


Dynamic microbial assembly processes correspond to soil fertility in sustainable paddy agroecosystems

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Abstract

1. Microbial community (diversity and functional traits) underpin the sustainability of agroecosystems. Assembly processes influence microbial community composition and in turn determine their functional traits. However we have a poor understanding of how assembly processes that govern microbial community interact through time to maintain soil fertility.
2. Flooded rice fields are one kind of agroecosystems with high level of sustainability. Ancient rice paddies have a history of cultivation spanning hundreds or thousands of years and thus provide an ideal opportunity to explore relationships between community assembly processes and agroecosystem sustainability. In this study, we used statistical null models to evaluate temporal patterns of bacterial community assembly processes along rice cultivation years and their associations with increases in soil fertility in paddy agroecosystems.
3. We found that stochasticity was a persistent force structuring paddy soil microbial communities, putatively due to frequent flooding that facilitates dispersal. However, determinism gradually increased over centuries of agriculture and corresponded to increases in soil fertility. Accordingly, multifunctionality, calculated using enzymes catalysing key soil functions in carbon, nitrogen, phosphorous and sulphur cycling significantly increased along rice cultivation years. We therefore propose that balanced assembly processes preserve a diverse array of traits that can dominate under various circumstances while also allowing for particular traits to be selected for by soil fertility, thus supporting multifunctionality that underpins agroecosystem sustainability.
4. In total, our results highlight that knowledge on temporal microbial assembly processes, community composition and ecological function in the context of soil fertility is key to understanding ecological mechanisms maintaining agroecosystem sustainability.

KEYWORDS

agroecosystem sustainability, assembly processes, determinism, microbial community, paddy soils, soil fertility, stochasticity

1 | INTRODUCTION

Micro-organisms play critical roles in soil fertility by facilitating organic matter decomposition, nutrient cycling, contaminant remediation and other processes that have direct implications for agroecosystem sustainability (Graham, Knelman, et al., 2016; Hall et al., 2018; van der Heijden, Bardgett, & van Straalen, 2008). We define agroecosystem sustainability here as the ability to meet society's current needs without compromising the needs of future generations (Liu et al., 2015; Tilman, Balzer, Hill, & Befort, 2011). Recent work has revealed that a dynamic balance of microbial community assembly processes facilitates ecosystem development in natural environments (Dini-Andreote et al., 2014; Ferrenberg et al., 2013). Yet, we lack knowledge on changes in microbial communities in agroecosystems over centennial time-scales and their relationship to soil fertility. Such knowledge is vital to sustainably maintaining agricultural yields for future generations.

Assembly processes govern changes in soil microbial community structure during ecosystem development and ecological succession, which in turn influence ecological function and ecosystem sustainability (Knelman & Nemergut, 2014). A vast body of investigations has explored the temporal dynamics of microbial communities along soil pedogenesis and development, including salt marshes (Dini-Andreote et al., 2014) and glacial landscapes (Castle, Lekberg, Affleck, Cleveland, & de Vries, 2016). Many of these works have used a community assembly framework developed by Vellend (2010) and modified by Stegen et al. (2013) to disentangle the ecological processes (variable and homogeneous selection, homogenizing dispersal, dispersal limitation and ecological drift) driving microbial community composition turnover through time (Dini-Andreote, Stegen, van Elsas, & Salles, 2015; Graham, Crump, et al., 2016; Yan et al., 2017). Variable selection causes divergence in community composition due to heterogeneous selective environments, whereas homogeneous selection leads to community convergence due to a consistent selective environment. Homogenizing dispersal leads to compositional similarity due to high rates of dispersal, whereas dispersal limitation results in dissimilarity in community composition due to a low rate of dispersal enabling ecological drift to occur (Stegen, Lin, Fredrickson, & Konopka, 2015). The balance of deterministic (e.g. variable and homogeneous selection) and/or stochastic processes (e.g. homogenizing dispersal, dispersal limitation and ecological drift) governs temporal community assembly (Graham, Crump, et al., 2016; Nemergut et al., 2013; Zhou & Ning, 2017). Deterministic processes are the dominant factor governing microbial compositional turnover along temporal gradients (Tripathi et al., 2018; Vanwonderghem et al., 2014); however, stochastic processes have been identified recently as another important driver for microbial community composition (Chase, 2010; Zhou & Ning, 2017) and can foster high rates of ecosystem functioning by maintaining biodiversity (Jangid, Whitman, Condon, Turner, & Williams, 2013).

