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Rare rather than abundant microbial communities drive the effects of long-term greenhouse cultivation on ecosystem functions in subtropical agricultural soils

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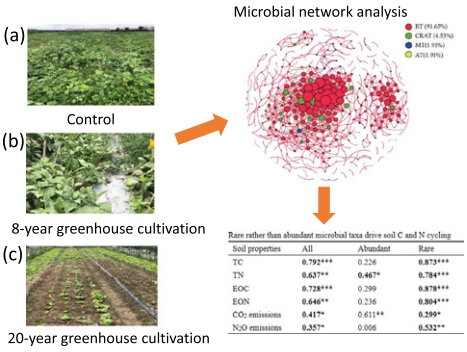
HIGHLIGHTS

- Long-term greenhouse cultivation (LG) significantly increased soil C and N pools.
- LG significantly affected greenhouse gas emissions potential.
- LG had no effects on soil microbial diversity.
- LG markedly affected rare microbial community composition.
- Rare rather than abundant microbial taxa drive the changes in ecosystem functions.

GRAPHICAL ABSTRACT



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ABSTRACT

Long-term greenhouse cultivation has an adverse effect on ecosystem functions such as soil carbon (C) and nitrogen (N) pools and greenhouse gas (GHG) emissions, but the underlying microbial mechanisms still remain unclear. Here, different sites under long-term greenhouse cultivation in a subtropical agricultural ecosystem were selected to measure soil C and N contents, extractable organic C (EOC) and N (EON) contents, and potential GHG emissions. Metagenomic analysis and *16S* rRNA high-throughput sequencing were used to measure microbial communities. The results showed that long-term greenhouse cultivation increased soil salinity, and significantly increased soil total C and N contents, EOC and EON contents, and N_2O emission potentials, although it significantly decreased CO_2 emission and CH_4 oxidation potential compared with the ambient control. Changes in soil CH_4 oxidation and N_2O emission potential exhibited similar patterns in the corresponding key functional genes based on according to our metagenomic analysis. In addition, long-term greenhouse cultivation did not change microbial diversity, although it clearly affected soil microbial community composition. Soil microbial communities were further classified into rare and abundant microbial taxa. Rare rather than abundant microbial taxa could adequately explain the changes in ecosystem functions, except for CH_4 oxidation potential across the treatments. To our knowledge, this is the first study to quantify the importance of microbial subcommunities to

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ecosystem functions on the basis of microbial co-occurrence network analysis under greenhouse cultivation in agricultural ecosystems. Overall, our results indicated that rare rather than abundant microbial taxa could act as indicators of variations in ecosystem functions under long-term greenhouse cultivation in subtropical agricultural soils, which might be useful for better management practices and improving crop yields in agricultural ecosystems.

1. Introduction

Over many years, to meet the increasing global demand for human food, greenhouse vegetable cultivation has been widely used worldwide (Ju et al., 2007). However, long-term greenhouse cultivation can cause increases in soil salinity, which, in turn, has adverse effects on ecosystem functions and crop yields (Miao et al., 2011). Given that ecosystem functions are determined by soil biodiversity and microbial communities (Delgado-Baquerizo et al., 2016; Wagg et al., 2014), more and more studies have focused on role and mechanisms of soil microbial communities under different management practices in agricultural ecosystems (Acosta-Martínez et al., 2003; Yao et al., 2006). However, the microbial mechanisms under long-term greenhouse cultivation in agricultural ecosystems still remain unclear.

It is known that soil microorganisms are extremely diverse with relatively few abundant species and a large number of rare species (Shade et al., 2012). Recent studies have increasingly emphasized the ecological importance of the rare microbial taxa, because these rare taxa can include more metabolically active microorganisms than abundant taxa (as measured by RNA to DNA ratios) and they may be keystone species in terrestrial ecosystems (Lynch and Neufeld, 2015). Recently, a few studies have investigated rare microbial taxa in aquatic ecosystems and found that rare rather than abundant microbial taxa play an important role in ecosystem functions such as nutrient cycling (Jousset et al., 2017), particularly after ecosystem disturbance (Xue et al., 2018). In addition, rare microbial taxa can act as part of the microbial "seed bank", which may become dominant after disturbance and thus drive ecosystem functions (Shade and Gilbert, 2015; Shade et al., 2014). A large number of studies have focused on the co-occurrence patterns of abundant and rare microbial taxa and their relative contributions in aquatic ecosystems (Galand et al., 2009; Xue et al., 2018). However, few studies have investigated the importance of rare and abundant microbial communities subjected to management practices in agricultural ecosystems, especially under long-term greenhouse cultivation.

Ecosystem functions can be indicated by several variables such as soil carbon (C) and nitrogen (N) pools and greenhouse gas (GHG) emissions (Chandregowda et al., 2018). On the one hand, in general, soil C and N pools are not sensitive to changes in different management practices, although the active components of soil C and N pools, (i.e., the soil extractable organic C (EOC) and extractable organic N (EON) pools) can rapidly respond to these changes (Haynes, 2005) and can provide a short-term reservoir of nutrients for crop growth in agricultural ecosystems (Ghani et al., 2003; Zhou et al., 2013). On the other hand, agricultural ecosystems are important sources of GHG emissions and contribute to about 10%–12% of total global GHG emissions (IPCC, 2013). Given that ecosystem functions in agricultural ecosystems are mediated by soil microbial communities (Andresen et al., 2015; Falkowski et al., 2008; Xiao et al., 2015), it is necessary to elucidate the mechanisms of rare and abundant microbial subcommunities under different greenhouse cultivation methods, which will help us better manage agricultural ecosystems and promote sustainable development.

Here, sites under long-term greenhouse vegetable cultivation in a subtropical agricultural ecosystem were selected to investigate ecosystem functions and their underlying microbial mechanisms. The objectives of this study were to (1) investigate the effect of long-term greenhouse cultivation on ecosystem functions such as soil C and N pools and potential GHG emissions, and (2) elucidate the mechanisms of rare and abundant microbial subcommunities and their contributions to ecosystem functions.

2. Materials and methods

2.1. Experimental site

The study site was a plastic greenhouse (width: 25 m; length: 30 m; height: 2.5 m) located at a crop cultivation farm in Fengxian district, Shanghai, southeastern China (30°52′N, 121°34′E). There was a typical subtropical monsoon climate in this region with a hot, humid summer and a drier, cold winter. The mean annual temperature is 15.8 °C and mean annual precipitation is 1149 mm (Cui and Shi, 2012).

