Ecological network analysis assesses the restoration success of disturbed mining soil in China

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Abstract

Understanding the interactions among soil microbial species and how they respond to land reclamation is essential to evaluate the success of ecological restoration actions in disturbed mining soil. In this study, we strived to reveal the interactions among soil bacterial communities along the reclamation timeline of a coal mine in Zoucheng, China. To do so, we investigated changes in the composition of soil bacterial over time and constructed molecular ecological networks (i.e. microbial network) following mining soil reclamation into agricultural land. The relationships between microbial networks and selected soil attributes (i.e. soil pH, electric conductivity, organic matter, soil nutrients and enzymatic activities) were also analyzed. The results showed that the composition of soil bacteria changed significantly along the reclamation timeline. The microbial network profile revealed that Acidobacteria, Planctomycetes and Proteobacteria were the key microbial populations. Soil pH, soil organic matter content, soil dehydrogenase and urease activities were significantly correlated (0.001 [?] $P < 0.05$) with the microbial network structure, suggesting that the microbial networks found influenced the provision of relevant soil ecological functions after reclamation. The variation in complexity of the microbial networks along the reclamation timeline revealed that microbial development was promoted by the shift in land use from mining into agriculture. Overall, our findings shed light on how soil microbial communities and networks change following mine reclamation into agricultural land. The results presented herein will undoubtedly aid in the establishment of success indicators of ecological restoration activities in disturbed mining soil.

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strived to reveal the interactions among soil bacterial communities along the reclamation timeline of a coal mine in Zoucheng, China. To do so, we investigated changes in the composition of soil bacterial over time and constructed molecular ecological networks (i.e. microbial network) following mining soil reclamation into agricultural land. The relationships between microbial networks and selected soil attributes (i.e. soil pH, electric conductivity, organic matter, soil nutrients and enzymatic activities) were also analyzed. The results showed that the composition of soil bacteria changed significantly along the reclamation timeline. The microbial network profile revealed that *Acidobacteria*, *Planctomycetes* and *Proteobacteria* were the key microbial populations. Soil pH, soil organic matter content, soil dehydrogenase and urease activities were significantly correlated (0.001 [?] P < 0.05) with the microbial network structure, suggesting that the microbial networks found influenced the provision of relevant soil ecological functions after reclamation. The variation in complexity of the microbial networks along the reclamation timeline revealed that microbial development was promoted by the shift in land use from mining into agriculture. Overall, our findings shed light on how soil microbial communities and networks change following mine reclamation into agricultural land. The results presented herein will undoubtedly aid in the establishment of success indicators of ecological restoration activities in disturbed mining soil.

Keywords : land reclamation; soil microbial community; ecological network; soil attributes; soil ecosystem

1 Introduction

China is a large country in coal resources and the largest coal consumer in the world. Underground mining, which has been the most common coal mining technique in the world, accounts for the most coal outputs in China (Hu et al., 2019). Large-scale underground coal mining in China has frequently resulted in soil collapse, damaging land resources irreversibly, and affecting agricultural production and the ecological integrity of the environment. At present, it is estimated that for each 1×10^4 t of coal that was mined, about 0.1 to 0.3 hm^2 of land was sinking in China (Hu and Luo, 2006). Moreover, in mining areas where water tables are near the ground surface, surface subsidence has led to surface water accumulation, provoking shifts from terrestrial to aquatic ecosystems with dramatic changes in the original ecological equilibrium. The latter issue has also led to the destruction of large farmlands, threatening food security, and triggering conflicts between rural people who once worked and lived from the land. To circumvent these issues, many subsided areas are currently being reclaimed into agricultural land.

Agricultural land provides essential resources related to food security worldwide, in general, and in China, in particular. As a result, land reclamation has become an important approach to increase the surface of agricultural land after the subsidence of the land overlying coal mines in China (Bian et al., 2018). It is estimated that about 32.6% of agricultural land has been reclaimed from coal-grain composite areas in China (Qu et al., 2018). The coal-grain composite areas in the eastern regions of China are vital to support food security in this area, but land subsidence as a result of coal mining activities are jeopardizing the integrity of its agroecosystems (Hu, 2019).

Land reclamation in subsided mining areas involves refilling the receded area with topsoil followed by compaction operations (Qu et al., 2017; Hu, 2019). As a result, the natural structure of the soil is severely altered (Bai et al., 2018). Moreover, key soil attributes are also modified after land reclamation. Reclaimed soils may present less soil organic matter, altered soil texture, lower soil water retention capacity, less soil nutrients, and lower native soil microbial diversity than the original soil (Ahirwal and Maiti, 2016; Luo, et al., 2020). All these changes may limit crop yield and land development following land reclamation (Zhao et al., 2015). Natural succession of reclaimed soils is slow and ecological equilibrium is normally reached after a long time following reclamation (Bai et al., 2018). During this time, the reclaimed land may also be exposed to wind and water erosion, as well as to contamination (Hu, 2019). Therefore, monitoring the status of key soil attributes after reclamation and over time is essential for gaining insights into soil development and land restoration following reclamation (Ngugi et al., 2018; Shrestha and Lal 2011). However, only few studies have addressed this issue (Qu et al., 2018, Min et al., 2017) and even fewer have focused on the existing ecological

networks between soil microbial communities (SMCs) after land reclamation in mining areas (Hu, 2019; Luo et al., 2020; Ma et al., 2020).

Soil microorganisms are an essential part of the soil ecosystem. SMCs play a key role in the processes of decomposing soil organic matter, nutrient cycling and utilization of nutrients by plants (Novianti et al., 2018; Yuan et al., 2018). After land reclamation, changes in the soil physical and chemical properties may provoke changes in key soil attributes (e.g. pH value) but also in the SMCs and their related functions in the soil ecosystem (Harris, 2009; Delgado-Baquerizo, et al., 2019). The reclamation of mining soil into farmland mostly focuses on restoring the integrity of the agroecosystem aboveground whilst the restoration of the integrity and function of the belowground compartments is largely overlooked (Liu et al., 2019). Detailed assessment of SMCs is essential to evaluate the success of soil reclamation and development (Dangi et al., 2012). In this regard, healthy soils will normally present diverse SMCs (Tu et al., 2020), indicating an adequate functioning of the soil ecosystem (Delgado-Baquerizo, et al., 2016) and an adequate provision of ecosystem services by the soil compartment (e.g. organic matter decomposition, nutrient cycling) (Griffiths and Philippot, 2013). However, little is known about the diversity of and the function provided by the SMCs found in soils subjected to reclamation after coal mining activities.

In recent years, high-throughput sequencing technology has become a common method to provide information related to SMCs. Nonetheless, the translation of the outputs from sequencing into new practical knowledge is challenging due to the size of the data generated throughout and the difficulty to correlate these with other environmental variables such as key soil attributes (Wang and Brose, 2018; Delgado-Baquerizo, et al., 2019). Molecular ecological networks (MENs), using the mathematical and bioinformatics methods to construct ecological association networks, and provide a conceptual framework to identify microbial interactions and key populations, and relate with key attributes and functions occurring in the soil compartment (Deng et al., 2016). Moreover, MENs can be useful to identify keystone taxa of soil microorganisms (Kitano, 2004). Keystone taxa play important roles in the microbiome, such as driving community composition and functionality, and their variation might cause changes in microbiome composition and functioning (Delgado-Baquerizo et al., 2018). While previous studies have reported keystone taxa in the soil under multiple environmental conditions, information on how keystone taxa may change over time following land reclamation in mining areas is severely lacking (Berry and Widder, 2014; Fierer, 2017; Ramirez et al., 2018; Banerjee et al., 2018 and 2019).

The aim of this study is to assess the restoration success of disturbed mine soil following land reclamation into arable land. To do so, we investigate changes in key soil attributes and in the composition of SMCs along a reclamation chronosequence of 17 years following land restoration. We also evaluate changes in the complexity of the ecological networks established between the SMCs and their interactions as an indicator of soil development after land reclamation. The results from our investigation will provide novel and practical insights into the ecological restoration of disturbed mine soils worldwide.

2 Materials and Methods

2.1 Study site

The study site was located in the Zoucheng (ZC) coal-mining area (35°8'12"N–35°32'54"N, 116°46'30"E–117°28'54"E; CGCS2000, Fig. 1) within the Shandong Province, China. The climate of the study site is within the warm temperate monsoon climate zone (Csa; Köppen, 1884). The mean annual rainfall over the period 1979-2018 was 777.1 mm, and the mean annual temperature was 14.1°C. The soil type is brown fluvo-aquic, with a 22.3% of sand (2-0.02mm), 65.9% of silt (0.02-0.002mm) and 11.8% of clay (<0.002mm). The soil bulk density is 1.48 g cm^{-3} (http://vdb3.soil.csdb.cn/).

The study site is subjected to subsidence (Fig. S1, see supplemental material) as a result of past coal mining activities. Since 2001, subsidence has been reverted in the study site through land reclamation, using coal

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gangue and topsoil fillings to a depth comprised between 200 and 400 cm, and with a topsoil layer 80 cm deep (Qu et al., 2017). Land reclamation in the study site was undertaken at different locations over time. As a result, four distinct reclamation zones can be distinguished on the basis of the time since reclamation, -i.e. (i) r17 - 17 years since reclamation; (ii) r14 - 14 years since reclamation; (iii) r11 - 11 years since reclamation; and (iv) r8 - 8 years since reclamation (Fig. 1). After reclamation, land use shifted to farmland, where crops of wheat and soybean are rotated following a one-year rotation scheme.