Flooded rice paddies are widely considered among the most sustainable agroecosystems, partially because they harbour large amounts of soil organic carbon (SOC) from exogenous inputs and

low anoxic decomposition rates that facilitate the accumulation of SOC (Zhang & Gong, 2003; Zhu et al., 2016). Soil fertility is the basis of agroecosystem sustainability as it maintains crop productivity and minimizes the need for synthetic fertilizer application (Doran & Zeiss, 2000; Rinot, Levy, Steinberger, Svoray, & Eshel, 2019; Wall, Nielsen, & Six, 2015). SOC is an integrated proxy of environment conditions associated with soil fertility that plays an important role in agroecosystem sustainability by influencing soil nutrient cycles, microbial characteristics and physical structure (i.e. aggregate formation; Lehmann & Kleber, 2015; Schmidt et al., 2011). Thus, high SOC content generally denotes high fertility and more sustainable soils (Baldivieso-Freitas et al., 2018; Cheng, Yang, Cao, Ci, & Yin, 2009; Lehndorff, Roth, Cao, & Amelung, 2014). Because of the vast amount of SOC stored in rice paddies and their importance in global crop production, microbial community composition in paddy soils has been extensively investigated (Ding, Su, Li, Zhu, & Cao, 2017; Zou, Fu, Cao, Ye, & Yu, 2015). However, we need a process-based understanding of how assembly governs microbial community composition turnover to predict and maintain agroecosystem sustainability for future generations.

The directionality and strength of shifts in assembly processes over long time-scales are poorly understood in most ecosystems (Dini-Andreote et al., 2015; Graham et al., 2017; Graham, Knelman, et al., 2016). Environmental (or niche) variations are widely recognized as a driver of ecological assembly processes, such that environmental changes through time induce fluctuations in the strength of stochastic versus deterministic assembly processes (Ferrenberg et al., 2013). Tripathi et al. (2018) and Stegen, Lin, Konopka, and Fredrickson (2012) posited that deterministic processes occur under drastic and/or sudden variation in environment conditions, whereas stochastic assembly is dominant when there are mild and/or gradual changes. Additionally, time itself can act as a driver of community assembly, for example, through the aggregation of dispersal events or selective pressures that cumulatively alter the strength of stochastic and deterministic processes (Brown & Jumpponen, 2015; Dini-Andreote et al., 2015). As stochastic or deterministic events accumulate through time, microbial communities may move towards a more stochastic or deterministic assemblage, reflecting the notion that the balance of stochastic and deterministic processes is time dependent (Tripathi et al., 2018; Zhang, Johnston, Liu, Li, & Han, 2016).

Here, we investigate assembly processes governing bacterial community composition through successional chronosequences in paddy agroecosystems (100- and 2,000-year rice cultivations) as well as their relationships with soil fertility. In spite of being influenced by repeated perturbation due to anthropogenic agricultural practices (Feng et al., 2017), paddy soils are characterized by consistent flooding that tempers environmental change and increases hydrologic connectivity. Thus, our first hypothesis is that stochastic processes dominate the assembly patterns of paddy agroecosystems across rice cultivation years. Furthermore, rice cultivation increases SOC (Wissing et al., 2011), and aggregation caused by increased SOC can hamper the hydrologic dispersal. These aggregates form compartmentalized

systems in which dissimilar physiochemical niches can persist (Trivedi et al., 2015). Therefore, we also hypothesize that deterministic processes, and variable selection in particular, increase with the duration of rice cultivation. We propose that this balance of assembly processes sustains a diverse array of traits while also allowing for particular traits to be selected for by soil fertility. Thus, a pattern of overarching stochasticity with increasing determinism may support multifunctionality that underlies the agroecosystem sustainability.