Three sites were selected. Two of them were subjected to different periods of vegetable greenhouse cultivation (Fig. S1): an 8-year mid-term greenhouse cultivation (hereafter referred to as MG) and a 21-year long-term greenhouse cultivation (hereafter referred to as LG), as well as a nearby ambient control site without greenhouse cultivation. All these sites have been subjected to similar fertilizer and cultivation treatments during the past 21 years. During the first 10 years, all sites received an application of compound fertilizer at a rate of ~450 g m^{-2} y^{-1}, and the ratio of N: phosphorus (P): potassium (K) in the compound fertilizer was 15%:15%:15%. During the past 10 years since 2009, all these sites have received organic manure (cow manure and crop residues) at a rate of 6000 g m^{-2} y^{-1}. The soil type in this region is a loamy soil according to international soil classifications. Particle sizes were similar among the treatments; the soil particle sizes were 53.41 ± 0.88% for sand, 22.01 ± 0.88% for silt and 24.58 ± 1.58% for clay.

2.2. Measurements of soil physiochemical properties

Within each site, three plots measuring 3 m × 3 m were randomly selected. Each plot was at least 2 m away from the others. Soil samples were collected in June 2018 by using a diagonal sampling pattern using a soil auger (5-cm in diameter) at a depth of 0–10 cm within each plot (Gu et al., 2019). After passing the samples through a 2-mm sieve to remove roots and stones, the soil samples were stored at 4 °C prior to analysis. Soil moisture contents were determined after the samples were oven-dried at 105 °C overnight. Soil pH was measured at a 1:2.5 dry soil/water ratio (Gu et al., 2019). Soil electrical conductivity (EC) and salinity were measured at a 1:5 dry soil/water ratio (Datta et al., 2019).

2.3. Measurement of ecosystem functions

In this study, soil total C (TC) and total N (TN) contents, extractable C and N contents (EOC and EON contents), as well as GHG emissions potential as indicators of ecosystem functions were measured in soils subjected to different greenhouse cultivation.

Regarding soil C and N contents, we first air-dried the soil samples and then finely ground them. Soil TC and TN contents were measured on a Vario MICRO cube elemental analyzer (Elementar, Germany) and a Smartchem Discrete Auto Analyzer (Smartchem200, AMS, Italy) respectively (Bu et al., 2018). Measurements of soil EOC and EON contents has been described previously by Zhou et al. (2013).

Regarding soil GHG emissions potential, CH_4, CO_2 and N_2O emission potential were measured via laboratory incubations. Briefly, about 10 g

(dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask in the dark at 22 °C for 7 days as described before Bu et al. (2018). The CH_4 and CO_2 and N_2O concentrations in the gas samples were analyzed on a gas chromatograph (GC7890B, Agilent). Soil potential CH_4 oxidation rates, and CO_2 and N_2O emissions were calculated from the differences in the concentrations in the gas samples between the two sampling times (Bu et al., 2018).

2.4. High-throughput sequencing of 16S rRNA and bioinformatics analysis

Soil genomic DNA was extracted from 0.5 g of each sample by following the procedures described previously (Zhou et al., 2010). The bacteria-specific *16S* rRNA primers of 515F and 907R were used to amplify the bacterial *16S* rRNA gene (Bu et al., 2018; Chen et al., 2016). The PCR products were run on a gel and the appropriate fragments were cut and purified with the Qiagen Gel Extraction kit (Qiagen Inc., Shanghai, China). The amplicons were sequenced on an Illumina Miseq platform (Illumina, Nanjing, China). Subsequently, QIIME and UPARSE were used to analyze the amplicons sequencing data as described before (Bu et al., 2018). Paired-end data from each sample were joined with FLASH (Magoc and Salzberg, 2011) using the default parameters. Operational taxonomic units (OTUs) were clustered at a similarity of 97%.

2.5. Definition of abundant and rare taxa

The definition of abundant and rare taxa depends on the cutoff level of their relative abundance, setting 0.01% as rare OTUs and 1% as abundant OTUs (Mangot et al., 2013; Pedrós-Alió, 2011; Xue et al., 2018). These classifications can neglect the intermediate taxa (i.e., relative abundance between 0.01 and 1%) and the oscillating taxa (i.e., rare and abundant under different conditions). In this study, all OTUs were classified into six categories following a recent study (Dai et al., 2016): (1) always abundant taxa with a relative abundance of ≥1% in all samples; (2) conditionally abundant taxa with a relative abundance of ≥0.01% in all samples and ≥1% in some samples; (3) always rare taxa with a relative abundance of <0.01% in all samples; (4) conditionally rare taxa with a relative abundance of <0.01% in some samples but never ≥1% in any sample; (5) moderate taxa with a relative abundance between 0.01% and 1% in all samples; and (6) conditionally rare and abundant taxa with a relative abundance ranging from rare (<0.01%) to abundant (≥1%). In the comparative study of abundant and rare taxa, the abundant taxa consisted of the always abundant and conditionally abundant taxa, and the rare taxa, which comprised always rare and conditionally rare taxa. Detailed and general descriptions of abundant and rare data sets are presented in Supplementary Tables S2.

2.6. Metagenomic analysis of soil genomic DNA

Total DNA extracted from the soil samples was sequenced with an Illumina HiSeq 4000 (Illumina). This produced an average of 109.83 Mb of high-quality reads for each sample, providing a total of 329.48 Mb of read data. The high-quality reads were assembled de novo into contigs with Megahit version 1.0.6 (Li et al., 2015) with the default parameters for all samples.