2.2 Soil sampling and analysis of key soil attributes

On 04/05/2019, 15 spatially-distributed, surface soil (i.e. 0-10 cm below the ground level; b.g.l) samples were retrieved from each reclamation zone -i.e. r17, r14, r11 and r8 (Section 2.1; Fig. 1), respectively, following a stratified random sampling approach (Ma et al., 2020). In addition, 15 surface soil samples were retrieved from three un-reclaimed plots and used as control (CK; Fig. 1).

A subsample of 20 g of unprocessed, fresh soil was retrieved from the bulk soil samples. The soil subsamples were frozen and stored at -20°C immediately after sampling for subsequent microbial analysis (Section 2.3). The remaining soil materials were air-dried, homogenized and sieved through a 2 mm sieve prior to analyzing eight key soil attributes. (i) Soil pH and (ii) electric conductivity (EC) were measured using a pH meter and conductivity meter, respectively (PHC-3C, DDS-307A, Shanghai leici, China), in a 1:2.5 soil:distilled water suspension. (iii) Soil organic matter content (SOM) was measured with a colorimetric method (Lu, 2000), using hydration heat during the oxidation of potassium dichromate. (iv) Soil total Kjeldahl nitrogen (TKN) was measured with a Kjeldahl analyzer (K9840, Shandong Hanon, China). (v) Soil extractable phosphorus (OP) was measured with the Olsen method (Lu, 2000). (vi) Soil available potassium (AK) was quantified with the ammonium acetate–flame photometric method (Lu, 2000). (vii) Soil enzymatic activity was assessed in the light of the soil dehydrogenase (DHG), urease (URA), and polyphenol oxidase (PPO) activities. These enzymatic activities were analyzed using the triphenyltetrazolium chloride (TTC) method, the sodium-hypochlorite phenol colorimetric method, and the pyrogallol colorimetric method, respectively (Guan, 1986). (viii) Microbial activity was quantified through measuring the activity of fluorescein diacetate hydrolase (FDA), using the fluorescein colorimetric method (Guan, 1986).

2.3 Soil microbial community (SMC) analysis

Soil microbial community (SMC) composition was analyzed through Illumina® MiSeq whole genome sequencing (Chen et al., 2017). Soil DNA was extracted from the fresh soil samples stored at -20°C (N=75; Section 2.2) using the FastDNA™ SPIN kit (MP Biomedicals, Solon, OH, USA) and following the manufacturer's instructions. DNA concentration and purity were measured with the NanoDrop One kit (Thermo Fisher Scientific, MA, USA). The V4–V5 region of the bacterial 16S rRNA genes were amplified using the primer sets 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (CCGTCAATTCMTTTRAGTTT) from Invitrogen (Invitrogen, Carlsbad, CA, USA). PCR reactions were carried out using the BioRad S1000 apparatus (Bio-Rad Laboratory, CA, USA). PCR products were mixed in ratios of equal density as specified by the GeneTools Analysis Software (Version4.03.05.0, SynGene). Accordingly, a 50 µl volume of PCR mix contained 25 µl Premix Taq (Takara Biotechnology, Dalian Co. Ltd., China), 1 µl of each rRNA primer (10 M) and 3 µl of DNA (20 ng µl⁻¹) template. The PCR mix was amplified following the thermos-cycling protocol: (i) 5 min at 94°C for initialization; (ii) 30 cycles of 30 s at 94°C for denaturation, 30 s at 52°C for annealing, and 30 s at 72°C for extension; (iii) 10 min at 72°C for final elongation. The PCR products were purified with the EZNA Gel Extraction Kit (Omega, USA).

A whole genome sequencing library was generated using the NEBNext® Ultra DNA Library Prep Kit for Illumina(r) (New England Biolabs, MA, USA) following the manufacturer's recommendations. The quality of the library was assessed with the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA) and the Agilent Bioanalyzer 2100 system (Agilent Technologies, Waldbron, Germany). Then, the generated library was sequenced on the Illumina Hiseq 2500 platform from which 250 paired-end, base pair (bp) reads were created (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou,

China). Paired-end raw reads were filtered and quality-checked following the Trimmomatic v0.33 tool (*http://www.usadellab.org/cms/?page=trimmomatic*). Paired-end clean reads were merged using the FLASH v1.2.11 software (*https://ccb.jhu.edu/software/FLASH/*). Genome sequences were assigned to each soil sample (Section 2.2) based on their unique barcode and primer using the Mothur v1.35.1 software (*http://www.mothur.org*).

The detection of potential chimaera and clustering of the retrieved rRNA sequences were performed with the usearch v10 software (*http://www.drive5.com/usearch/*). Operational taxonomic unit (OTU) were defined as clusters of sequences with [?]97% similarity. For each sequence, the silva database (https://www.arb-silva.de/) was used to annotate taxonomic information, setting the confidence threshold to default at [?]0.5. According to the taxonomic results, OTU relative abundance diagrams were built from which rich infrared images were obtained using Origin 9.1 (OriginLab, Northampton, MA, USA).

Data availability: The raw sequence data for 16S rRNA gene have been deposited under NCBI Sequence Read Archive with accession number PRJNA674491.

2.4 Molecular ecological networks (MENs)

To investigate the effects of reclamation time on interactions and key species of SMC, phylogenetic molecular ecological networks (MENs; Zhou et al., 2011) were established per reclamation site (r8, r11, r14 and r17) using the information retrieved from the SMC sequence data (Section 2.3). MENs were built using the computational pipeline MENA (*http://ieg4.rccc.ou.edu/mena*) and plotted using the Cytoscape v3.7.1 software (Doncheva et al., 2018) for further analysis and characterization. Only those OTUs detected in all the analyzed soil samples were considered to build the MENs. Each MEN was characterized in the light of its topology (Tu et al., 2020) – i.e. the total number of nodes (species or OTUs) and links between the nodes. The quantity of nodes within a network was quantified by establishing a power-law distribution (Deng et al., 2012), for which we quantified the coefficient of determination (R^2, between 0 and 1) to classify the network into scale-free (i.e. most nodes have few neighbors; Girvan and Newman 2002; Tu et al., 2020). The links between the nodes were characterized in the light of the average node degree or average connectivity (avgK), which was used to classify the network into a small-world (i.e. the average distance between two nodes is short, meaning that the nodes in the network are closely related with each other; Amaral et al., 2000; Zhou et al., 2010). The average geodesic distance (GD) between nodes was also calculated (Deng et al., 2012). On one hand, AvgK refers to the number of nodes directly connected to a particular node, where 'higher average degree' (connectivity) indicates a more complex network (Deng et al., 2012). Nodes with higher degrees were considered to be the central nodes in the network structure (Layeghifard et al., 2017). Accordingly, nodes with higher connectivity (degree) in the network were defined as top nodes. On other hand, GD is the shortest path between two nodes. When GD is low indicates that the nodes within a network are close to each other, indicating that the network is complex (Deng et al., 2012). We used the metric 'average clustering coefficient' (avgCC) to describe how well a node was connected to its neighbors using a scale ranging from 0 to 1, where 1 denotes a fully connected node and 0 an unconnected node (Deng et al., 2012). Further MEN attributes were retrieved by establishing/detecting 'Modules' within each MEN. A 'Module' was defined using the metric 'modularity' (i.e. ranging for 0 to 1, it is the degree to which a network can be divided into communities or modules) as a group of OTUs with high connectivity within a MEN but with few connections outside the group. The higher the modularity is, the more modules a network can be divided into and, thus, the less complex a network is (Olesen et al., 2007; Deng et al., 2012). To estimate 'modularity', we used the 'greedy modularity optimization approach (Deng et al., 2012). This approach enabled us to retrieve module connectivity metrics such as 'within-module' connectivity (Zi) and 'among-module' connectivity (Pi), considering all the nodes within the modules. These metrics were used to classify the nodes within a given MEN according to their role in the network (Newman, 2006; Deng et al., 2012) -i.e. peripherals (Zi [?] 2.5, Pi [?] 0.62), connectors (Zi [?] 2.5, Pi > 0.62), module hubs (Zi > 2.5, Pi [?] 0.62), and network hubs (Zi > 2.5, Pi > 0.62).

2.5 Statistical Analysis

Statistically significant differences between reclamation sites in terms of the studied key soil attributes were evaluated with the one-way ANOVA test at the 95 % and 99 % confidence levels, following normality testing through Shapiro-Wilk test and T test. The correlation between the OTU's abundance profile and the studied key soil attributes (Section 2.2.) was assessed through Pearson's correlation tests (r^2).

Differences between the MEN retrieved for each reclamation site were evaluated through similarity matrices (Zhang and Horvath, 2005; Horvath and Dong, 2008). We also generated similarity matrices to evaluate the correlation between different MENs. To do so, Spearman's rank correlation coefficients were used and similarity thresholds ranging between 0.01 and 0.99 were tried to then choose an optimum similarity threshold (Newman, 2004). The optimum similarity threshold was found through an iterative process that evaluated whether the statistical distribution of the eigenvalues' spacing contained within the eigenvectors originated from each similarity matrix followed a Poison distribution (Newman, 2006; Deng et al., 2012). This test was carried out using the computational pipeline MENA (Deng et al., 2012 and 2016). We also investigated the relationship between modules (with >5 nodes) of MENs and soil attributes through Pearson's correlation tests (r^2) (Guimera et al., 2007). All other statistical tests were undertaken using the statistical software SPSS v20 (IBM, USA).