2 | MATERIALS AND METHODS

2.1 | Dataset description

We compiled two datasets from paddy agroecosystems: one paddy chronosequence in Yingtan (YT) with a cultivation history of 100 years (Li et al., 2017) and another in Cixi (CX) with a cultivation history of 2,000 years (Ding et al., 2017). Both of these ancient rice paddies provided an ideal platform to study the temporal dynamics of agroecosystem sustainability due to their well-characterized chronosequential records of soil physicochemistry and microbial information along hundreds to thousands of years of rice cultivations. In addition, the uploaded raw sequences and metadata were suitable for the downstream assembly processes analyses. Detailed information on each experiment site is shown in Table 1.

Briefly here, YT is located in Jiangxi Province, China (28°15'N, 116°55'E). This region is a typical subtropical monsoon climate with a mean annual precipitation of 1795 mm and mean annual temperature of about 17.6°C. Soils are derived from Quaternary red clay. The sample plots were several hundreds of metres apart and were used to cultivate double-cropping rice over one century. Ages of the 15-, 30- and 100-year soils were assessed with published literature, and ages of the 5-year soil were identified based on the historical information with much help from experienced soil

experts (Li, Zhang, Han, & Felix-Henningsen, 2005). Further information on YT is available in Li et al. (2017). CX is located in the south bank of Hangzhou Bay, Zhejiang Province, China (30°15'N, 121°20'E). This region has a north subtropical monsoon climate with a mean annual precipitation of 1,325 mm and mean annual temperature of about 16.3°C. The soils in this area are mainly originated from coastal sediments from the Yangtze River Delta. Sites with different durations of rice cultivation are located within an area of 40 km². The samples collected from 50-, 100-, 300-, 500-, 700-, 1,000- and 2,000-year duration of rice cultivation were assessed based on the record in the County Annals of the Zhejiang Province, as well as in Cixi Counties (<http://www.cixi.gov.cn>). Additionally, sediment samples (0 year) were collected from the tidal wetland which typically stands for the parent materials of agricultural land reclamation through sea dike building. More details about the study area such as geography, geochemistry and groundwater level are given in Cheng et al. (2009).

2.2 | Calculation of multifunctionality index

Several enzymes related to carbon, nitrogen, phosphorous and sulphur cycling were used to calculate microbial-driven soil multifunctionality. The activities of several extracellular enzymes were measured using standard assays detailed in Li et al. (2017) and Liu (2016). Based on the local site characteristics and known biogeochemical cycles, β -xylosidase, β -glucosidase, *N*-acetyl-glucosaminidase and acid phosphatase enzymes were chosen to represent processes enhancing soil fertility for YT (Li et al., 2017), and urease, arylsulfatase, catalase, dehydrogenase and phosphatase enzymes were chosen for CX (Liu, 2016). We calculated the multifunctionality index described by Maestre et al. (2012) within each experimental study as the average Z-score for all enzymes. This index measures all functions on a common scale of standard deviation units.

TABLE 1 Summary of the datasets included in this investigation

Dataset	Description	Rice cultivation years	Number of samples	Primer pair and targeted variable region	Sequence platform	Rarefied sequence depth	Enzymes involved in multifunctionality index	Data source
YT	Ecological Experimental Station of Red Soil, Jiangxi Province, CHN	5, 15, 25, 30 and 100 years	15	519F/907R (V4-V5)	Illumina Miseq	45,198	β -xylosidase, β -glucosidase, <i>N</i> -acetyl-glucosaminidase and acid phosphatase	Li et al. (2017)
CX	South bank of Hangzhou Bay, Cixi city, Zhejiang Province, CHN	0, 50, 100, 300, 500, 700, 1,000 and 2,000 years	24	27F/534R (V1-V3)	454-Pyrosequencing	4,039	Urease, arylsulfatase, catalase, dehydrogenase and phosphatase	Ding et al. (2017) and Liu (2016)

Abbreviations: CX, Cixi; YT, Yingtan.

