.723	0.171	**0.047**
	η2	0.006	0.003	0.011	0.017
Site × Fertilization	*p*	0.784	**0.001**	**0.001**	**0.017**
	η2	0.062	0.231	0.193	0.093

Control: without fertilization; NPK: mineral NPK fertilizers; OM: mineral NPK fertilizers plus organic amendments. Data are means of 3 replicates ± standard deviation (n = 3). The significance of differences for diazotrophic α-diversities were analyzed by two-way ANOVA, significant values are shown in bold ($p < 0.05$)

a decisive role in diazotroph assembly; at the same time, the contribution of ecological drift, probabilistic dispersal, and/or random births/deaths cannot be ignored (Luan et al., 2020). In other studies, deterministic processes always dominated at large scales (Shi et al., 2018; Wang et al., 2017a, 2017b), explained by fact that spatial increase; in addition, the greater the geographical distance, the smaller the impact of dispersal and random events on community assembly (Hanson et al., 2012). The distance-decay pattern (Fig. 5) verifies the contribution of geographical factors to diazotroph assembly processes (Morlon et al., 2008). The negative correlation between community similarity and geographical distance and chemical distance indicates that environmental selection and drift have an important contribution to the diazotroph assembly process (Jiao et al., 2020). However, the slopes were not significantly different between the various fertilization strategies, indicating a relative contribution between geographical factors and fertilization; obviously, geographical factors rather than fertilization are the most important contributors to the distance-decay pattern. The PLS-PM model and statistical analysis (two-way ANOVA, $p < 0.05$ and higher η^2 for the main factor of the geographical factors) of diazotroph composition and diversity (Table 2) provide support that geographical factors play a decisive role.

However, this does not mean that the effect of long-term fertilization can be ignored. Long-term fertilization shifts diazotrophic community turnover from stochastic processes to heterogeneous deterministic processes, suggesting that fertilization changes local environmental properties and increases environmental heterogeneity, in line with hypothesis 1. The PLS-PM model showed that fertilization affected the diazotroph assembly processes by indirectly affecting the soil properties ($p < 0.05$), which increased phylogenetic turnover and adjusted the community composition (Fig. 1b and Table 3). On the one hand, fertilization resulted in an extreme decrease in soil pH and an increase in TN and NO_3^- and also increased the relative abundance of *Bradyrhizobium* at most sites; the mechanism is similar to that of the geographical factors. Similarly, Lin et al. (2018) found that fertilization (inorganic fertilizer plus pig manure) increased soil pH while shifting the diazotroph composition from one dominated by *Bradyrhizobium* to one dominated by *Azospirillum*, similar to our results. On the other hand, fertilization shifted the linear regression curve of NTI and soil pH upward within any single site, providing sound proof that fertilization shifted the diazotrophic community from phylogenetic evenness to phylogenetic clustering (Fig. S2).

4.2. How do geographical factors and fertilization cooccurrence cooperatively affect diazotroph activity?

In line with our second hypothesis, two-way ANOVA analysis of N fixation rate showed that geographic factors played a decisive role in diazotrophs activity. This was supported by PLS-PM model that showed the geographic factors, directly and indirectly, altered N fixation rate (Fig. 6b). Geographical location determined the participants (i.e., diazotroph assembly processes and composition) and environment (i.e., environmental variables). The dominant taxa (i.e., *Bradyrhizobium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Anabaena* and *Nodosiline*), which are considered as non-symbiotic nitrogen-fixing bacteria (Berthrong et al., 2014; Bothe et al., 2010; Kennedy, 2004; Okubo et al., 2012), shown obvious geographic distribution patterns. In addition, a suitable environment is also more conducive to N fixation, such as a neutral pH (Hsu and Buckley, 2009) and sufficient nutrients (Ma et al., 2019).

Table 3
Relationship between environmental variables and the diversity indices of diazotrophs.

		α-diversity				β-diversity	
		Richness	Shannon	MNTD	NTI	Unifrac	βMNTD
MAT	R	0.44	0.461	0.615	−0.648	0.046	−0.076
	p	**0.001**	**0.001**	**0.001**	**0.001**	0.051	0.983
MAP	R	0.326	0.249	0.772	−0.816	0.062	0.021
	p	**0.004**	**0.028**	**0.001**	**0.001**	**0.012**	0.282
pH	R	−0.104	0.052	−0.62	0.748	0.073	0.066
	p	0.363	0.65	**0.001**	**0.001**	**0.003**	**0.025**
TN	R	0.415	0.298	0.387	−0.484	0.005	0.087
	p	**0.001**	**0.008**	**0.001**	**0.001**	0.438	**0.035**
NH_4^+	R	0.255	0.13	0.212	−0.31	0.116	0.054
	p	**0.024**	0.256	0.058	**0.006**	**0.002**	0.131
NO_3^-	R	−0.3	−0.355	−0.262	0.131	0.156	0.137
	p	**0.008**	**0.001**	**0.019**	0.252	**0.001**	**0.013**
SOM	R	0.341	0.272	0.379	−0.458	0.034	0.061
	p	**0.002**	**0.016**	**0.001**	**0.001**	0.105	0.066
AP	R	−0.057	0.099	0.374	−0.266	0.055	0.066
	p	0.618	0.388	**0.001**	**0.019**	**0.046**	0.107

The significances were tested based on 10 000 permutations. Partial Mantel tests for the correlation between β-diversity metric and the explanatory variable using Pearson correlation. Significant values are shown in bold ($p < 0.05$).