3 Results

3.1 Key soil attributes

The time since reclamation had a substantial effect on the studied soil key attributes (*Fig. 2*). Soil organic matter (SOM) significantly increased after 17 years since reclamation (Fig. 2A; χ^2=0.68, p=0.410). Similarly, soil pH and EC increased significantly in the reclaimed plots with respect to the control plot (*Figs. 2* B-C; χ^2=22.432, p<0.001; χ^2=19.557, p<0.001). Soil available potassium (AK) was significantly higher in the plots under reclamation than in the control plots (Fig. 2D; χ^2=4.369, p=0.037). However, it was not clear whether the time since reclamation had an effect on the AK concentration in the soil. Both soil extractable phosphorus (OP) and total nitrogen (TN) were substantially lower in the reclaimed than in the control plots (Fig 2E-F; χ^2=11.938, p=0.001; χ^2=0.22, p=0.639). The enzymatic activity of soil urease (URA) in the soil was significantly higher in the plots under reclamation than in the control plots (Fig. 2G; χ^2=16.518, p<0.001). Soil polyphenol oxidase (PPO) activity showed a clear increasing trend along the reclamation timeline (Fig. 2H; χ^2=43.014, p<0.001). Yet, it was not clear whether the time since reclamation led to an increase in enzymatic activities of URA, dehydrogenase (DHG) and fluorescein diacetate hydrolase (FDA) in the soil (Fig. 2G and Figs 2I-J; χ^2=1.16, p=0.281; χ^2=0.065, p=0.799).

3.2 Taxonomic composition of the soil microbial communities (SMCs)

The most abundant phylum and genera identified in this study are shown in Figs. 3A and 3B, respectively. The phyla *Thaumarchaeota* ,*Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Patescibacteria* , *Planctomycetes* ,*Proteobacteria* and *Verrucomicrobia* accounted for more than 95% of the total genetic sequences retrieved for each analyzed soil sample (Fig. 3). The relative abundance of the two most abundant phylum - i.e. *Bacteroidetes* and *Proteobacteria* decreased with increasing time after reclamation (Fig. 3A; χ^2=18.339, p<0.001; χ^2=26.530, p<0.001). The abundance of the third and fourth most abundant phylum i.e. *Acidobacteria* and *Chloroflexi*increased following reclamation (Fig. 3A; χ^2=15.437, p<0.001; χ^2=42.547, p<0.001). Moreover, the abundance of the phylum *Thaumarchaeota* ,*Actinobacteria* and *Planctomycetes* also increased with the time after reclamation (Fig. 3A; χ^2=0.000, p=0.993; χ^2=9.862, p=0.002; χ^2=1.346, p=0.246), but the abundance of the phylum *Verrucomicrobia, Cyanobacteria* , *Dependentiae* , *Patescibacteria* , *FBP*and *Firmicutes* decreased. The relative abundance of other identified phylum is shown in supplementary material – Fig. S2.

Regarding the most abundant SMCs' genera found (Fig. 3B), the abundance of the genera *RB41* and *UTCFX1* increased significantly with the time after reclamation (Fig. 3B; χ^2=18.110, p<0.001; χ^2=43.022, p<0.001). Nonetheless, other genera, such as *Flavobacterium*, *Pedobacter*, *Sphingomonas* and *Luteimonas* (Fig. 3B; χ^2=21.616, p<0.001; χ^2=13.077, p<0.001; χ^2=25.592, p<0.001; χ^2=12.872, p<0.001) showed the opposite trend. The abundance of the less abundant genus is shown in supplementary material – Fig. S2.

3.3 Molecular ecological networks (MENs)

3.3.1 Topological features

MENs topological features (i.e. number of nodes, number of links, avgK, avgCC, geodesic distance and modularity) for the different reclamation plots are shown in Table 1. The five retrieved MENs were modular, especially for the reclaimed sites, which had modularity values above 0.6 (Table 1). The time after reclamation varied the topological features of the retrieved MENs (Table 1). A power-law model explained successfully (i.e. $R^2 > 0.8$) the connectivity of the retrieved MENs (Table 1; Fig. S3, see supplemental material), showing that the five MENs in these microbial communities exhibited scale-free behavior. A longer time after reclamation led to higher avgK (Table 1), but the control had the highest avgK. In addition, the MEN retrieved for CK had the closest nodes (i.e. GD was the lowest; Table 1), being GD different for the CK with respect to the plots under reclamation (Table 1). The module number was not statistically different between reclaimed and control plots (Table 1), yet plots under reclamation had higher values for the module number.

3.3.2 Dominant microbial taxa

The five retrieved MENs had a distinct microbial structure and the nodes connectivity changed with the time after reclamation (Fig. 4, Table 2, Fig. S4, see supplemental material). However, each MEN had at least nine main phyla with a node degree above 1. The nine main phyla were *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Planctomycetes*, *Proteobacteria, Thaumarchaeota* and *Verrucomicrobia*.

In the MEN belonging to r8 (Fig. 4A, Table 2), the phylum *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes* and *Proteobacteria* had the highest node degree (i.e. node degree between 10 and 20) within this MEN. Within r8's MEN, high degree nodes were primarily clustered into modules 2 and 3, and the phyla *Acidobacteria* (OTUs 2 and 178) was detected as the module hub. In the Fig 4A, the interactions between OTUs showed as positive, with few pink red color edges.

In the MEN belonging to r11 (Fig. 4B, Table 2), the nodes with the highest node degree (i.e. node degree between 14 and 25) belonged to the phylum *Acidobacteria*, *Chloroflexi*, *Planctomycetes* and *Proteobacteria*. These nodes were clustered into modules 1 and 2.

In the MEN belonging to r14 (Fig. 4C, Table 2), the node degrees were similar to those in r8 and r11, but smaller than in CK. The phylum *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Nitrospirae*, *Planctomycetes* and *Proteobacteria* had the highest node degree (i.e. node degree between 11 and 20). The nodes with the highest node degree were clustered into modules 3 and 4. The phyla *Nitrospirae* (OTU375) was detected as the module hub.

In the MEN belonging to r17 (Fig. 4D, Table 2), the node degrees (i.e. node degree between 22 and 37) were higher than in r8, r11 and r14. The phylum *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Planctomycetes* and *Proteobacteria* (OTU146) had the highest node degree. The nodes with the highest node degree were clustered into modules 2, 3 and 8. The phyla *Gemmatimonadetes* was detected as the module hub, while the phylum *Acidobacteria* and *Planctomycetes* were detected as connectors within the network. In the Fig. 4D, although most interactions between OTUs showed as positive (light blue edges), more negatively interactions (pink red edges) than those in Figs. 4A, B and C.

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In the MEN belonging to CK (Fig. 4E, Table 2), the top five nodes (i.e. node degree between 35 and 55) belonged to the phylum *Acidobacteria* , *Planctomycetes* and *Proteobacteria* . Nodes with higher node degree were clustered into modules 1 and 3, which included *Acidobacteria* , *Bacteroidetes, Chloroflexi* ,*Plancto-mycetes* and *Proteobacteria* . OTUs 128, 46 and 948 were detected as the module hub. In the Fig 4E, the interactions between OTUs became more complicated, with much more negatively interactions (pink red edges) than those in Figs. 4A, B, C and D.

3.3.3 Keystone species: Nodes and hubs

The five retrieved MENs had peripheral species that occupied more than 97% of the total nodes (Fig. 5, Files S1-S5, see supplemental material). Three module hubs were detected for r8 (i.e. OTU 178, OTU2, OTU5380) and r11 (*i.e.* OTU225, OTU47, OTU7) which belonged to the phylum *Acidobacteria and Bacteroidetes, respectively.* However, four module hubs were detected for r14 (*Acidobacteria* ,*Bacteroidetes* , *Nitrospirae* and *Planctomycetes*), r17 (*Bacteroidetes* , *Gemmatimonadetes* and*Planctomycetes*) and CK (*Acidobacteria* , *Chloroflexi* ,*Planctomycetes* and *Proteobacteria)* (Fig. 5). With regard to connector species, over 16 connectors were detected, being CK the treatment that presented the highest number (Fig. 5). Both module hub and connector species had a wider distribution across microbial species. No network hubs were identified (Fig. 5).