2.3 | Processing of the high-throughput sequencing data

Raw sequences and metadata were directly downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>). Although two different sequencing platforms (Illumina Miseq vs. 454-pyrosequencing) were used in respective studies (Table 1), congruence in main results from the two independent studies (e.g. temporal bacterial community successions along rice cultivations) was observed, which supported that the difference in sequencing platform had minimal influence on our results. A similar approach was taken by Graham, Knelman, et al. (2016). The downloaded bacterial 16S rRNA gene sequences for each dataset were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010). Briefly, sequences with a quality score below 20 and the length fewer than 200 bp were trimmed and then assigned to soil samples based on unique 5-bp barcodes. After denoising and chimera filtering with the 'usearch' pipeline, the quality reads were then binned into operational taxonomic units (OTUs) using a 97% identity threshold, and the most abundant sequence from each OTU was selected as the representative sequence. Taxonomy was then assigned to bacterial OTUs with reference to a subset of the SILVA 119 database (<http://www.arb-silva.de/download/archive/qiime/>). A phylogenetic tree was then constructed using FastTree to support phylogenetic diversity calculations (Price, Dehal, & Arkin, 2009). Representative sequences were aligned with QIIME default PyNAST algorithm (Caporaso et al., 2010), and sequences that failed to align were removed. Details on sequences and OTUs per samples number and rarefaction depth at each site are displayed in Table 1.

2.4 | Calculation of community assembly processes

Prior to null model construction, we verified the phylogenetic conservatism of communities in each dataset by testing the correlation coefficient relating between-OTU phylogenetic distances to between-OTU niche distances (Mantel correlograms, 999 randomizations, 'mantel.correlog' in 'vegan' v2.0-2; <http://vegan.r-forge.r-project.org>; Figure S1; Diniz-Filho, Terribile, da Cruz, & Vieira, 2010; Stegen et al., 2013; Warren, Glor, & Turelli, 2008).

To evaluate community assembly processes at each site, we first calculated the standardized effect size measure of the mean nearest taxon distance (ses.MNTD) using the 'taxa.labels' function (999 randomization) in PICANTE R package (Kembel et al., 2010). Low values of ses.MNTD (<0) denote phylogenetic clustering (i.e. co-occurring OTUs are more closely related than expected by chance). In contrast, high values (>0) indicate phylogenetic over dispersion (i.e. co-occurring OTUs are less closely related than expected by chance; Webb, Ackerly, McPeck, & Donoghue, 2002).

We also quantified the turnover of phylogenetic composition between samples (phylogenetic β -diversity) using between-community

mean nearest taxon distance (β MNTD) and nearest taxon index (β NTI) as per Stegen et al. (2012, 2013, 2015). β MNTD was calculated using the R function 'comdistnt' (abundance.weighted = TRUE; package PICANTE). The difference between observed β MNTD and mean of the null distribution of β MNTD generated by 1,000 randomizations by its standard deviation is referred as β NTI. The values between -2 and +2 indicate the expectation under stochastic community assembly while the individual values below -2 or above +2 indicate that the observed difference in phylogenetic community composition is the result of deterministic selection (Stegen et al., 2013). We quantified these metrics for pairwise comparisons among each site's samples using any species present in a single sample as part of the regional species pool. This was done to specifically relate temporal dynamics of community composition to the soil pedogenesis and development.

In addition to β NTI, the Bray-Curtis-based Raup-Crick (RC_{bray}) null model was further used to quantify dispersal-based stochastic ecological processes generating the turnover of community composition (Stegen et al., 2015). RC_{bray} was based on a comparison between observed and expected levels of turnover, but without using phylogenetic information. The deviation between empirically observed Bray-Curtis and the null distribution was then standardized to vary between -1 and +1, and the resulting metric is referred to as RC_{bray} . Values of RC_{bray} below -0.95 or above +0.95 indicate significant deviations from the null model expectation.

The relative contributions of ecological processes governing community turnover that are determined by homogeneous and variable selection are denoted by β NTI < -2 and β NTI > +2 respectively. The relative contributions of dispersal limitation and homogenizing dispersal processes are estimated by $|\beta$ NTI| < 2 but RC_{bray} > +0.95 and RC_{bray} < -0.95 respectively. Furthermore, if $|\beta$ NTI| < 2 and $|RC_{bray}| < 0.95$, then undominated fraction drives compositional turnover process (Stegen et al., 2015).