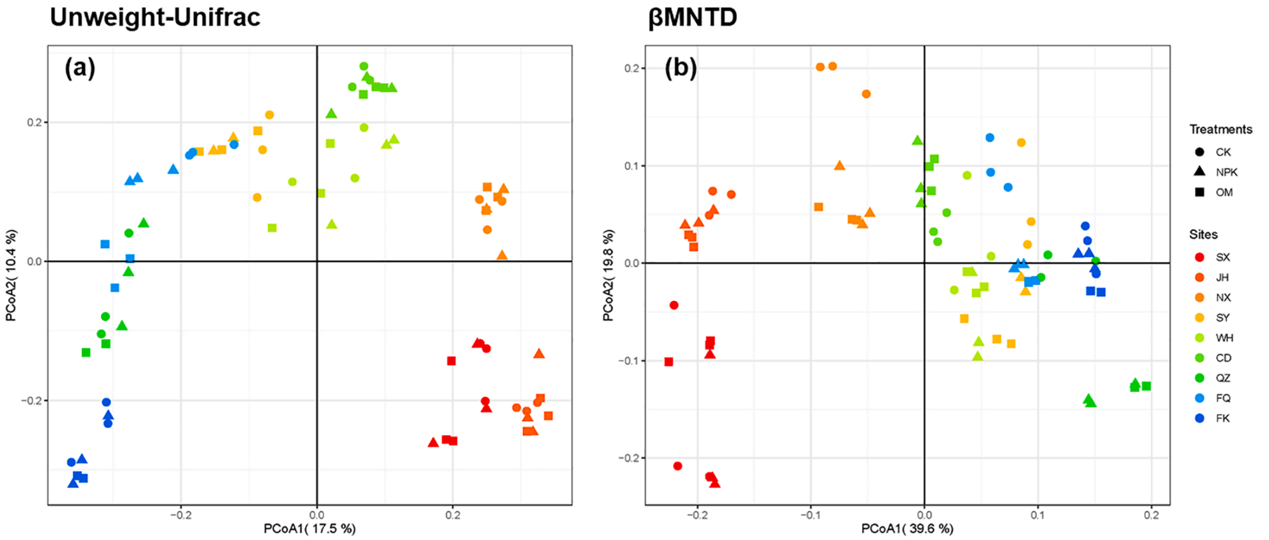


Fig. 2. principal coordinates analysis (PCoA) plots based on Unweighted-UniFrac (a) and βMNTD (b) of diazotrophic communities as affected by geographic factors and fertilization strategies.

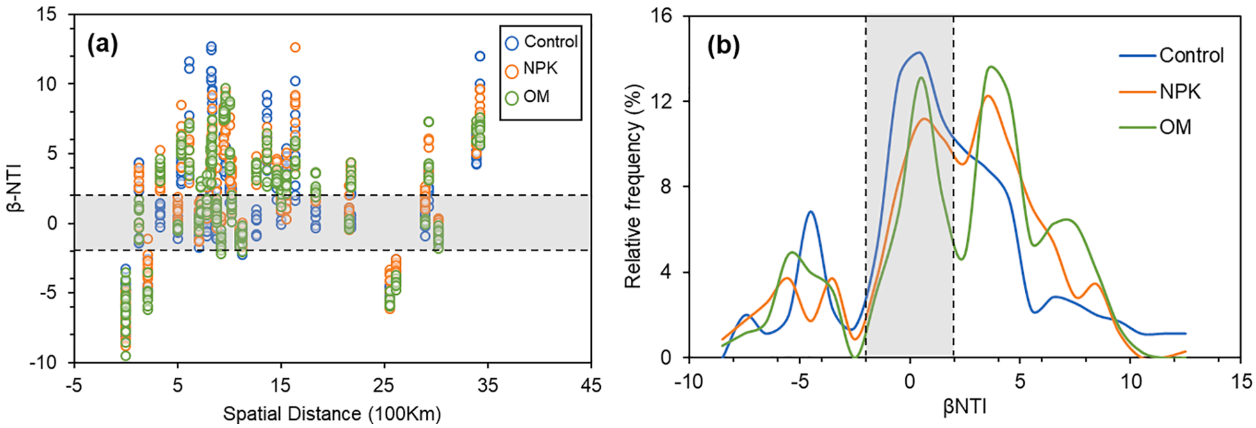
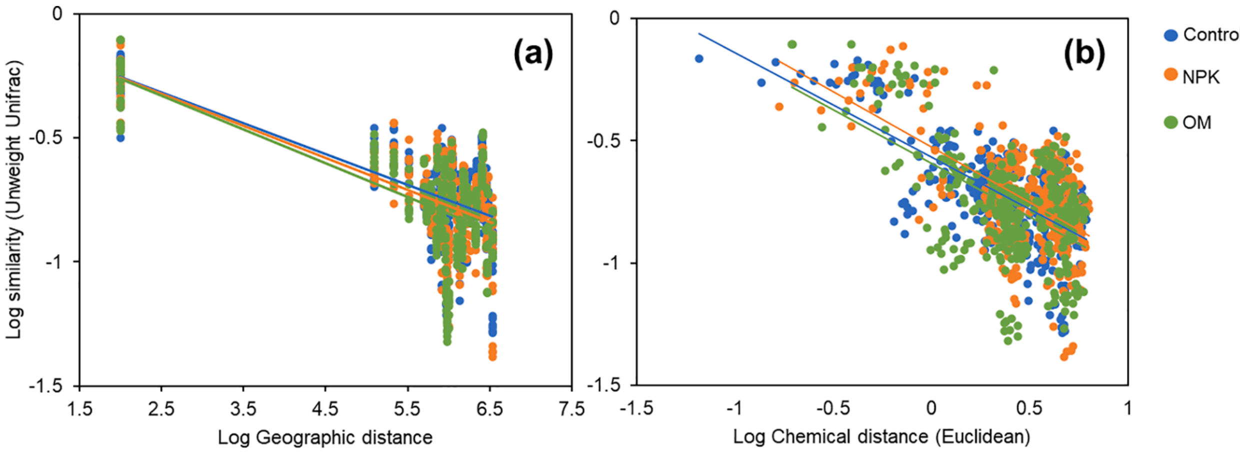