Then genes were predicted from the contigs with lengths of >500 bp in MetaGeneMark (version 3.26) (Karlsson et al., 2013). A non-redundant gene catalogue was constructed with CD-HIT using a sequence identity cut-off of 0.95 and a minimum coverage cut-off of 0.9 for the contigs (Li, 2015). The non-redundant catalogue contained 1,470,368 microbial genes. To assess the abundance of genes, high

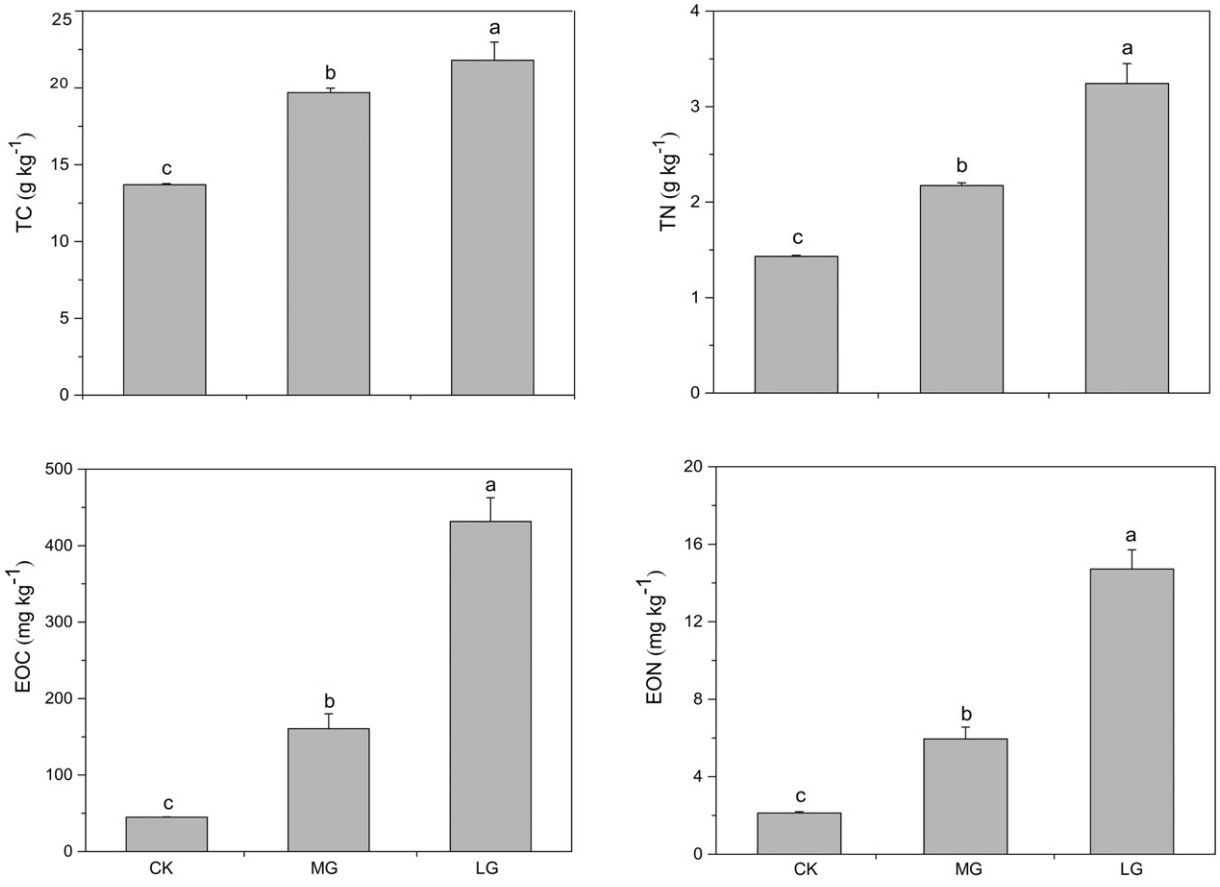

Fig. 1. Variations in soil C and N contents under long-term greenhouse cultivation in a subtropical agricultural farm. Data represent the means and standard errors ($n = 3$). Different lowercase letters indicate significant differences at $P < .05$ among the treatments. TC, total C; TN, total N; EOC, extractable organic C; EON, extractable organic N; CK, control; MG, mid-term greenhouse cultivation; LG, long-term greenhouse cultivation.