3.3.4 Relationship between MEN and key soil attributes

The connectivity of module 1 was significantly positively correlated with OP (r=0.59, p < 0.05) in r8, while module 5 was negatively correlated with PPO (r=-0.59, p < 0.05) in this plot (Fig. 6A). Module 4 was positively correlated with AK (r=0.63, p<0.05) in r11 (Fig. 6B), while module 5 was positively correlated with URA (r=0.58, p<0.05). However, no positive significant correlations were observed between modules and soil attributes in r14, whereas the negative correlations were found between SOM and module 6, 13 (r=-0.57, p < 0.05; r=-0.65; p < 0.01) (Fig. 6C). In r14, module 3 had the negative correlation with OP (r=-0.53, p < 0.05), while module 10 with PPO (r=-0.55, p < 0.05) (Fig. 6C). AK was negatively correlated with module 1, 11 and 12 (r=-0.57, p < 0.05; r=-0.53, p < 0.05; r= -0.55, p < 0.05) (Fig. 6C). In r17, significant correlations were observed between module 2 and SOM, DHG and URA (r=-0.56, p < 0.05; r=0.71, p < 0.01; r=0.73, p < 0.01) (Fig. 6D). In addition, Module 3 had a positive correlation with pH and DHG (r=0.59, p < 0.05; r=0.85, p < 0.001) (Fig. 6D) in r17, and module 5 was positively correlated with SOM and TN (r=0.61 and 0.63; p < 0.05) but negatively correlated with DHG (r=-0.71; p < 0.01) (Fig. 6D). Moreover, Module 6 had a positive correlation with DHG and URA (r=0.63 and 0.64; p < 0.05), whereas module 7 had a negative correlation with DHG (r=-0.68; p < 0.01) (Fig. 6D). Module 8 was positively correlated with pH, DHG and URA (r=0.58, p < 0.001; r=0.87, p < 0.05; r= 0.76, p < 0.01), while negatively correlated with SOM, AK and TN (r=-0.79, p < 0.001; r=-0.57, p < 0.05; r=-0.56, p < 0.05) (Fig. 6D). Module 9 had a negative correlation with SOM, OP and AK (r=-0.73, p < 0.01; r=-0.56, p < 0.05 and r=-0.61, p < 0.05), but a positive correlation with PPO significantly (r=-0.57, p < 0.05) (Fig. 6D). In the control plot (CK), significant correlations were found between modules 1, 3, 4, 7, 8 and soil pH (r=0.67, p < 0.01; r=-0.78, p < 0.001; r=0.87, p < 0.001; r=-0.57, p < 0.05; r=-0.76, p < 0.05), while modules 1, 3, 4, 7 were correlated with the OP value significantly (r=-0.55, p < 0.05; r=0.64, p < 0.05; r=-0.54, p < 0.05; r=0.57, p < 0.05) (Fig. 6E). Module 5 was negatively correlated with SOM and FDA (r=-0.67, p < 0.01; r=-0.57, p < 0.05) (Fig. 6E), while modules 6 and 7 were also significantly related with DHG, respectively (r=-0.53, p < 0.05; r=0.57, p < 0.05) (Fig. 6E).

4 Discussion

The results showed that land reclamation had a positive effect on the provision of soil ecosystem functions, which were measured in the light of the enzymatic activities (Figs. 2G-J). All the investigated plots subjected

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to land reclamation showed higher enzymatic activities than the control plots. The results suggested that URA, DHG and FDA increased rapidly after reclamation (Figs. 2G, 2I and 2J), indicating that long-time reclamation may change soil functions such as the conversion of soil nitrogen, or the soil respiratory metabolism (Delgado-Baquerizo, et al., 2016). Yet, an increase in the activity of PPO was only noticeable after 11 years from reclamation, increasing steadily subsequently (Fig. 2H), which might be related with the microbial activity of *Chloroflexi*. In the Fig. S5 (see supplemental material), the PPO showed the positive relation with phylum*Chloroflexi*, whereas had negative relationships with*Proteobacteria* and *Bacteroidetes*. In the Fig. 3A, the abundance of increased *Chloroflexi* and declined*Proteobacteria* and *Bacteroidetes* along the reclamation time. In addition, the activities of DHG and FDA presented some fluctuations over time, suggesting that continuous land management (such as crop planting, irrigation, or fertilization) is needed to maintain their activities levels high (Xiao et al., 2019; Hu et al., 2016). All the variations of soil enzyme activities in the study might suggest that the reclaimed soil became healthier, which might be related with the continuous agricultural production activities, as well as the natural successional processes (Dick, 1994; Sarathambal et al., 2016).

The effect of land reclamation on the studied key soil attributes was uncertain. Only the concentration of AK in the soil seemed to increase following reclamation (Fig. 2D), while the levels of soil N and P were lower in the reclaimed than in the control plots (Figs. 2E and 2F). The increasing AK in this study (Fig. 2D) might be related with coal gauges which contain potassium, and those coal gauges partially dissolving over time (Hu et al., 2015; Spargo and Doley, 2016). This observation could be also related to the fertilization schemes implemented in the studied plots by the local farmers as well as to the depletion of soil nutrients by the crops rotated in the studied plots (Yu et al., 2012; Wang et al., 2016). In addition, SOM showed a decreasing trend along the reclamation timeline and only 17 years after reclamation an increase in SOM was noticeable (Fig. 2A). The latter observation could be related to the accumulation of crop litter, leaves and roots over time, facilitating the formation of humus in the soil (Ezeokoli et al., 2019; Mukhopadhyay et al., 2016). It is also worth noting that the observed decreasing trend in SOM could be related to higher mineralization levels of SOM provided by a changing soil microbial community following reclamation, as we found herein (Zhong et al., 2015; Duval et al., 2016). The reclaimed soil composition has influenced the soil organic matter, which thus might affect the change of total N and Olsen-P contents, which indicated that the soil phosphorus content might be positively correlated with soil organic matter (Fig. S5, see supplemental material).

Contrariwise, the soil pH and EC levels increased over the reclamation timeline, indicating that soil alkalinity increased after reclamation. These observations contradicted earlier studies (Mukhopadhyay et al., 2014, Adeli et al., 2013) and were likely produced by the presence of a water table near the ground surface and with high concentration in salt (Qu et al., 2018) resulting from the release of cations (e.g. Ca^{2+}, Mg^{2+}) present in the coal gangue from past mining activities (Hu et al., 2015). Another possible source for the increase in soil pH and EC following land reclamation could be the application of inorganic fertilizers by the local farmers (Liu et al., 2018), or the soil materials used for reclaiming the land. The latter stresses that careful monitoring of the attributes and origin of the soil materials used in land reclamation should be considered to avoid worsening the attributes of the soil following restoration (Mukhopadhyay et al., 2016; Bai, et al., 2018).

In general, our results seemed to differ from previous studies indicating that land reclamation led to decrease the soil pH and to increase SOM and TN (Shrestha and Raj, 2010; Spargo and Doley, 2016; Li et al., 2014a; Sheng et al., 2015). However, as the plots under study have been subjected to intensive farming activities since they were reclaimed, it was hard to acknowledge which changes in the key soil attributes studied herein were influenced by land reclamation or by the existing farming practices. Future research will aim at disentangling the effects of farming and land reclamation on key soil attribute by identifying if key soil attributes played an important role in driving the multifunctionality in soil ecosystem (Delgado-Baquerizo, et al., 2016).

The results from analyzing the taxonomic composition of the SMCs (Fig. 3) indicated that the distributions

at the phylum level differed among the reclamation plots (Fig. 3A), which evidenced the effect of land reclamation on the SMCs composition (Li, et al., 2014b). The relative abundance of phyla such as *Bacteroidetes* , *Proteobacteria* and *Verrucomicrobia* declined along the reclamation timeline, whereas the relative abundance of the phyla *Acidobacteria* ,*Actinobacteria* , *Chloroflexi* , *Planctomycetes* and *Thaumarchaeota* increased (Fig. 3A). These findings are somehow in disagreement with the results gathered in Li et al. (2014c), who suggested a decrease in the abundance of *Actinobacteria* and *Chloroflexi* in old, restored sites, and who also reported a negative effect of land reclamation on the abundance of *Actinobacteria* . However, the increasing abundance of *Acidobacteria* along the reclamation timeline was interpreted herein as a good indicator of soil health, provided the role that this phylum plays in relevant soil ecosystem processes (e.g. soil acidification; Tringe et al., 2005; Liu et al., 2016). In fact, we observed that *Acidobacteria* became the dominant phylum in all the reclaimed plots (Fig. 3A and Fig. 5), which is in agreement with previous studies evaluating the abundance of SMCs after land restoration (Li et al., 2014bc). Nonetheless, we detected a positive correlation between the abundance of *Acidobacteria* and soil pH (Fig. S5, see supplemental material), which differs from the findings reported before, that the phylum *Acidobacteria* decreased in relative abundance as soil pH increased (Chu et al., 2010; Griffiths et al., 2011). On other hand, the increasing levels of alkalinity found in the reclaimed plots (Fig. 2B) likely explained the increasing abundance of *Actinobacteria* (Fig. 3A), which has a great affinity for alkaline, arid soils. Yet this phylum was the seventh most abundant of the detected phyla, which is in opposition with previous studies reporting *Actinobacteria* as the most abundant phylum in reclaimed mine soil in semiarid climates (Bastida et al., 2013). Our finding may thus be related to the high soil moisture encountered during sampling (i.e. 35%, there was a five-day thunder and heavy rain prior to sampling;). In fact, the observed increase in abundance for the phylum *Chloroflexi* was indicative of water tables present near the ground surface (Chen et al., 2017), and it correlates with the high soil moisture levels found at sampling time and with the relatively high abundance of the phylum *Planctomycetes* - i.e. aquatic bacteria commonly found in brackish water (Lindsay et al., 2001). Still, the steady increase in abundance of *Chloroflexi* along the reclamation timeline (Fig. 3A) may be related to the increase in the water retention capacity of the soil resulting from the observed increase in SOM in the plot assessed 17 years after reclamation - i.e. r17 (Fig. 2A).