Fig. 3. Variation of beta Nearest Taxon Index (βNTI) for all pairwise community comparisons along spatial distance between communities (a); and the distributions of βNTI in different fertilization strategies (b). The horizontal dotted line (above +2 or below −2 are statistically significant) shows the 95% confidence intervals around the expectation under neutral community assembly. Control: without fertilizers; NPK: mineral NPK fertilizers; OM: mineral NPK fertilizers plus organic amendments.



(c)

Unweight-Unifrac	Similarity-Geographic distance			Similarity-Chemical distance		
	Control	NPK	OM	Control	NPK	OM
slope	-0.125	-0.128	-0.136	-0.428	-0.453	-0.441
r	0.70	0.70	0.69	0.67	0.59	0.55
p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
Permutation analysis						
Control v.s. NPK	0.692			0.545		
Control v.s. OM	0.239			0.762		
NPK v.s. OM	0.432			0.805		

Fig. 4. The relationships between unweighted-Unifrac distance and geographic distance (a) or chemical distance (b). A list of the correlation and slopes of distance-decay curves (c). The slopes were pairwise compared among 3 fertilization strategies by permutation tests. Significant values are shown in bold ($p < 0.05$). Control: without fertilization; NPK: mineral NPK fertilizers; OM: mineral NPK fertilizers plus organic amendments.

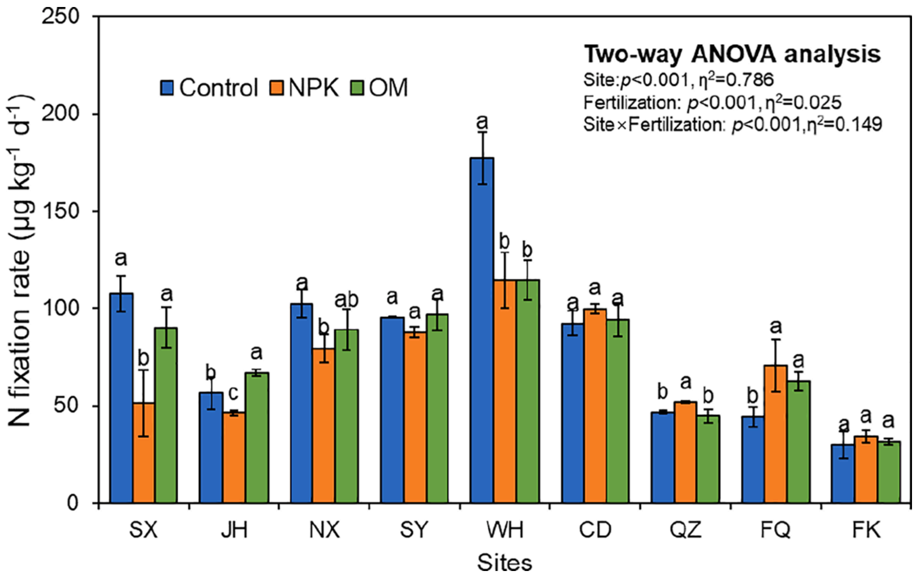


Fig. 5. Nitrogen fixation rates affected by sites and fertilization strategies. Control: without fertilization; NPK: mineral NPK fertilizers; OM: mineral NPK fertilizers plus organic amendments, *p* values were obtained after two-way ANOVA. Data are means of 3 replicates with standard deviation. Values with different lowercase letters within a site are statistically significantly different at $p < 0.05$ after Tukey HSD test.