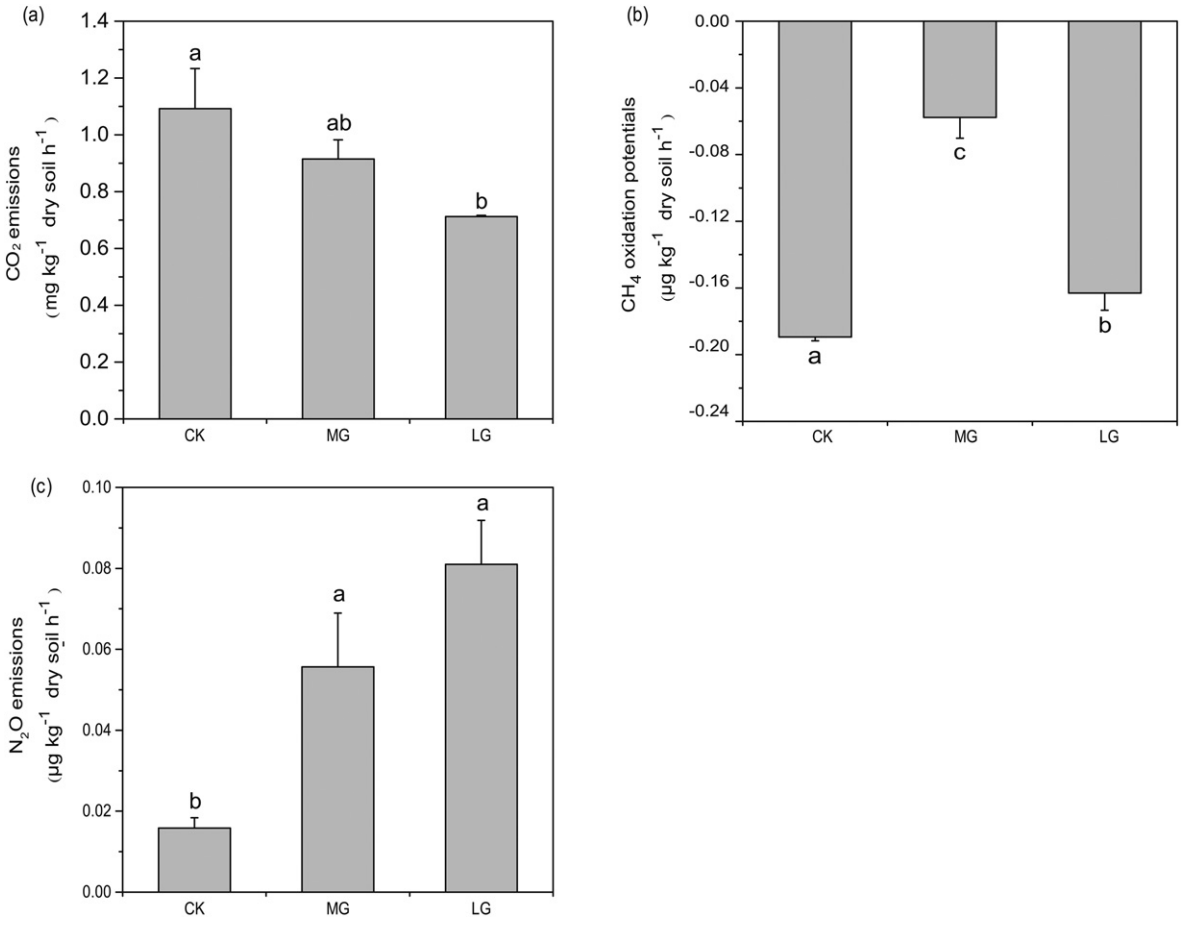

Fig. 2. Variations in soil CO_2 emissions (a), CH_4 oxidation potential (b) and N_2O emissions (c) under long-term greenhouse cultivation in a subtropical agricultural farm. Data represent the means and standard errors ($n = 5$). Different lowercase letters significant differences at $P < .05$ among the treatments. CK, control; MG, mid-term greenhouse cultivation; LG, long-term greenhouse cultivation.

quality reads were aligned to the gene catalogue with Soapaligner (version 2.21) with the following parameters: *-m* 100, *-x* 500, identity ≥95% (Le Chatelier et al., 2013).

To determine the gene taxonomy classifications, protein sequences of the predicted genes in our catalogue were searched against the NCBI-NR database (June 2017 version) and the lowest common ancestor algorithm was used on alignments with an e-value of 10^{-5} or lower (Huson et al., 2007). For functional annotation, protein sequences of the predicted genes were searched against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (version 59), with DIAMOND blastp and an e-value threshold of 10^{-5} (Buchfink et al., 2015). Each protein was assigned a KEGG ortholog based on the best hit gene in the KEGG database. The abundance of a KEGG ortholog was calculated by summing the abundance of genes annotated to a feature.

2.7. Statistical analyses

One-way analysis of variance (ANOVA) was used to determine the effects of different greenhouse cultivation on soil moisture contents, pH, TC and TN contents, EOC and EON contents, NH_4^+–N and NO_3^-–N contents, soil EC and salinity. For the incubation experiment, one-way ANOVA was used to examine the effects of different treatments on soil potential CH_4 oxidation rates and CO_2 and N_2O emissions. After statistical analysis via ANOVA in the "stats" R package (R Foundation for

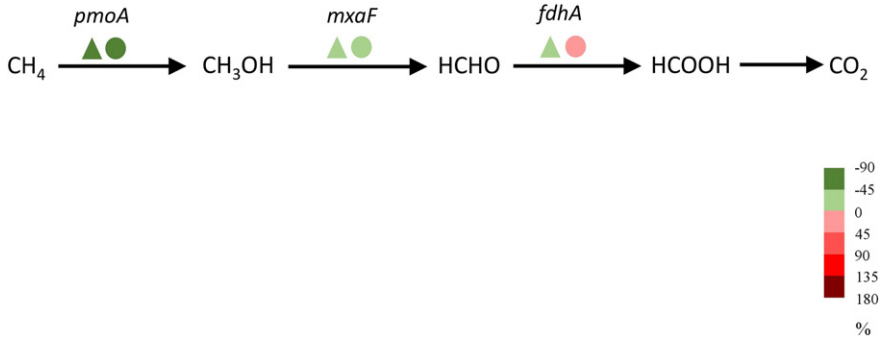

Fig. 3. Relative changes (%) in key genes involved in soil methane oxidation according to metagenomic sequencing in mid-term greenhouse cultivation (△) and long-term greenhouse cultivation (○) compared with the ambient control in a subtropical agricultural farm.

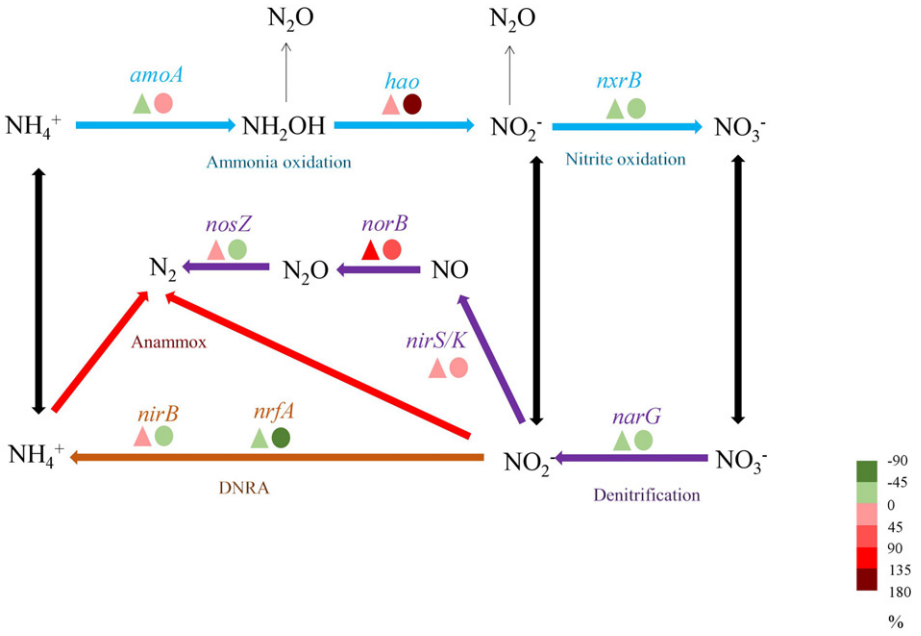

Fig. 4. Relative changes (%) in key genes involved in the soil cores' nitrogen cycle according to metagenomic sequencing in mid-term greenhouse cultivation (△) and long-term greenhouse cultivation (○) compared with the ambient control in a subtropical agricultural farm. DNRA, dissimilatory nitrate reduction to ammonium.

Statistical Computing, Vienna, Austria), Tukey's HSD test was used to compare the significant differences among the treatments.