Organic manure has been used as soil fertilizer in Eastern China since a long time ago. This popular farming practice could have distinctly affected the composition of the SMC found herein (Geng et al., 2008). The long-term application of organic fertilizer in the plots subjected to reclamation may have affected the soil physicochemical properties (Liang et al., 2014), which in turn may have affected the microbial microenvironments harboring different microbial communities (Jangid et al, 2008). For example, the increasing trend observed for the phylum *Thaumarchaea* (Fig. 3A) could be related to the fertilization schemes implemented by the local farmers in the studied plots. It is worth noting that *Thaumarchaea* is an important ammonia oxidizer in aquatic and terrestrial environments, and that ammonia is a common form of nitrogen in organic manure (Brochier-Armanet et al., 2008). In fact, *Thaumarchaea* is the first archaea identified as being involved in the nitrification process (Brochier-Armanet et al., 2012). Hence, the observed abundance trend for *Thaumarchaea* may explain why the levels of soil TN decreased over the reclamation timeline, as discussed above (Fig. 2F) -i.e. more nitrification (Nitrification, the two-step oxidation of ammonia to nitrate via nitrite – is a critical component of the global nitrogen cycle. Biller et al., 2012) triggered by *Thaumarchaea* derived into lower levels of soil N. Contrariwise, the decreasing trend observed for the phyla *Proteobacteria* can be related to the reduction in mine-based, soil pollution in the reclaimed plots over time (Banning et al., 2011; Lewis et al., 2012; Ma et al., 2017), stressing additional benefits of land reclamation on soil health. To clarify further the latter, we will investigate the levels of soil pollution (e.g. Polycyclic Aromatic Hydrocarbon (PAHs)), as well as the relationship between PAHs and phyla *Proteobacteria* in the study site in the future. As well known that, *Protobacteria* are the best bacteria to PAH degradation, containing PAH-degrading genes (e.g. naphthalene 1,2-dioxygenase, extradiol dioxygenase, Martin et al., 2012; Guarino et al. 2019). Guarino et al. (2019) have also reported that *Proteobacteria* was a phylum with higher proportions (82%), containing known species of PAH degraders (e.g. *Pseudomonas* ,*Burkholderiales*) in the contaminated soil of Bagnoli brownfield site (Southern Italy). Other phyla, however, showed a relatively stable abundance trend along the reclamation timeline (Fig. S2, see supplemental material), as

indicated in Li et al. (2014b). This can be due to having detected those SMCs at a stable stage in their succession at the time sampling was conducted (Novianti et al., 2018; Sun et al., 2019). Anyhow, the array of phyla for the SMCs detected in this study agrees with previous studies focusing on land reclamation (e.g. Li et al., 2014b; Chen et al., 2016; Luo et al., 2019), reflecting the eight major bacterial phyla commonly encountered in the soil (Fig. 3A, Fig. S2, see supplemental material; Chen et al., 2017). Moreover, the most abundant genera of SMCs found herein (i.e. RB41 and UTCFX1; Fig. 3B) were attributed to the high abundance of *Acidobacteria* and *Chloroflexi*, given the demonstrated affinity between these phyla and the most abundant genera (Ngugi et al., 2018). *Flavobacterium* and *Pedobacter* classified to *Bacteroidetes*, as well as *Sphingomonas* and *Luteimonas* belonged to *Proteobacteria* presented the similar phenomenon (Fig. 3B).

In this study, we identified strongly significant or significant correlations between the modules of the microbial ecological networks (MEN) and the studied key soil attributes, such as soil pH, OP, or DHG (Fig. 6), supporting that land reclamation had an effect on the SMC provided through changes in the key soil attributes. In this regard, Ngugi et al. (2018) have investigated the soil bacterial characteristics of 21 coal-mining sites, and found that soil bacterial structure was significantly correlated with soil OP value, which was similar to the conditions identified in the r8 network (Figs. 2 and 6). Phosphorus can be easily fixed in the soil, but its utilization rate is slow, making the concentration of available phosphorus (OP) in the soil small (Ngugi et al.,2018). Yet, some soil microbes, such as *Acidobacteria* might produce acidic substances through metabolism, which then dissolve some insoluble phosphates and utilize them for their own metabolism (Chu et al., 2010), reducing the concentration of soil P. In the network identified for the plot sampled 11 years after reclamation (r11; Fig. 6B), we observed that the differences in terms of SMC were mostly affected by AK. Sun et al. (2019) pointed out similar findings, in which the distribution of bacteria was primarily affected by AK in coal-mining areas, although SOM and OP were also identified as key drivers of SMC change. We also identified a significant correlation between soil pH and the structure of SMC (Fig. 6), which is in agreement with earlier studies (e.g. Li et al., 2018; Hartman et al., 2008; Lauber et al., 2009), and which was mainly attributed to the role that *Acidobacteria* may play on the soil pH (Table 2; Fig. 6). Although our result suggested that the key soil attributes present a complex relationship with soil microbial communities, which need further research to be disentangled, our results also confirmed that network analysis was an effective and feasible tool to analyze the relationship between environmental factors and microbial community structures.

The features of the microbial ecological networks (MEN) established between the detected SMCs varied across the five study sites (Tables 1-2; Fig. 4; Fig. S4, see supplemental material), suggesting that the time after reclamation substantially affected SMC stability and MEN complexity. MEN were more complex in the control plots (CK) than in the plots undergoing reclamation (Fig. 4). The latter may be related to the presence of more stable SMCs in the CK plots due to the absence of operations provoking soil disturbance as opposed to the plots that were reclaimed (Hunt and Ward, 2015; Helingerová et al., 2010). This is further supported by the higher MEN complexity found in r17 -i.e. higher SMC stability, and thus MEN complexity, is expected along the reclamation timeline (Luo et al., 2020; Ma et al., 2020). These findings may be directly related to the effect of time after reclamation on soil ecosystem health and stability, as more stable and complex SMCs are more resilient to environmental stress and ecosystem disturbance (Hunt and Ward, 2015; Dimitriu et al., 2010). Furthermore, the microbial species involved in each network was different, suggesting shifts in the dominant phyla along the reclamation timeline (Figs. 4 and 5). For example, the phyla *Acidobacteria*, *Planctomycetes* and *Proteobacteria* were always present while the keystone phyla changed from plot to plot (Figs. 4 and 5, File S1-S5, see supplemental material). The variation here may cause changes in soil ecological functioning (Delgado-Baquerizo et al., 2018). Therefore, the observed changes in the composition of the SMCs and their interaction through MENs in relation to the time after reclamation is another indicator of the evolution of the soil ecosystem towards a more complex but stable system (Odum, 1969; Gonzalez-Ollauri and Mickovski, 2017) as time from reclamation (i.e. disturbance) progresses in spite of the soil disturbance triggered by past mining activities.

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5. Conclusion

This study investigated the variation of soil attributes and microbial community composition along a land reclamation timeline. In the light of the results gathered in this study, it can be concluded that:

(1) The activities of soil enzymes such as urease, dehydrogenase and polyphenol oxidase increased with the time after reclamation, indicating that land reclamation can promote relevant soil ecosystem functions like N and P mineralization and soil respiration.

(2) The composition of the SMC varied across the reclamation timeline, reflecting changes in the soil attributes brought by land reclamation and/or by the current land practices.

(3) The SMCs and the Molecular Ecological Networks were more stable and less complex along the reclamation timeline, suggesting that land reclamation encourages the stability of the soil ecosystem, and that it thus has a positive impact on the soil.

However, we could not disentangle the effect of land reclamation on the studied key attributes. In particular, we could not verify whether some of the observed trends in the studied soil attributes were due to land reclamation or to the current land use practices. As a result, we animate future research striving to untwine the effect of land reclamation on the soil from that from farming or other land use practices taking place after reclamation. Yet, this study provided a new approach to study soil development through microbial network interactions in agricultural land, which is readily reproducible in studies seeking to evaluate the effect of land reclamation on the soil.

Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Author Contributions

Conceptualization, J.M. and F.C.; Data curation, Q.Z., D.X. and J.M.; Formal analysis, J.M.; Funding acquisition, J.M. and F.C.; Investigation, Q.Z., D.X. and J.M.; Methodology, A.G.O. and J.M.; Project administration, F.C. and J.M.; Resources, F.C., D.X.; Software, J.M. and A.G.O.; Supervision, F.C.; Validation, J.M., A.G.O. and F.C.; Visualization, J.M. and Q.Z.; Writing—original draft, J.M.; Writing—review and editing, J.M. and A.G.O.

Supplementary Materials

Fig. S1: Typical subsidence areas formed by mining activities in Eastern China. Fig. S2: Taxonomic distributions of soil samples on the phylum (A) and genus levels (B). Fig. S3: Power law models of the MENs in five studied plots: (A) r8, (B) r11, (C) r14, (D) r17 and (E) CK. Fig. S4: MEN retrieved modules for study sites (A) r8, (B) r11, (C) r14, (D) r17 and (E) CK where the nodes are portrayed in colors. Fig. S5: RDA and heatmap analysis of major phyla and environmental factors. File S1: The node attribute for each node in the r8 network. File S2: The node attribute for each node in the r11 network. File S3: The node attribute for each node in the r14 network. File S4: The node attribute for each node in the r17 network. File S5: The node attribute for each node in the CK network.