Fertilization also significantly changed the diazotroph activity (Fig. 5); however, this does not mean that all fertilization types suppressed diazotroph activity, refuting hypothesis 2. Chemical fertilization alone inhibited the N fixation rate at nearly half of the sites; in contrast, chemical fertilizer plus organic amendment maintained or even increased the N fixation rate at most sites. Current researchers have reported conflicting results about whether fertilization reduces the N fixation rate (Fan et al., 2019; Liao et al., 2018). For example, while it has been found that various fertilization strategies reduced N fixation rates (Fan et al., 2019). Liao et al. (2018) found that fertilization increased N fixation rate. However, all of these studies also believed that the effects of fertilization on the diazotroph community composition and soil properties were related to a change in the N fixation rate, consistent with the results of the PLS-PM model in the present study (Fig. 6b),

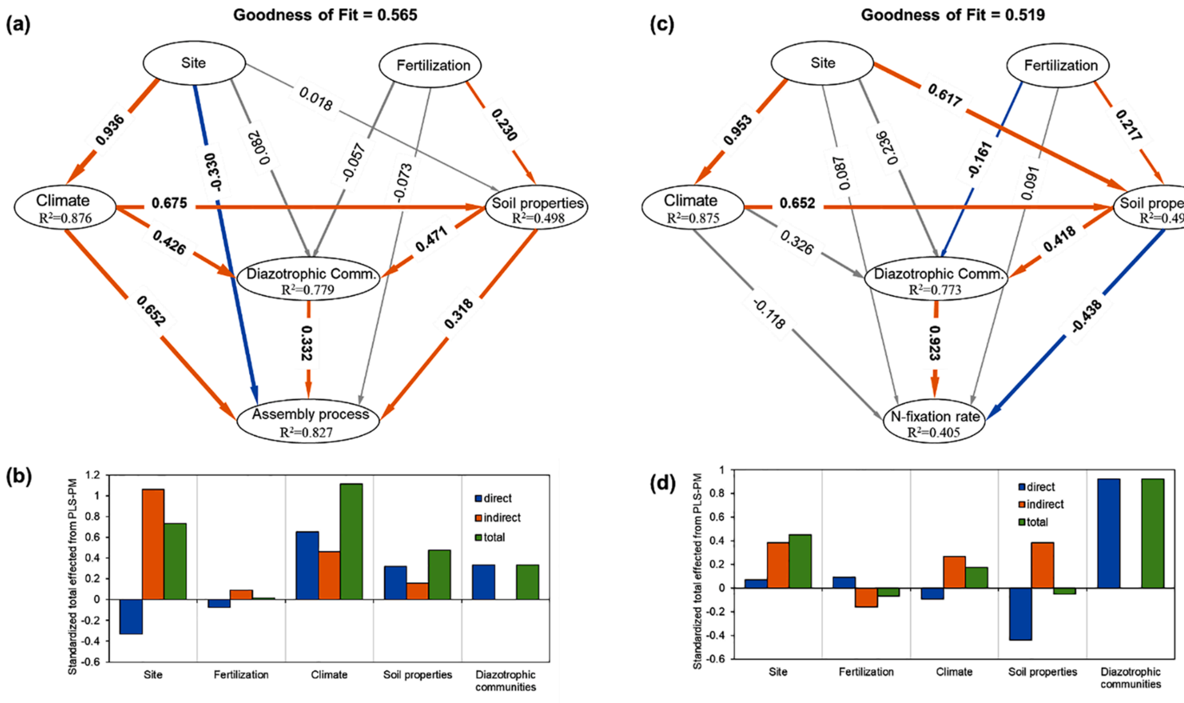


Fig. 6. PLS-PM describing the relationships among Geographic factor, Fertilization, Climate, Soil properties, and diazotrophic communities with respect to assembly processes (a) and N-fixation rate (c). Larger path coefficients are shown as wider arrows, and significance positive and negative effects are indicated by orange and blue colors, respectively. Path coefficients and coefficients of determination (R^2) were calculated after 999 bootstraps. Standardized direct and indirect mean effects on assembly processed (b) and N-fixation rate (d), respectively, derived from the PLS-PMs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

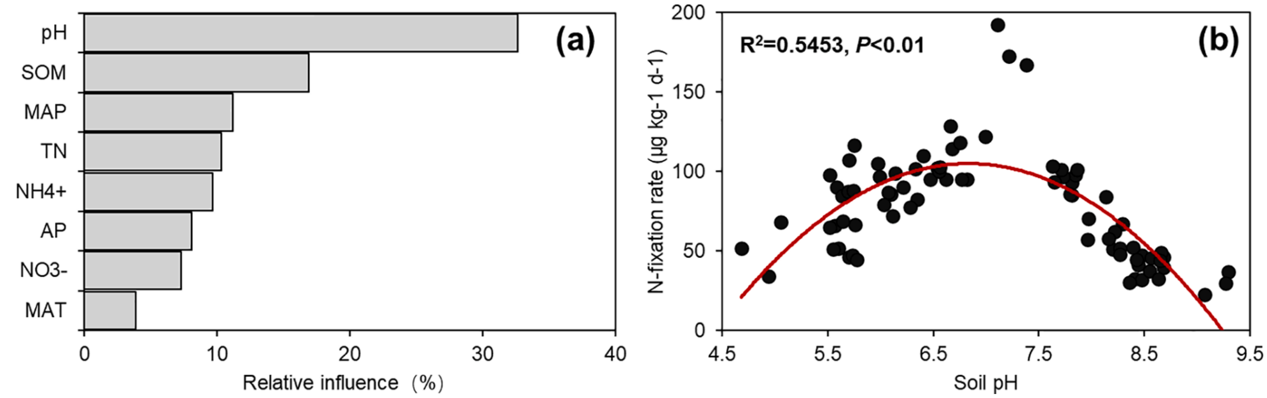


Fig. 7. Relative influence of environmental variables on nitrogen fixation rates. Partial correlation analysis between the nitrogen fixation rates and soil pH by ABT models (a). Relationship between N fixation rate and soil pH (b).

which showed that fertilization affected the N fixation rate by indirectly affecting the diazotroph community and soil properties. For example, fertilization significantly decreased the relative abundance of cyanobacteria from 26% to 10% (Fig. S1).