Venn diagram analysis was used to calculate the shared and unique OTUs among the treatments. Alpha-diversity indices were computed via the diversity function in the "vegan" R package (Xue et al., 2018). For soil microbial community structure, beta-diversity was measured as Bray–Curtis dissimilarity. The soil microbial community composition was visualized via non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities. Mantel correlations between community composition and either environmental variables or GHG emissions were also calculated. All ANOVA and correlation analyses were performed in R (R Core Team, 2014).

To reduce the complexity of the datasets, OTUs present in more than six samples were retained for the construction of networks. A total of 888 OTUs were used for the network analyses. Statistical differences in measured node-level attributes across different taxa were determined by the nonparametric Mann–Whitney *U* test. Nodes with high degree (>10) and low betweenness centrality values (recognized as keystone species in co-occurrence networks(Berry and Widder, 2014).

2.8. Data availability

All raw sequences data from this study have been deposited in the DNA Data Bank of Japan (DDBJ) biosample database with the accession numbers of PRJDB8545.

3. Results

3.1. Ecosystem functions in soils under different treatments

As we expected, LG and MG had significantly higher soil salinity than the ambient control site, and LG had significantly higher soil salinity than MG (Table S1). Changes in soil EC showed the same patterns as soil salinity across the treatments. LG had significantly higher soil moisture contents and lower pH than the ambient control site, although there were no marked differences in these attributes between MG and LG (Table S1).

LG and MG had significantly higher soil TC and TN contents, EOC and EON contents, and NH_4^+–N and NO_3^-–N contents compared with the ambient control (Fig. 1 and Table S1), whereas LG had significantly higher soil TC and TN contents, EOC and EON contents than MG. In addition, LG and MG had significantly lower CO_2 emission potential, CH_4 oxidation potential, but had significantly higher N_2O emission potential than the ambient control, except for CO_2 emission potential between MG and the control site (Fig. 2). LG had significantly higher CH_4 oxidation potential than MG (Fig. 2). Metagenomic analysis showed that LG and MG had relatively lower *pmoA* (a key functional gene in CH_4 oxidation) genes (Fig. 3 and Table S4), although they had more *nirS*/*nirK* genes but less *nosZ* genes (key denitrification genes linked to N_2O emissions) compared with the ambient control (Fig. 4 and Table S5).

3.2. Soil microbial subcommunities and their co-occurrence network under different treatments

Overall, the sequencing of *16S* rRNA genes yielded 99,486 high-quality sequences and 2437 OTU numbers at 97% similarity level (Table S2). LG and MG had significantly higher richness, ACE and Chao1 index of rare microbial taxa than the ambient control, whereas LG had significantly lower values for these characteristics than MG. Rare and abundant microbial taxa were clearly separated across the treatments (Fig. 5a), which was partly attributed to differences in 13 OTUs of abundant microbial taxa and OTUs of rare microbial taxa, as shown in the Venn diagram (Fig. 5b). Compared with the abundant microbial taxa, rare microbial taxa showed a striking separation, which was confirmed by a comparison of between-group distances among the different treatments (Fig. 5c).

Microbial taxa were classified further into four groups (abundant taxa, moderate taxa, conditionally rare and abundant rare taxa, and rare taxa). Of them, the abundant taxa category includes two subgroups (always abundant taxa and conditionally abundant taxa) and only accounts for a fraction of the total number of OTUs (0.53%), whereas the rare taxa category, which includes two subgroups (always rare taxa and conditionally rare taxa), accounts for the majority of total bacterial OTU numbers (97.62%) (Table S2). Based on the four subcommunities' correlation relationships, a meta-community co-occurrence network was built (Fig. 6a). This network consisted of 418 nodes linked by 1072 edges and was mainly composed of Proteobacteria and Planctomycetes (Fig. S3). The network obtained scale-free characteristics (power-law: $R^2 = 0.941$), indicating that it was non-random. Non-rare taxa (abundant taxa, moderate taxa and conditionally rare