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Table 1 Topological features of the molecular ecological networks (MENs) of the soil microbial communities detected in this study. CK: control; r8: eigth years after reclamation; r11: eleventh years after reclamation; r14: fourteenth years after reclamation; r17: seventeenth years after reclamation

Network indexes	r8	r11	r14	r17	CK
Similarity threshold	0.82	0.82	0.82	0.82	0.82
R2 of power law	0.890	0.885	0.899	0.882	0.822
Total nodes	248	298	358	397	396
Total links	426	572	487	972	1642
Average degree (avgK)	3.435	3.839	2.721	4.897	8.293
Average clustering coefficient (avgCC)	0.238	0.224	0.152	0.261	0.314
Average geodesic distance (GD)	4.853	6.205	8.038	6.049	4.126
Modularity	0.687	0.710	0.798	0.614	0.487
Module number	41	32	59	45	28
Module number (with >5 nodes)	8	12	14	9	13

Table 2 Key node attributes in the ecological networks retrieved from the plot undergoing land reclamation 8 (r8), 11 (r11), 14 (r14), and 17 (r17) years before sampling. CK: control plot.

Plot	OTU number	node degree	No. module	Phylum	module hub or connector
	OTU178	18	2	Acidobacteria	module hub
	OTU2	16	3	Acidobacteria	module hub
	OTU474	14	3	Acidobacteria	/
	OTU686	12	3	Acidobacteria	/
r8	OTU284	16	3	Actinobacteria	/
	OTU22	13	2	Chloroflexi	/
	OTU98	16	2	Chloroflexi	/
	OTU244	13	2	Gemmatimonadetes	/
	OTU198	11	2	Proteobacteria	/
	OTU54	11	3	Proteobacteria	/
	OTU1	15	2	Chloroflexi	/
	OTU16	19	2	Acidobacteria	/
	OTU200	19	2	Acidobacteria	/
	OTU25	13	2	Acidobacteria	/
	OTU293	12	2	Acidobacteria	/
	OTU35	16	2	Acidobacteria	/
	OTU70	17	2	Acidobacteria	/
	OTU48	13	1	Acidobacteria	/
	OTU5	12	1	Acidobacteria	/
r11	OTU41	13	2	Bacteroidetes	/
	OTU172	22	1	Chloroflexi	/
	OTU22	11	2	Chloroflexi	/
	OTU948	15	2	Chloroflexi	/
	OTU291	12	1	Planctomycetes	/
	OTU164	13	1	Planctomycetes	/
	OTU39	13	2	Planctomycetes	/
	OTU40	24	2	Planctomycetes	/
	OTU33	12	2	Proteobacteria	/
	OTU90	15	2	Proteobacteria	/
	OTU376	12	3	Acidobacteria	

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Plot	OTU number	node degree	No. module	Phylum	module hub or connector
	OTU453	15	3	Acidobacteria	
	OTU120	12	3	Actinobacteria	
	OTU346	11	3	Actinobacteria	
	OTU7041	15	3	Actinobacteria	
	OTU249	14	3	Chloroflexi	
r14	OTU300	12	3	Chloroflexi	
	OTU663	12	3	Chloroflexi	
	OTU387	11	3	Gemmatimonadetes	
	OTU375	18	3	Nitrospirae	module hub
	OTU306	12	4	Planctomycetes	
	OTU2602	11	4	Proteobacteria	
	OTU282	11	4	Proteobacteria	
	OTU33	12	4	Proteobacteria	
	OTU101	20	8	Acidobacteria	
	OTU122	32	8	Acidobacteria	
	OTU189	21	8	Acidobacteria	
	OTU19	36	8	Acidobacteria	
	OTU253	23	8	Acidobacteria	
	OTU3453	26	8	Acidobacteria	connector
	OTU63	27	3	Acidobacteria	
	OTU41	24	3	Bacteroidetes	
	OTU412	25	3	Bacteroidetes	
	OTU251	22	8	Chloroflexi	
r17	OTU1146	27	8	Gemmatimonadetes	module hub
	OTU337	21	8	Gemmatimonadetes	
	OTU702	29	8	Gemmatimonadetes	
	OTU138	20	2	Gemmatimonadetes	
	OTU191	21	3	Planctomycetes	
	OTU51	28	3	Planctomycetes	
	OTU208	22	3	Planctomycetes	
	OTU203	34	2	Planctomycetes	
	OTU442	23	2	Planctomycetes	connector
	OTU146	23	8	Proteobacteria	
	OTU33	22	3	Proteobacteria	
	OTU1120	37	3	Acidobacteria	
	OTU1299	40	3	Acidobacteria	
	OTU128	44	1	Acidobacteria	module hub
	OTU5	33	1	Acidobacteria	
	OTU7	33	1	Bacteroidetes	
	OTU2544	34	1	Bacteroidetes	
CK	OTU179	34	2	Bacteroidetes	
	OTU22	35	1	Chloroflexi	
	OTU948	36	1	Chloroflexi	module hub
	OTU1068	34	3	Planctomycetes	
	OTU46	44	3	Planctomycetes	module hub
	OTU154	38	1	Planctomycetes	
	OTU6478	50	3	Proteobacteria	

Figure captions

Fig. 1. Sampling plots distributed within the study site -i.e. CK: control; r8: 8 years since reclamation; r11: 11 years since reclamation; r14: 14 years since reclamation; and r17: 17 years since reclamation. Figs. A, B and show the study site in Zoucheng, China. The D pictures is from the subsidence area in eastern China.

Fig. 2. Soil key attributes in the reclaimed (i.e. r8, r11, r14 and r17) and control (iCK) plots. Mean ± standard deviation (SD) (n = 15). Letters indicate significant differences among the 5 sites. A: SOM; B: soil pH value; C: soil EC value; D: soil AK content; E: soil OP content; F: soil TN content; G: soil URA activity; H: soil PPO activity; I: soil DHG activity; J: soil FDA activity. The values in the picture represent the significance of T-test; when the number < 0.05, it means significant; the value < 0.01: very significant; the value < 0.001: extremely significant.

Fig. 3. Taxonomic distributions of the most abundant (A) phylum and (B) genera levels of the SMCs found in the studied sites after reclamation (i.e. r8, r11, r14, r17) and control sites (CK).

Fig. 4. Molecular ecological networks (MENs) retrieved for the sampling plots subjected to land reclamation 8 (r8; A), 11 (r11; B), 14 (r14; C), and 17 (r17; D) years before sampling and control plot (CK; E). The nodes are plotted in circles of different sizes. The established nodes are portrayed in colors (i.e. purple, yellow, blue, green, ...). Each node corresponds to one microbial population of the most abundant phylum. The identified modules are labeled with numbers. A pink connector indicates a negative correlation, while a light-blue connector indicates a positive correlation between modules. Please, see online version for colors. CK: control; r8: eighth years after reclamation; r11: eleventh years after reclamation; r14: fourteenth years after reclamation; r17: seventeenth years after reclamation

Fig. 5. Z-P plot showing the keystone species (i.e. module hubs and connectors) in the different molecular ecological networks (MENs). Different symbols with special colors represent different networks as follows: green square for the CK network, red star for the r8 network, blue upward-facing triangles for the r11 network, magenta downward-facing triangles for the r14 network and cross cirle for the r17 network. The module hubs and connectors are labeled with phylogenetic affiliations (*Acido-Acidobacteria, Actino- Actinobacteria, Bacteroi-Bacteroidetes, Chloro-Chloroflexi, Depen-Dependentiae, Elusi-Elusimicrobia Gemma-Gemmatimonadetes, Nitro-Nitrospirae, Patesci-Patescibacteria, Plancto-Planctomycetes, Proteo-Proteobacteria and Verru-Verrucomicrobia*). CK: control; r8: eighth years after reclamation; r11: eleventh years after reclamation; r14: fourteenth years after reclamation; r17: seventeenth years after reclamation.