4.3. What are the dominant influential factors and the biological mechanisms of diazotroph assembly processes and activity?

Evidently, environmental variables were among the dominant influential factors that also directly affected species sorting and biotic interactions (Fig. 6), in line with the niche-based theory in the historical view (Baas Becking, 1934). Among many environmental variables, soil pH played a key role in the diazotrophic community (Table 3, Figs. 1 and 7). We observed pronounced soil pH trends in the diazotrophic community within any single site (α-diversity) and community turnover (β-diversity), consistent with other studies (Fan et al., 2018; Wang et al., 2017a, 2017b). A number of other studies using various methods to characterize microbial communities confirmed that soil pH was the best predictor of the bacterial community composition and diversity (Bååth and Anderson, 2003; Cookson et al., 2007; Lauber et al., 2009). Soil pH was significantly linearly related to the dominant diazotrophic taxa (*Bradyrhizobium* and *Azospirillum*), phylogenetic diversities and phylogenetic community structure in the pH range of 4.5 – 9.5. There may be two reasons, on the one hand, dominant diazotrophic taxa had apparently different pH-relative abundance patterns, for example, as soil pH increased, *Azospirillum* replaced *Bradyrhizobium* and gradually became the most dominant taxa. *Bradyrhizobium* may have higher acid tolerance and may be dominant in the pH range of 4.5 – 7.8 while *Azospirillum* may be dominant in the pH range of 8.0 – 9.5. On the other hand, soil pH often covaries with other environmental variables such as the climate, N content and SOM, and jointly determines the diazotrophic community (Williams et al., 2013). Neutral and acidic environments have high MAT, MAP and nutrient content and provide a more suitable growth environment for microorganisms. the result of the diversity and community structure that were significantly positively correlated with MAT, MAP, TN and SOM support this point (Carrell et al., 2019; Han et al., 2019; Wang et al., 2017a, 2017b).

Soil pH was observed to be a key factor involved in diazotroph activity (Fig. 7). Correlation analysis showed that the N fixation rate increased at a nearly neutral pH, consistent with a previous study (Hsu and Buckley, 2009; Lauber et al., 2009). Moreover, soil pH changes caused by geographical factors or fertilization have the same effect on the trend of the N fixation rate, because chemical fertilization causes acidification of neutral or acidic soils, which is equivalent to worsening the living environment of diazotrophic soil (Feng et al., 2018; Lin et al., 2018), however, chemical fertilization causes acidification of alkaline soils equivalent to improving the living environment of diazotrophs. Furthermore, the addition of organic amendments retains a neutral pH and maintains the N fixation rate at a high level (Liu et al., 2020).

Remarkably, soil pH was linearly correlated with diazotroph-dominant taxa, diversity and community turnover, but the more neutral the soil pH, the higher the N fixation rate. Moreover, the contribution of fertilization to diazotroph activity was greater than that of fertilization to diazotroph assembly processes. The microbial activity reflects the result of contemporary selection, and different regions may have other keystone taxa, but the same activity is performed (Zhang et al., 2020). In addition, species abundance is related to disproportionate types of functions (Jia et al., 2018). Increasing evidence has confirmed that rare taxa (relative abundance $< 1\%$) serve as limitless reservoirs of genetic diversity and play a more important role than dominant taxa in community construction and function (Chen et al., 2017a, 2017b; Xue et al., 2018).

Notably, the contribution of geographical factors to diazotroph assembly processes and activity is far greater than that of fertilization. This may be the result of the influence of historical processes and contemporary selection (Hanson et al., 2012). Historical processes determined specific soil types and regional species pool, which limited species sorting and biotic interactions (Ricklefs, 2004; Zober, 1997) and shaped a clear geographical distribution pattern (Morlon et al., 2008). Regardless of how the environment changes, historical processes will leave an indelible mark on the composition of the community, which is confirmed by the existence of endemic taxa (Gao et al., 2019; Vellend, 2010). For example, it is difficult to find *Dechloromonas* (~18.7% in Jinhua) and *Halorhodospira* (~5.8% in Ningxiang) elsewhere, and previous studies showed that *Mesorhizobium* (~12%) was present in a Qinghai-Tibet alpine ecosystem, China (Wang et al., 2017a, 2017b), and *Geobacter* (~15%) was reported in Mengcheng, Anhui Province, China (Fan et al., 2019), but both were rare taxa in the present study.