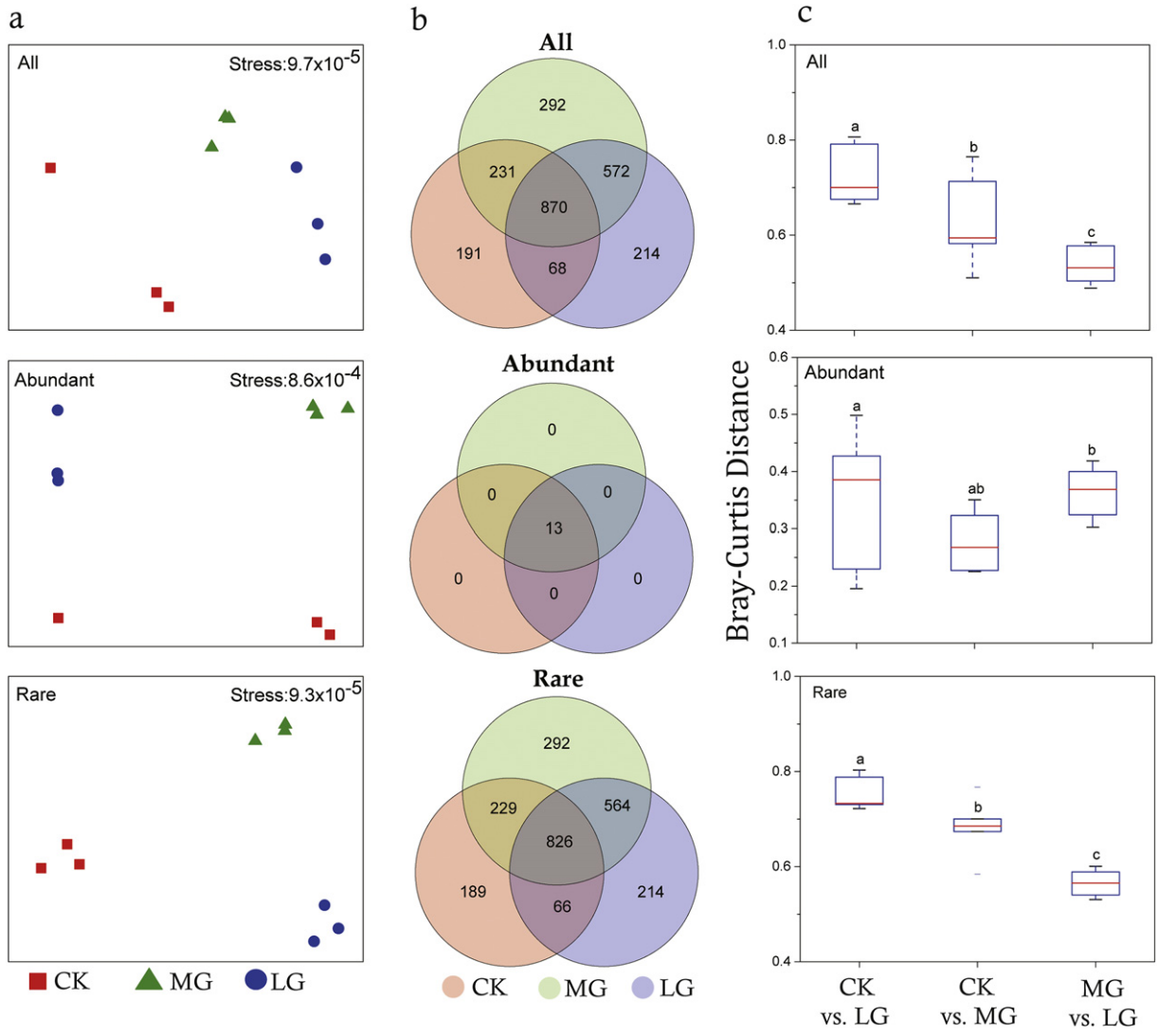

Fig. 5. Variations in soil microbial community structure under long-term greenhouse cultivation (LG) and mid-term greenhouse cultivation (MG) in a subtropical agricultural farm. (a) Non-metric multidimensional scaling (NMDS) ordination of soil bacteria communities based on Bray–Curtis distances. (b) Venn diagram showing the number of unique and shared operational taxonomic units (OTUs) in three MG, LG and the control. (c) The pairwise Bray–Curtis dissimilarity of soil bacteria communities in three MG, LG and the control. The top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively, and the lines within each box represent the median values ($n = 3$). Different letters above bars indicate a significant difference at the $P < .05$ level according to nonparametric Mann–Whitney U test. All, total soil bacteria communities; Abundant, abundant soil bacteria subcommunities; Rare, rare soil bacteria subcommunities. CK, control.

and abundant rare taxa) had a strong connection with rare microbial taxa (Fig. 6a). In general, rare microbial taxa had similar node-level topological features such as degree, betweenness centrality and eigenvector centrality values to abundant microbial taxa (Fig. 6b and c and d), but rare microbial taxa had significantly lower closeness centrality than abundant microbial taxa (Fig. 6e). There were 32 keystone species of microbial taxa, most of which belonged to the rare microbial taxa category (Table S3).

3.3. Relationship between microbial subcommunities and ecosystem functions

The Mantel test results indicated that all taxa combined and rare microbial taxa rather than abundant microbial taxa were correlated with soil moisture contents, pH, EC, salinity, TC, EOC and EON contents, and rare microbial taxa exhibited stronger correlation relationships with these properties than all taxa combined and abundant microbial taxa (Table 2). Both rare and abundant microbial taxa showed significant correlations with TN and NO_3^-–N contents, but the former exhibited stronger correlations with them than the latter (Table 2).

Total, abundant and rare microbial taxa exhibited significant correlations with CO_2 emission potential, with abundant microbial taxa having the highest correlation coefficient. Rare and total rather than abundant microbial taxa had significant correlations with N_2O emission potential, with rare taxa having the highest correlation coefficient. However, any significant relationships between total, abundant or rare microbial taxa, and soil CH_4 oxidation potential were not found (Table 3).

4. Discussion

Soil microbial communities and their related ecosystem function properties are considered to be one of the indicators of management practices as they show a fast response to environmental changes (Larkin, 2015; Yao et al., 2006). Rare and abundant microbial taxa exhibited different responses to long-term greenhouse cultivation. Therefore, it is necessary to elucidate the mechanisms of rare and abundant microbial subcommunities under different greenhouse cultivation methods, which is crucial for better management practices and crop yields in agricultural ecosystems.