Fig. 6. Correlation between the identified molecular ecological networks (MEN) modules and the key soil attributes for the five study plots: (A) r8; (B) r11; (C) r14 and (D) r17; (E) CK. CK: control; r8: eighth years after reclamation; r11: eleventh years after reclamation; r14: fourteenth years after reclamation; r17: seventeenth years after reclamation. * mean significant difference at 5% (p<0.05), ** mean significant difference at 1% (p<0.01), *** mean extremely significant difference at 1% (p<0.001).
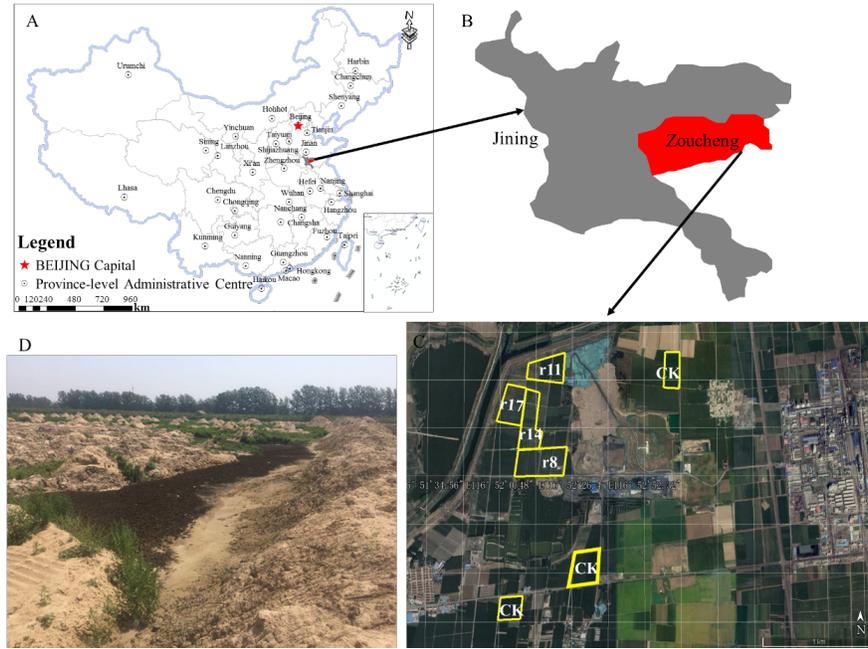
Fig. 1.