Overall, we found that diazotrophs assembly processes are influenced by both stochastic and deterministic processes simultaneously in large geographic scales and various fertilization strategies. While fertilization increased the proportion of deterministic processes, soil pH once again proved to be a reliable predictor of diazotrophs assembly processes and activity. Soil pH is a key factor affecting diazotrophic activity; however, N fixation rate increased as pH nearly neutral. These results revealed that soil diazotrophs assembly process and activity were shaped by both geographical factor and fertilization; hence, expand current understanding of diazotroph community affected by fertilization strategies across a large geographic scale.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Prof. Bo Sun, Chengyi Zhao, Gang Wang, Haiyan Chu, Jian Ma, Jianwei Lu, Junwei Ma, Wantai Yu and Weixin Ding for providing soil samples; Yongxiang Yu, Xing Li, Jiantao Xue, Ningguo Zheng and Yizu Zhu for revising the manuscript; Xinxin Wang and Ziyi Feng for technical assistance. This work was supported by the National Natural Science Foundation of China [Grant Numbers 41525002, 41761134085, 41877051], the National Key R & D Program of China [Grant Number 2017YFD0200102], and Ningbo Municipal Science and Technology Bureau (202002N3079).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114923.

References

Baas Becking, L., 1934. Geobiologie of inleiding tot de milieukunde. Van Stockum & Zoon, The Hague, the Netherlands.

Bååth, E., Anderson, T.-H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol. Biochem. 35, 955–963. https://doi.org/10.1016/S0038-0717(03)00154-8.

Berthrong, S.T., Yeager, C.M., Gallegos-Graves, L., Steven, B., Eichorst, S.A., Jackson, R. B., Kuske, C.R., Lovell, C.R., 2014. Nitrogen fertilization has a stronger effect on soil nitrogen-fixing bacterial communities than elevated atmospheric CO $_2$. Appl. Environ. Microbiol. 80, 3103–3112.

Bothe, H., Schmitz, O., Yates, M.G., Newton, W.E., 2010. Nitrogen fixation and hydrogen metabolism in cyanobacteria. MMBR 74, 529–551. https://doi.org/10.1128/MMBR.00033-10.

Carrell, A.A., Kolton, M., Glass, J.B., Pelletier, D.A., Warren, M.J., Kostka, J.E., Iversen, C.M., Hanson, P.J., Weston, D.J., 2019. Experimental warming alters the community composition, diversity, and N_2 fixation activity of peat moss (*Sphagnum fallax*) microbiomes. Glob. Change Biol. 25, 2993–3004. https://doi.org/10.1111/gcb.14715.

Chen, W., Pan, Y., Yu, L., Yang, J., Zhang, W., 2017b. Patterns and processes in marine microeukaryotic community biogeography from Xiamen coastal waters and intertidal sediments, Southeast China. Front. Microbiol. 8, 1912. https://doi.org/10.3389/fmicb.2017.01912.

Chen, R., Zhong, L., Jing, Z., Guo, Z., Li, Z., Lin, X., Feng, Y., 2017a. Fertilization decreases compositional variation of paddy bacterial community across geographical gradient. Soil Biol. Biochem. 114, 181–188. https://doi.org/10.1016/j.soilbio.2017.07.013.

Cookson, W.R., Osman, M., Marschner, P., Abaye, D.A., Clark, I., Murphy, D.V., Stockdale, E.A., Watson, C.A., 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biol. Biochem. 39, 744–756. https://doi.org/10.1016/j.soilbio.2006.09.022.

De'ath, G., 2007. Boosted trees for ecological modeling and prediction. Ecology 88, 243–251.

Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. U.S.A. 112, E1326–E1332. https://doi.org/10.1073/pnas.1414261112.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930. https://doi.org/10.1658/1100-9233(2003)014[0927:VAPORF]2.0.CO;2.

Dixon, R., Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. Nat. Rev. Microbiol. 2, 621–631. https://doi.org/10.1038/nrmicro954.

Dong, J., 2017. Diversity and Structure of Diazotrophic Communities in Mangrove Rhizosphere, Revealed by High-Throughput Sequencing. Frontiers in Microbiology 8, 11.

Fan, K., Delgado-Baquerizo, M., Guo, X., Wang, D., Wu, Y., Zhu, M.o., Yu, W., Yao, H., Zhu, Y.-G., Chu, H., 2019. Suppressed N fixation and diazotrophs after four decades of fertilization. Microbiome 7, 143. https://doi.org/10.1186/s40168-019-0757-8.

Fan, K., Weisenhorn, P., Gilbert, J.A., Shi, Y., Bai, Y., Chu, H., 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biology and Biochemistry 121, 185–192. DOI:10.1016/j.soilbio.2018.03.017.

Feng, M., Adams, J.M., Fan, K., Shi, Y.u., Sun, R., Wang, D., Guo, X., Chu, H., 2018. Long-term fertilization influences community assembly processes of soil diazotrophs. Soil Biol. Biochem. 126, 151–158. https://doi.org/10.1016/j.soilbio.2018.08.021.

Gaby, J.C., Buckley, D.H., 2012. A Comprehensive Evaluation of PCR Primers to Amplify the nifH Gene of Nitrogenase. PLoS ONE 7, e42149. DOI:10.1371/journal.pone.0042149.

Gaby, J.C., Buckley, D.H., 2014. A comprehensive aligned nifH gene database: a multipurpose tool for studies of nitrogen-fixing bacteria. Database 2014. DOI:10.1093/database/bau001.

Gaby, J.C., Rishishwar, L., Valderrama-Aguirre, L.C., Green, S.J., Valderrama-Aguirre, A., Jordan, I.K., Kostka, J.E., 2018. Diazotroph Community Characterization via a

High-Throughput nifH Amplicon Sequencing and Analysis Pipeline. Applied and Environmental Microbiology 84. DOI:10.1128/aem.01512-17.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science 320, 889–892. DOI:10.1126/science.1136674.