2.5 | Identification of bacterial biomarkers for rice cultivation year

To decipher bacterial taxa driving community turnover across rice cultivation year and/or uniquely characterizing a successional stage (termed 'biomarker taxa', succession stage information is shown in Section 3.1), the relative abundance of bacterial taxa at the order level was regressed against rice cultivation years using random forests (RF; R package RANDOMFOREST, ntree = 1,000, using default mtry of p/3 where p is the number of taxa; Zhang, Tang, Zhu, Lin, & Feng, 2019). Lists of taxa ranked by RF in order of feature importance were determined over 100 iterations. Biomarker taxa were identified using 10-fold cross-validation, implemented using R 'rfcv' function. The top 15 bacterial biomarkers were chosen according to the stabilized cross-validation error curve. The majority of biomarker taxa showed high relative abundance in the corresponding successional stages.

2.6 | Statistical analysis

Nonmetric multidimensional scaling was performed to visualize the dissimilarities of bacterial composition along the chronosequences based on β MNTD distances. ANOVA was used to test the significance of variation in diversity indices among eras. PERMANOVA test was conducted to confirm the significance of dissimilarity. The above analyses were conducted using VEGAN package in R software (VEGAN package, Version 3.1.2). SPSS16.0 (Statistical Product and Service Solutions) calculated the correlation between ses.MNTD as well as assembly processes within each successional year with environment index. To unravel the drivers of community composition, a Mantel test with 999 permutations was conducted to explore the relation between β NTI and SOC. For all the statistical tests, significance and high significance were determined as $p < 0.05$ and $p < 0.01$ respectively.

3 | RESULTS

3.1 | Variation in soil fertility, microbial community composition and microbial multifunctionality across rice cultivation years

Soil organic carbon content increased along rice cultivation years from 10.74 g/kg (5 years) to 20.45 g/kg (100 years) at YT (Li et al., 2017) and from 16.96 g/kg (0 year) to 45.98 g/kg (2,000 years) at CX (Ding et al., 2017). SOC was positively correlated with time at YT ($r = 0.896$, $p < 0.001$) and CX ($r = 0.937$, $p < 0.001$), indicating SOC

accumulation with rice cultivation years. In addition, nutrient pools, such as total N (TN; from 1.10 to 2.65 g/kg for YT and from 0.85 to 4.11 g/kg for YZJ) and available P (AP; from 19.54 to 74.55 mg/kg and from 13.0 to 23.4 mg/kg) contents also increased with longer rice cultivation (Ding et al., 2017; Li et al., 2017).

Grouping of microbial communities based on dissimilarity (β MNTD) at YT (Figure S2a) and CX (Figure S2b) was consistent with groupings in Li et al. (2017) and Ding et al. (2017) respectively. Specifically, in the 100-year YT dataset, we grouped samples by rice cultivation years resulting in five stages: 5, 15, 25, 30 and 100 years, while in the 2,000-year CX dataset, samples were grouped into seven rice cultivation stages: 50, 100, 300, 500, 700, 1,000 and 2,000 years. Each group had triplicate samples. All groupings were confirmed by global PERMANOVA (all $p < 0.001$).

Multifunctionality for soil fertility significantly increased with rice cultivation years ($p < 0.05$). In particular, the highest multifunctionality index was found at 100 years at YT (Figure 1A) and 2,000 years at CX (Figure 1B) respectively.

3.2 | Phylogenetic structuring in each rice cultivation stage

When investigating assembly within each sample, we observed determinism that increased with soil fertility and rice cultivation years. The ses.MNTD values were significantly less than zero in all datasets ($p < 0.05$; Figure 2), indicating that in each paddy agroecosystem most microbial communities were more phylogenetically clustered than expected by chance. Significant negative correlations were observed