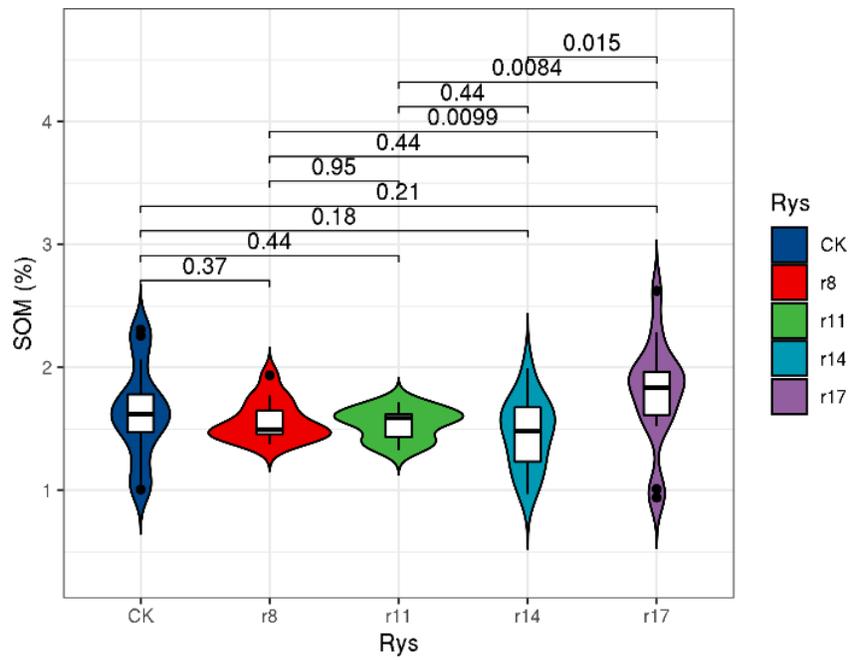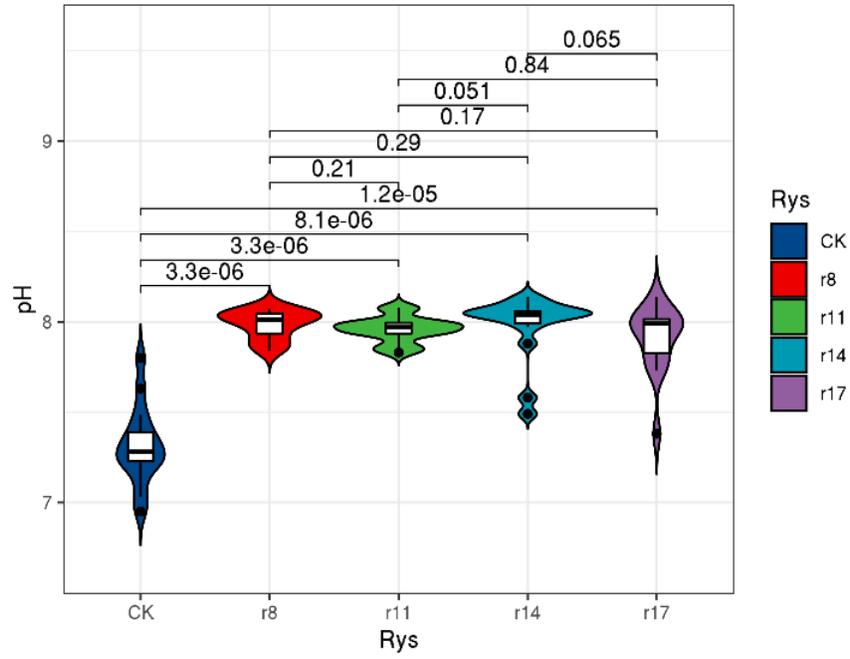
A B
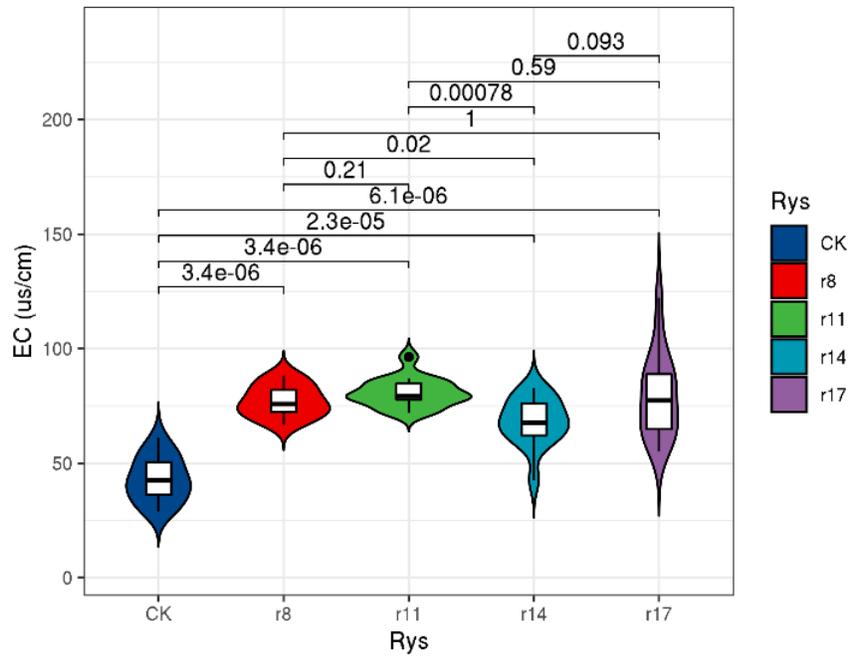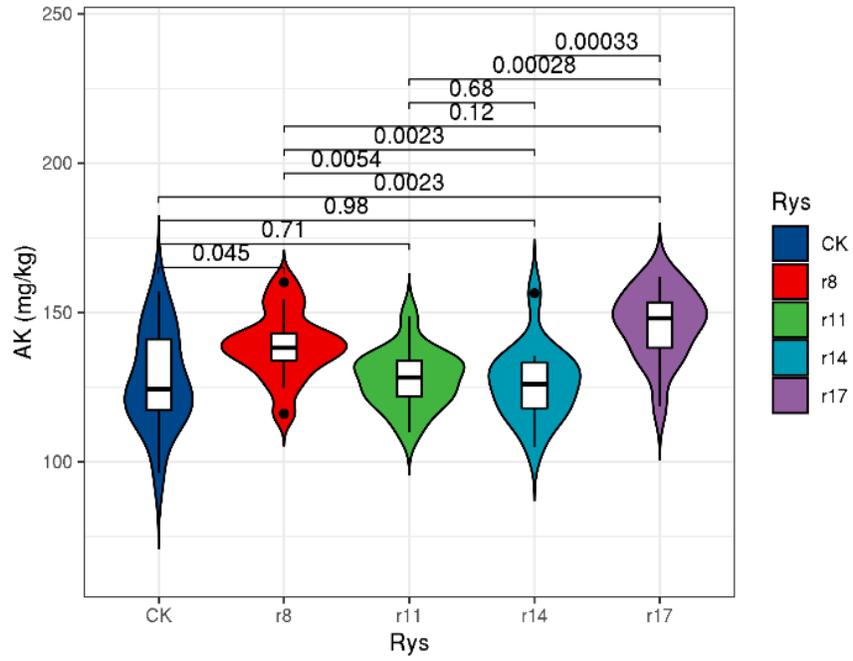
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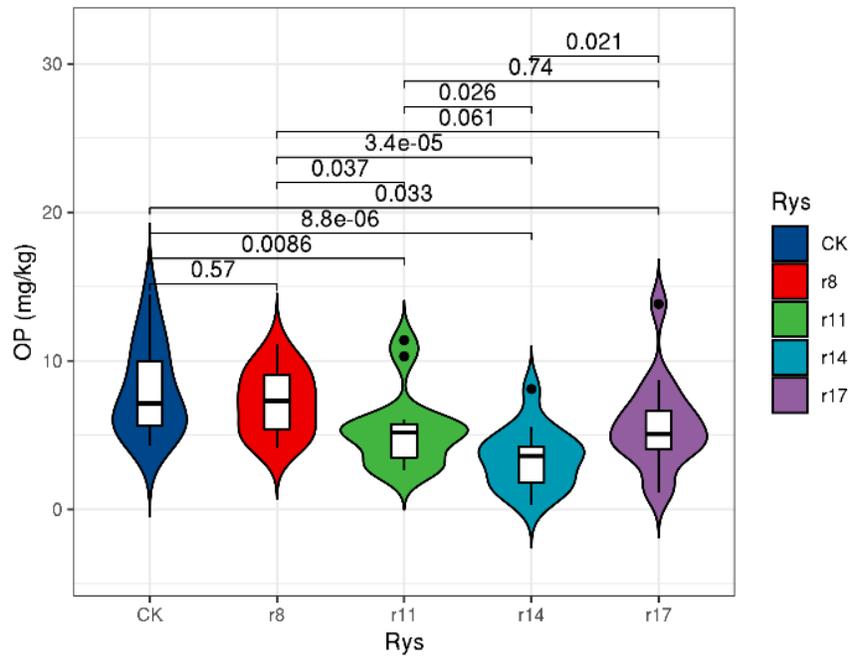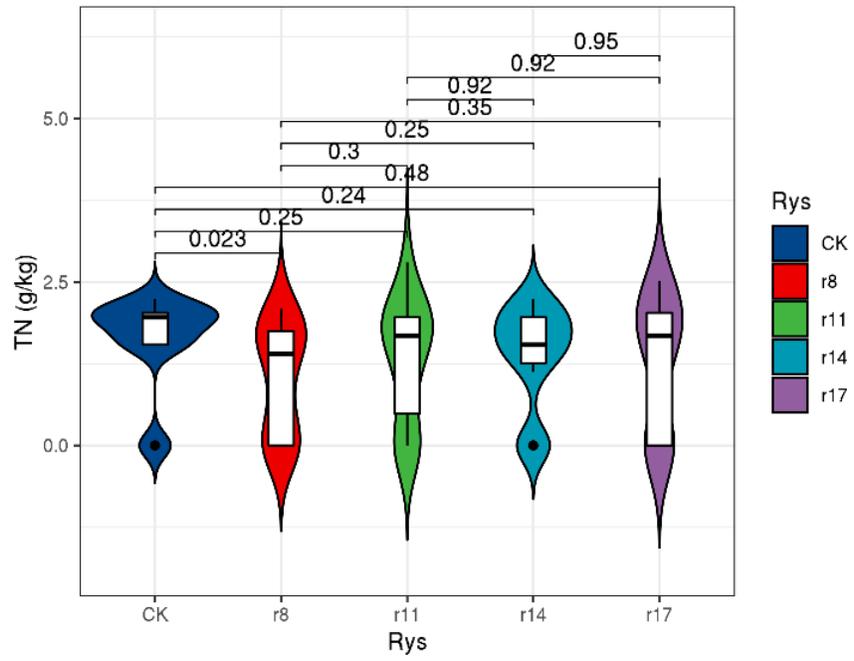
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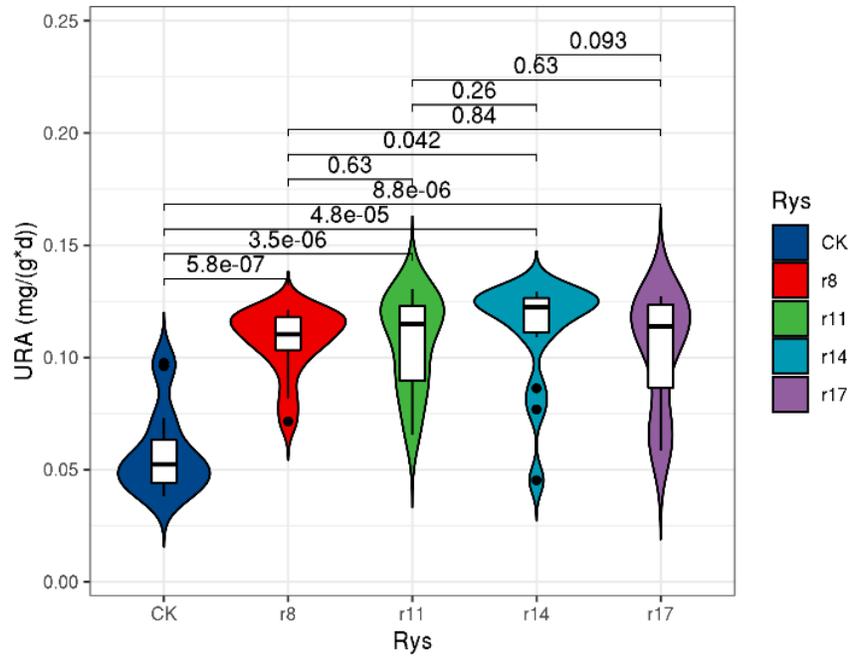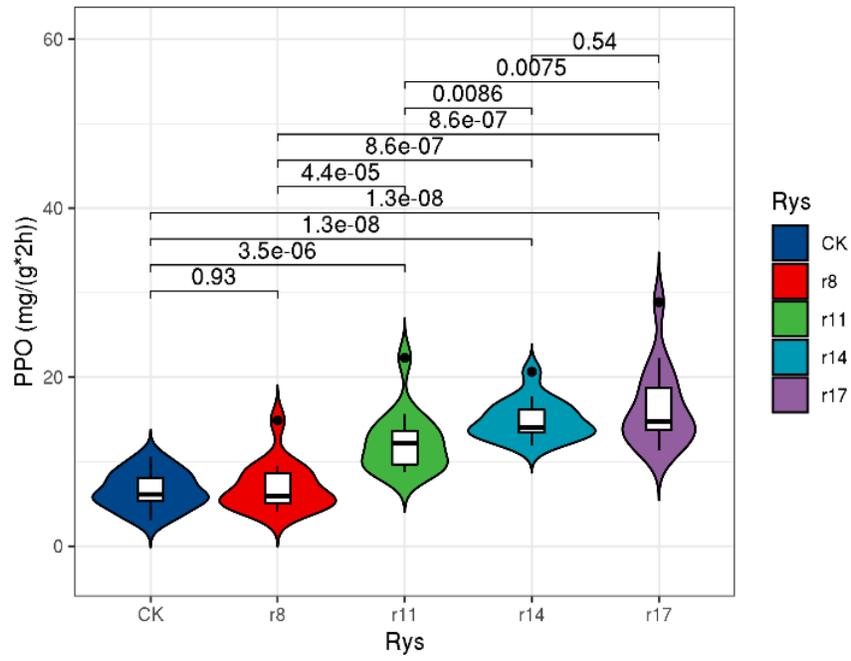
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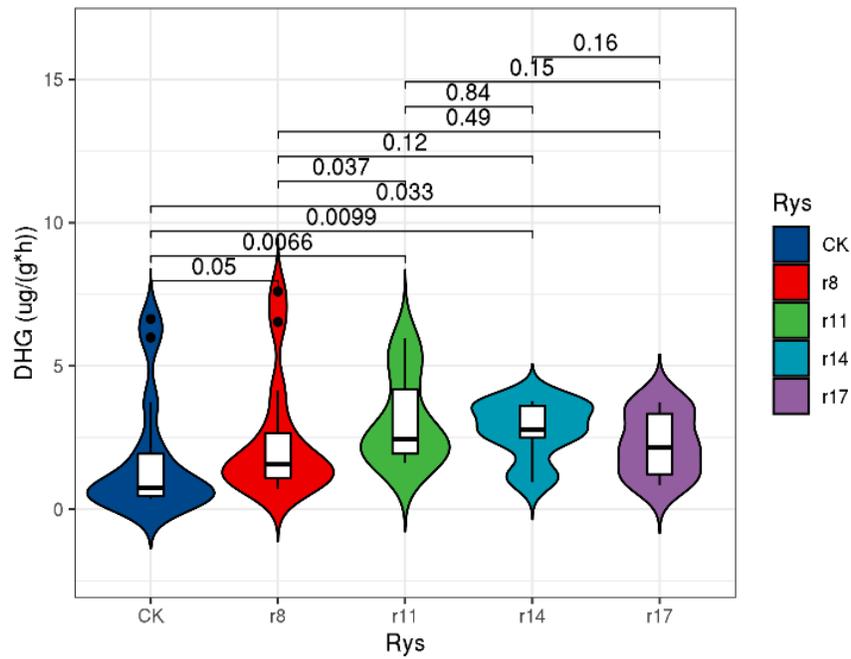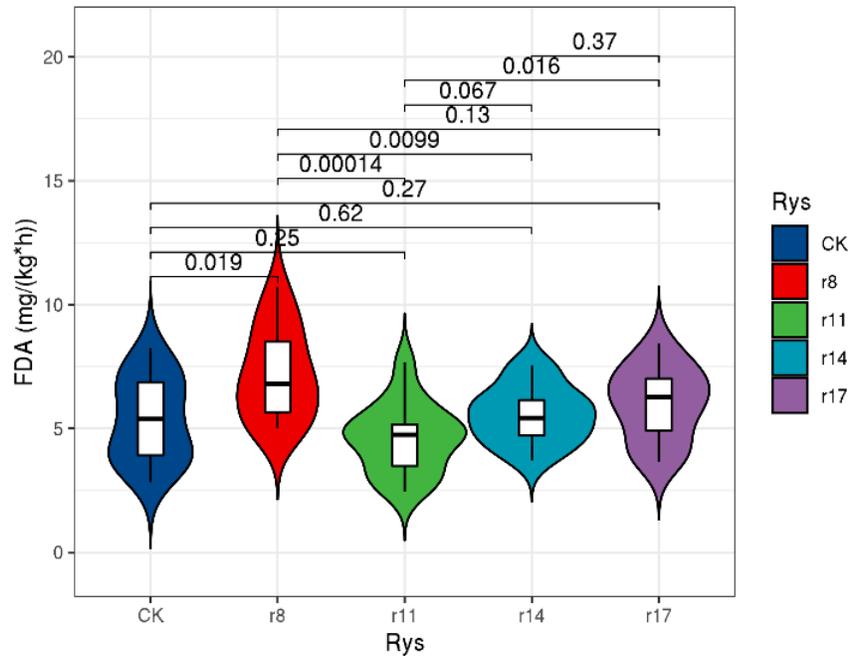
I J

Fig. 2.

A

B

Fig. 3.

Fig. 4.

Fig. 5.

A B

C D

E

Fig. 6.