Gao, Q., Yang, Y., Feng, J., Tian, R., Guo, X., Ning, D., Hale, L., Wang, M., Cheng, J., Wu, Linwei, Zhao, M., Zhao, J., Wu, Liyou, Qin, Y., Qi, Q., Liang, Y., Sun, B., Chu, H., Zhou, J., 2019. The spatial scale dependence of diazotrophic and bacterial community assembly in paddy soil. Global Ecol Biogeogr geb.12917. DOI:10.1111/geb.12917.

Gause, G.F., 1934. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. Science 79, 16–17.

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293–296. DOI:10.1038/nature06592.

Han, L.-L., Wang, Q., Shen, J.-P., Di, H.J., Wang, J.-T., Wei, W.-X., Fang, Y.-T., Zhang, L.-M., He, J.-Z., 2019. Multiple factors drive the abundance and diversity of diazotrophic community in typical farmland soils of China. FEMS Microbiology Ecology fiz113. DOI:10.1093/femsec/fiz113.

Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., Martiny, J.B.H., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat. Rev. Microbiol. 10, 497–506. https://doi.org/10.1038/nrmicro2795.

Hsu, S.-F., Buckley, D.H., 2009. Evidence for the functional significance of diazotroph community structure in soil. ISME J. 3, 124–136. https://doi.org/10.1038/ismej.2008.82.

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography: MacArthur and Wilson's Radical Theory. Princeton University Press.

Jia, X., Dini-Andreote, F., Falcão Salles, J., 2018. Community assembly processes of the microbial rare biosphere. Trends Microbiol. 26, 738–747. https://doi.org/10.1016/j.tim.2018.02.011.

Jiao, S., Yang, Y., Xu, Y., Zhang, J., Lu, Y., 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. ISME J. 14, 202–216. https://doi.org/10.1038/s41396-019-0522-9.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166.

Kennedy, I., 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol. Biochem. 36, 1229–1244. https://doi.org/10.1016/j.soilbio.2004.04.006.

Kuypers, M.M.M., Marchant, H.K., Kartal, B., 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16, 263–276. https://doi.org/10.1038/nrmicro.2018.9.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. AEM 75, 5111–5120. https://doi.org/10.1128/AEM.00335-09.

Li, T., Zhang, W., Yin, J., Chadwick, D., Norse, D., Lu, Y., Liu, X., Chen, X., Zhang, F., Powlson, D., Dou, Z., 2018. Enhanced-efficiency fertilizers are not a panacea for resolving the nitrogen problem. Glob. Change Biol. 24, e511–e521. https://doi.org/10.1111/gcb.13918.

Liao, H., Li, Y., Yao, H., 2018. Fertilization with inorganic and organic nutrients changes diazotroph community composition and N-fixation rates. J. Soils Sediments 18, 1076–1086. https://doi.org/10.1007/s11368-017-1836-8.

Lin, Y., Ye, G., Liu, D., Ledgard, S., Luo, J., Fan, J., Yuan, J., Chen, Z., Ding, W., 2018. Long-term application of lime or pig manure rather than plant residues suppressed diazotroph abundance and diversity and altered community structure in an acidic Ultisol. Soil Biol. Biochem. 123, 218–228. https://doi.org/10.1016/j.soilbio.2018.05.018.

Liu, S., Wang, J., Pu, S., Blagodatskaya, E., Kuzyakov, Y., Razavi, B.S., 2020. Impact of manure on soil biochemical properties: a global synthesis. Sci. Total Environ. 745, 141003. https://doi.org/10.1016/j.scitotenv.2020.141003.

Luan, L., Liang, C., Chen, L., Xu, Q., Jiang, Y., Sun, B., et al., 2020. Coupling bacterial community assembly to microbial metabolism across soil profiles. mSystems. https://doi.org/10.1128/mSystems.00298-20.

Ma, J., Bei, Q., Wang, X., Lan, P., Liu, G., Lin, X., Liu, Q., Lin, Z., Liu, B., Zhang, Y., Jin, H., Hu, T., Zhu, J., Xie, Z., 2019. Impacts of Mo application on biological nitrogen fixation and diazotrophic communities in a flooded rice-soil system. Science of the Total Environment 649, 686–694. DOI:10.1016/j.scitotenv.2018.08.318.

Morlon, H., Chuyong, G., Condit, R., Hubbell, S., Kenfack, D., Thomas, D., Valencia, R., Green, J.L., 2008. A general framework for the distance-decay of similarity in ecological communities. Ecology Letters 11, 904–917. DOI:10.1111/j.1461-0248.2008.01202.x.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77, 342–356. https://doi.org/10.1128/MMBR.00051-12.