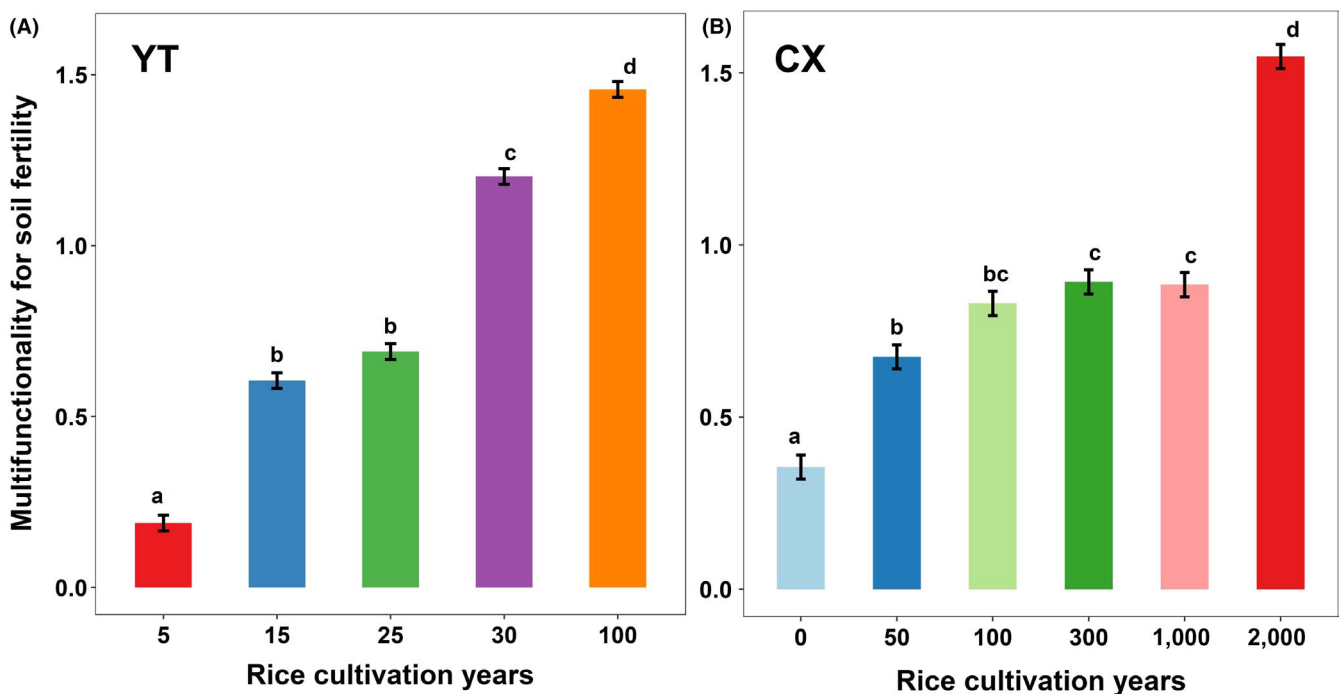


FIGURE 1 The microbial-driven multifunctionality for soil fertility along rice cultivation years for Yingtian (YT; A) and Cixi (CX; B) respectively. The multifunctionality index was calculated using several enzymes after Z-score transformation

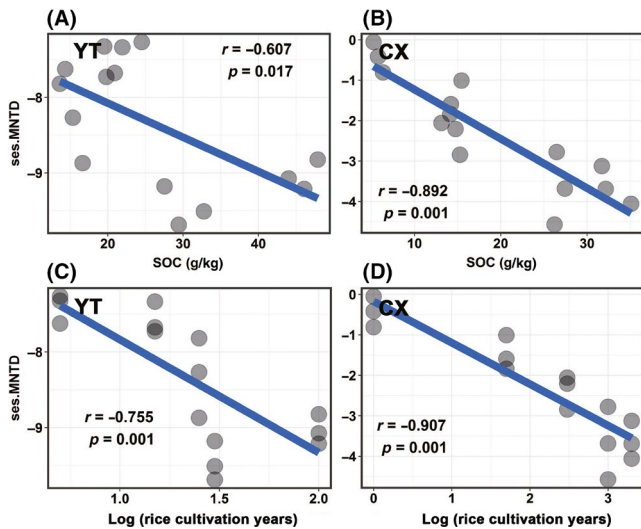


FIGURE 2 Correlations between standardized effect size measure of the mean nearest taxon distance (ses.MNTD) and soil organic carbon (SOC; A and B) as well as rice cultivation years (C and D) along Yingtan (YT) and Cixi (CX) rice cultivation histories