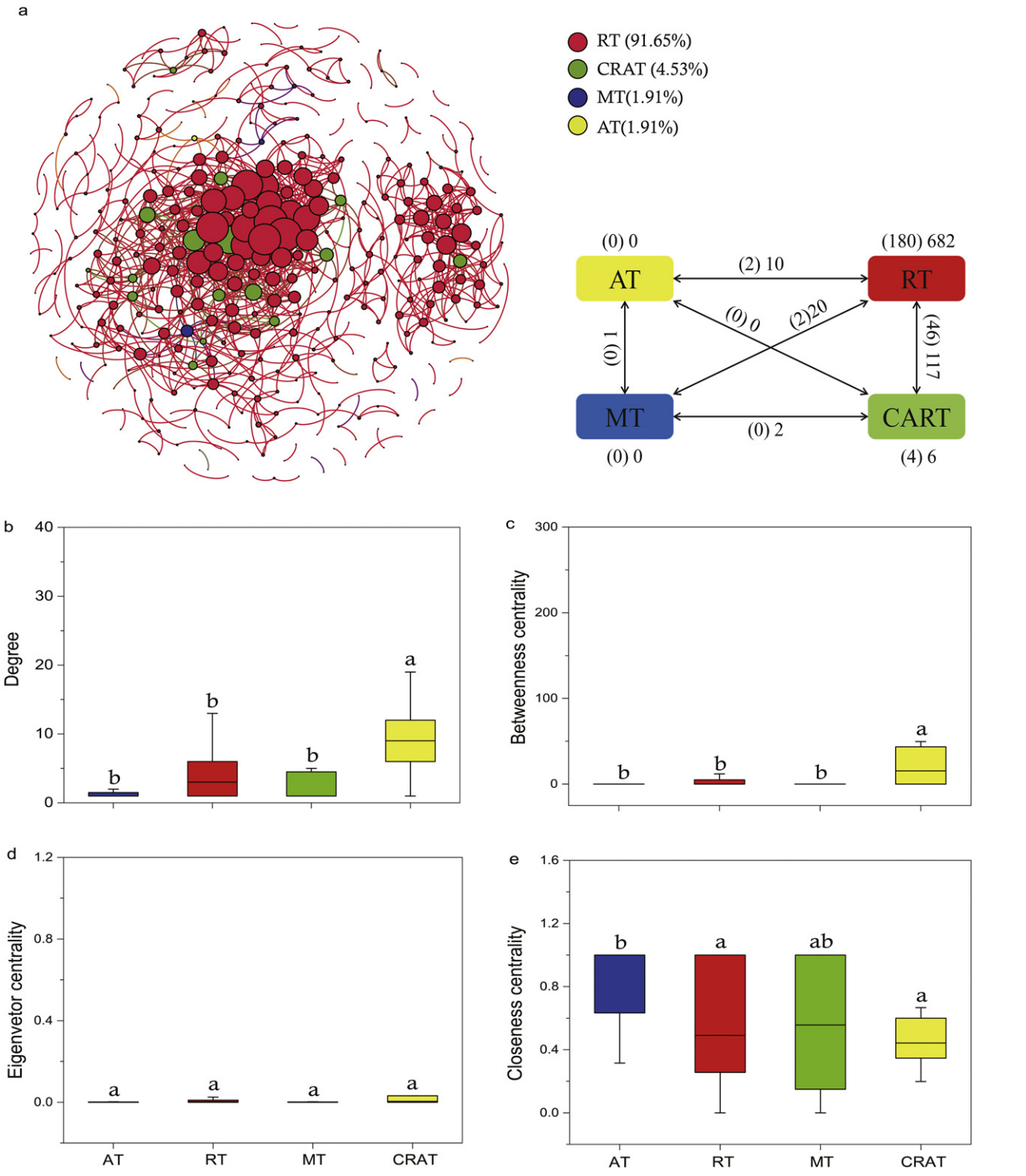

Fig. 6. Properties of microbial correlation-based network under long-term greenhouse cultivation in a subtropical agricultural farm. (a) Network analysis showing the intra-associations within each subcommunity and inter-associations between different subcommunities. A connection stands for a strong (Spearman's $r > 0.8$ or $r < -0.8$) and significant (P-value < .01) correlation. The size of each node is proportional to the number of connections (i.e., degree). Numbers outside and inside parentheses represent positive edge numbers and negative edge numbers, respectively. (b–e) Comparison of node-level topological features among four different subcommunities. The top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively, and lines within each box represent the median values. Different letters above bars indicate a significant difference at the $P < .05$ level. AT abundant taxa; RT rare taxa; MT moderate taxa; CRAT conditionally rare and abundant taxa.

4.1. Ecosystem functions under long-term greenhouse cultivation

Long-term greenhouse cultivation can cause an accumulation of salt in agricultural soils (Miao et al., 2011; Yao et al., 2006). Here, mid-term and long-term greenhouse cultivation had 9 times and 13 times higher saline concentrations than the ambient control, respectively (Table S1); these values are much higher than those in previous studies (Kim et al., 2016; Miao et al., 2011). Higher soil EC under long-term greenhouse

Table 1
Variations of long-term greenhouse cultivation on microbial richness, ACE and the Chao1 index, and the Shannon–Wiener diversity in a subtropical agricultural farm.

	Richness	ACE	Chao1	Shannon–Wiener
All				
CK	1057.33 ± 83.72c	1267.50 ± 42.99c	1258.11 ± 46.32c	7.85 ± 0.83
MG	1522.33 ± 32.74a	1869.94 ± 41.22a	1877.56 ± 41.28a	9.14 ± 0.08
LG	1260.33 ± 33.34b	1596.06 ± 37.46b	1603.96 ± 33.93b	8.66 ± 0.10
AT				
CK	NA	NA	NA	3.41 ± 0.02a
MG	NA	NA	NA	2.91 ± 0.06b
LG	NA	NA	NA	3.36 ± 0.02a
RT				
CK	1011.33 ± 84.20c	1218.36 ± 45.03c	1207.74 ± 47.67c	9.12 ± 0.16b
MG	1471.00 ± 32.97a	1816.05 ± 40.99a	1823.21 ± 43.06a	9.64 ± 0.05a
LG	1210.33 ± 34.42b	1544.18 ± 39.33b	1551.41 ± 35.80b	9.21 ± 0.05b

Values are means ± standard error. Different lowercase letters in the same row indicate significant differences at $P < .05$ among the treatments.
Note that abundant OTUs were persistent across all samples, and the means and standard error values of richness, ACE and Chao 1 indices cannot be calculated.
All, total soil bacteria; Abundant, abundant soil bacteria; Rare, rare soil bacteria; CK, control; MG, mid-term greenhouse cultivation; LG, long-term greenhouse cultivation.

cultivation supported the abovementioned changes in soil salinity (Table S1).

Many studies have demonstrated that soil salinity and pH can shape differences in soil microbial communities, thus influencing ecosystem functions (Fierer et al., 2007; Kim et al., 2016; Shen et al., 2016; Yao et al., 2006). In this study, long-term greenhouse cultivation produced clear differences in soil bacterial communities from the other treatments (Fig. 5). To our surprise, significantly differences in bacterial diversity were not observed across the treatments (Table 1), indicating that bacterial diversity was, to some extent, not sensitive to salinity in this agricultural soil.

In general, it is difficult to detect significant changes in soil C and N contents after management practices. However, in this study, long-term greenhouse cultivation had significantly higher soil C and N contents. This is attributed to higher salinity under long-term greenhouse cultivation, which inhibited soil microbial activities, such as CO_2 emission potential (Fig. 2a), and thus increased the accumulation of soil C and N contents. Soil EOC and EON contents act as active fractions of soil C and N contents and are more sensitive to management practices (Zhou et al., 2013). Our results showed higher soil EOC and EON contents under long-term greenhouse cultivation, which was consistent with previous findings and exhibited similar patterns to soil C and N contents (Fig. 1).

It is known that soil CH_4 oxidation and N_2O emissions are mediated by specific microorganisms (Singh et al., 2010; Tate, 2015). The former is mediated by soil methane-oxidizing bacteria (Kolb, 2009), which are aerobic microorganisms belonging to the methanotrophs. In general, soil CH_4 oxidation is sensitive to soil moisture contents, but not other environmental changes (Tate, 2015; Zhou et al., 2014). However, long-term greenhouse cultivation significantly decreased soil CH_4 oxidation potentials, which was supported by lower *pmoA* numbers, a key CH_4 oxidation gene involved this process (Fig. 3 and Table S4). On the other hand, N_2O emission are mediated by soil nitrifiers and denitrifiers; in general, N_2O emissions are mainly a result of denitrification in agricultural soil (Wang et al., 2018). A previous study showed that the abundance of the *nirS*/*nirK* minus *nosZ* gene can act as a good indicator of N_2O emissions (Morales et al., 2010). In this study, the *nirS*/*nirK* minus *nosZ* gene abundance was calculated across the treatments. Long-term greenhouse cultivation had 1.62 times higher abundance than these gene abundances than the ambient control (Fig. 4 and Table S5), which might support higher N_2O emission potential under long-term greenhouse cultivation (Fig. 2c).