Okubo, T., Tsukui, T., Maita, H., Okamoto, S., Oshima, K., Fujisawa, T., Saito, A., Futamata, H., Hattori, R., Shimomura, Y., Haruta, S., Morimoto, S., Wang, Y., Sakai, Y., Hattori, M., Aizawa, S.-I., Nagashima, K.V.P., Masuda, S., Hattori, T., Yamashita, A., Bao, Z., Hayatsu, M., Kajiya-Kanegae, H., Yoshinaga, I., Sakamoto, K., Toyota, K., Nakao, M., Kohara, M., Anda, M., Niwa, R., Jung-Hwan, P., Sameshima-Saito, R., Tokuda, S.-I., Yamamoto, S., Yamamoto, S., Yokoyama, T., Akutsu, T., Nakamura, Y., Nakahira-Yanaka, Y., Takada Hoshino, Y., Hirakawa, H., Mitsui, H., Terasawa, K., Itakura, M., Sato, S., Ikeda-Ohtsubo, W., Sakakura, N., Kaminuma, E., Minamisawa, K., 2012. Complete Genome Sequence of Bradyrhizobium sp. S23321: insights into symbiosis evolution in soil oligotrophs. Microb. Environ. 27, 306–315. https://doi.org/10.1264/jsme2.ME11321.

Olsen, S.R., Cole, C.V., Watanabe, E.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circ. 939, US Dept. Agric, Washington DC.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reganold, J.P., Glover, J.D., Andrews, P.K., Hinman, H.R., 2001. Sustainability of three apple production systems. Nature 410, 5.

Ricklefs, R.E., 2004. A comprehensive framework for global patterns in biodiversity. Ecol. Lett. 7, 1–15. https://doi.org/10.1046/j.1461-0248.2003.00554.x.

Rösch, C., Mergel, A., Bothe, H., 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. AEM 68, 3818–3829. https://doi.org/10.1128/AEM.68.8.3818–3829.2002.

Sanchez, G., 2013. PLS Path Modeling with R. Trowchez Editions. Berkeley. URL http://www.gastonsanchez.com/PLS Path Modeling with R.pdf.

Shi, Y.u., Li, Y., Xiang, X., Sun, R., Yang, T., He, D., Zhang, K., Ni, Y., Zhu, Y.-G., Adams, J.M., Chu, H., 2018. Spatial scale affects the relative role of stochasticity versus determinism in soil bacterial communities in wheat fields across the North China Plain. Microbiome 6, 27. https://doi.org/10.1186/s40168-018-0409-4.

Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J 6, 1653–1664. DOI:10.1038/ismej.2012.22.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. 7, 2069–2079. https://doi.org/10.1038/ismej.2013.93.

Vitousek, P.M., Menge, D.N.L., Reed, S.C., Cleveland, C.C., 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philosophical Transactions of the Royal Society B: Biological Sciences 368, 20130119–20130119. DOI:10.1098/rstb.2013.0119.

Wang, Y., Li, C., Kou, Y., Wang, J., Tu, B., Li, H., Li, X., Wang, C., Yao, M., 2017b. Soil pH is a major driver of soil diazotrophic community assembly in Qinghai-Tibet alpine meadows. Soil Biol. Biochem. 115, 547–555. https://doi.org/10.1016/j.soilbio.2017.09.024.

Wang, Q., Wang, J., Li, Y., Chen, D., Ao, J., Zhou, W., Shen, D., Li, Q., Huang, Z., Jiang, Y., 2018. Influence of nitrogen and phosphorus additions on N2-fixation activity, abundance, and composition of diazotrophic communities in a Chinese fir plantation. Sci. Total Environ. 619, 1530–1537. https://doi.org/10.1016/j.scitotenv.2017.10.064.

Wang, C., Zheng, M., Song, W., Wen, S., Wang, B., Zhu, C., Shen, R., 2017a. Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in an acidic soil in southern China. Soil Biol. Biochem. 113, 240–249.

Webb, C.O., Ackerly, D.D., McPeek, M.A., Donoghue, M.J., 2002. Phylogenies and community ecology. Annu. Rev. Ecol. Syst. 33, 475–505.

Williams, M.A., Jangid, K., Shanmugam, S.G., Whitman, W.B., 2013. Bacterial communities in soil mimic patterns of vegetative succession and ecosystem climax but are resilient to change between seasons. Soil Biol. Biochem. 57, 749–757. https://doi.org/10.1016/j.soilbio.2012.08.023.

Xue, Y., Chen, H., Yang, J.R., Liu, M., Huang, B., Yang, J., 2018. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. ISME J. 12 (9), 2263–2277. https://doi.org/10.1038/s41396-018-0159-0.

Zehr, J.P., Jenkins, B.D., Short, S.M., Steward, G.F., 2003. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ. Microbiol. 5, 539–554. https://doi.org/10.1046/j.1462-2920.2003.00451.x.

Zhang, X., Johnston, E.R., Liu, W., Li, L., Han, X., 2016. Environmental changes affect the assembly of soil bacterial community primarily by mediating stochastic processes. Glob. Change Biol. 22, 198–207. https://doi.org/10.1111/gcb.13080.

Zhang, X., Kuzyakov, Y., Zang, H., Dippold, M.A., Shi, L., Spielvogel, S., Razavi, B.S., 2020. Rhizosphere hotspots: root hairs and warming control microbial efficiency, carbon utilization and energy production. Soil Biol. Biochem. 148, 107872. https://doi.org/10.1016/j.soilbio.2020.107872.

Zober, M., 1997. The relative role of species pools in determining plant species richness. An alternative explanation of species coexistence? Trends Ecol. Evol. 12, 266–269.