Table 2
Spearman's correlations of soil microbial community and physiochemical factors, based on Mantel tests.

Soil properties	All	Abundant	Rare
Soil moisture	**0.634****	0.002	**0.771*****
pH	**0.686*****	0.042	**0.776*****
TC (g kg^{-1})	**0.792*****	0.226	**0.873*****
TN (g kg^{-1})	**0.637****	**0.467***	**0.784*****
EOC (mg kg^{-1})	**0.728*****	0.299	**0.878*****
EON (mg kg^{-1})	**0.646****	0.236	**0.804*****
NH_4^+–N (mg kg^{-1})	0.198	−0.011	**0.292***
NO_3^-–N (mg kg^{-1})	0.320	**0.340***	**0.449***
EC (μs cm^{-1})	**0.751*****	0.268	**0.878*****
Salinity (mg g^{-1})	**0.744*****	0.231	**0.864*****

TC, total C; TN, total N; EOC, extractable organic C; EON, extractable organic N; EC, electrical conductivity.
All, total soil bacteria communities; Abundant, abundant soil bacteria subcommunities; Rare, rare soil bacteria subcommunities.
The significances are tested on the basis of 999 permutations.
Bold text indicates statistical significance.
* $P < .05$ significance level.
** $P < .01$ significance level.
*** $P < .001$ significance level.

Table 3
Spearman's correlations between soil microbial community with greenhouse gas, emissions based on Mantel tests.

	All	Abundant	Rare
CH_4 oxidation potentials (μg kg^{-1} dry soil h^{-1})	−0.113	0.001	0.042
CO_2 emissions (mg kg^{-1} dry soil h^{-1})	**0.417***	**0.611****	**0.299***
N_2O emissions (μg kg^{-1} dry soil h^{-1})	**0.357***	0.006	**0.532****

All, total soil bacteria communities; Abundant, abundant soil bacteria subcommunities; Rare, rare soil bacteria subcommunities.
The significances are tested on the basis of 999 permutations.
Bold text indicates statistical significance.
* $P < .05$ significance level.
** $P < .01$ significance level.

4.2. Mechanisms of microbial subcommunities under long-term greenhouse cultivations

Ecosystem functions are controlled by soil biodiversity and microbial communities (Yao et al., 2006; Larkin, 2015). In this study, rare microbial taxa were more sensitive to long-term greenhouse cultivation than abundant microbial taxa (Table 1). The reason for this might be differences in ecological strategies between them (Jousset et al., 2017; Lynch and Neufeld, 2015). In general, abundant microbial taxa can competitively utilize a broad array of resources, whereas rare microbial taxa have less competitive ability and are restricted to few samples (Jousset et al., 2017; Logares et al., 2015). This suggests that long-term greenhouse cultivation can act as a kind of environmental filtering, which can have a strong impact on the composition of soil microbial communities. Our results showed that long-term cultivation did not affect total microbial diversity (Table 1) but it markedly altered microbial community structures (Fig. 5a), which supported the previous statement.

Network analyses can potentially provide deeper information on the interactions of microbial subcommunities than simple diversity indices and community structure descriptions (Ziegler et al., 2018). To our knowledge, this is the first time correlation-based network analysis has been applied to explore the co-occurrence patterns of rare and abundant microbial subcommunities after long-term greenhouse cultivation. The topology of the network can reflect interactions among

microorganisms (Hu et al., 2017). For example, the degree value describes the level of connectedness between OTUs, and the betweenness centrality value provides information on how critical an OTU is to the connectedness of a network (Ma et al., 2016). Our results showed that rare microbial taxa (i.e., rare OTUs) had higher degree values than abundant microbial taxa (5.0 vs 1.6, respectively), although there were no other differences between them (Fig. 6b). Previous studies have shown that keystone nodes commonly occur initially as components in networks with higher degree values (Berry and Widder, 2014). In this study, the majority of keystone species (87%) in the co-occurrence network were affiliated to rare taxa (Table S3). This suggests that rare species play an irreplaceable role in maintaining ecosystem functions.

In general, positive interactions in the network can be considered as indicators of cooperation (Ma et al., 2016). Rare microbial taxa showed more positive interactions with non-rare microbial taxa than negative ones in this study. Interactions between microorganisms can support ecosystem function (Lynch and Neufeld, 2015; Ziegler et al., 2018). A previous study demonstrated that less abundant taxa can act as important keystone species in the rhizosphere networks (Shi et al., 2016). The disappearance of these key species may cause the microbial network to break apart, so rare microbial taxa can be as important as abundant microbial taxa in maintaining ecosystem functions (Shi et al., 2016). Our results showed that rare microbial taxa play an important role in maintaining ecosystem functions, which might be further useful for better management practices and improving crop yields in agricultural ecosystems.

5. Conclusion

Compared with the ambient control, long-term greenhouse cultivation caused increase in soil salinity and pH, which then inhibited soil microbial activity and increased soil TC and TN contents, EOC and EON contents, and N_2O emission potential, but decreased CH_4 oxidation potential. Changes in CH_4 oxidation and N_2O emission potential showed similar patterns to key CH_4 oxidation genes and key denitrifier genes, respectively, according to our metagenomic analysis under long-term greenhouse cultivation. In addition, there were no marked differences in soil microbial diversity across the treatments, but long-term greenhouse cultivation clearly separated soil microbial communities. Rare rather than abundant microbial taxa could adequately explain changes in the majority of ecosystem functions. To our knowledge, this was the first time that the role of microbial subcommunities has been quantified through microbial co-occurrence network analysis in response to greenhouse cultivations. Overall, our results indicate that long-term greenhouse cultivation influences ecosystem functions via rare rather than abundant microbial taxa in soils, which might be useful for better management practices and improving crop yields in agricultural ecosystems.

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Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.136004.

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