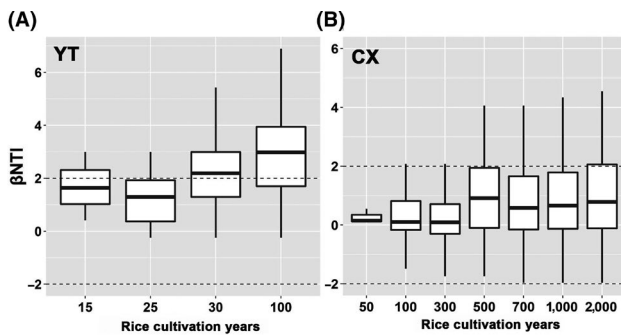


FIGURE 3 Patterns of between-community nearest taxon index (β NTI) along rice cultivation years for Yingtan (YT; A) and Cixi (CX; B). Horizontal dashed lines indicate upper and lower significance thresholds at β NTI = +2 and -2 respectively

between ses.MNTD and SOC (Figure 2A,B) as well as between ses.MNTD and rice cultivation years (Figure 2C,D) for YT and CX, indicating that longer rice cultivation, and concomitantly SOC accumulation, increased the phylogenetic clustering of soil micro-organisms.

3.3 | The balance of deterministic and stochastic assembly processes shifts with rice cultivation years and soil fertility

We calculated β NTI values across samples within each successional stage to quantify the relative contributions of stochasticity versus determinism to community structure. At both YT and CX, most β NTI values were between -2 and +2, indicating a large influence of stochasticity across rice cultivation years (38% to 73% in YT, 66% to 93% in CX; Figure 3). As rice cultivation years increased, β NTI values either became larger than +2 (YT; Figure 3A) or positively deviated from 0 (CX; Figure 3B), indicating a shift in assembly processes towards determinism. When combining the outcomes of β NTI and RC_{bray} null models, we disentangled four ecological processes governing temporal successions of bacterial community (Table 2). At CX, dispersal limitation (from 53% to 64%) largely contributed to the stochasticity. Similarly, the contribution of dispersal limitation to stochasticity (more than 27%) was higher than homogenizing dispersal in all successional stages at YT, except at 15 years. In addition, for deterministic processes, variable selection was exclusively observed without any homogeneous selection, regardless of sites and rice cultivation years (Table 2).

To understand the association of determinism versus stochasticity with soil fertility, we first performed Mantel tests of β NTI with measured soil variables and rice cultivation years for YT and CX (Table 3). We consistently found significant relationships between β NTI and SOC, which is further confirmed by linear regressions (Figure S3). We observed a significant ($p = 0.024$; YT) and a marginally significant correlation ($p = 0.082$; CX) relationships between

TABLE 2 The relative contributions of ecological assembly processes across rice cultivation years for two paddy chronosequences

	Rice cultivation years	Variable selection	Homogeneous selection	Deterministic	Dispersal limitation	Homogenizing dispersal	Stochastic	Undominated process
YT	15	0.13	0	0.13	0	0.73	0.73	0
	25	0.17	0	0.17	0.39	0.33	0.72	0.06
	30	0.47	0	0.47	0.29	0.18	0.47	0.03
	100	0.58	0	0.58	0.27	0.11	0.38	0.02
CX	50	0.07	0	0.07	0.53	0.40	0.93	0
	100	0.11	0	0.11	0.64	0.25	0.89	0
	300	0.06	0	0.06	0.62	0.18	0.80	0.14
	500	0.20	0	0.20	0.57	0.14	0.71	0.09
	700	0.16	0	0.16	0.60	0.12	0.72	0.12
	1,000	0.20	0	0.20	0.58	0.10	0.68	0.12
	2,000	0.24	0	0.24	0.58	0.08	0.66	0.10

Abbreviations: CX, Cixi; YT, Yingtan.

β NTI and rice cultivation years (Table 3), suggesting that the SOC accumulation due to long rice cultivation history governs general ecological assembly processes.

Then, relationships between the contributions of individual assembly process and rice cultivation years were further calculated for YT and CX (Table 4). At CX, deterministic processes increased with successional year ($r = 0.836$, $p = 0.019$), largely due to variable

TABLE 3 Mantel tests between β NTI and soil chemical properties as well as rice cultivation years for Yingtan (YT) and Cixi (CX) agroecosystems

Mantel test	YT	CX
SOC	0.314 (0.025)	0.214 (0.046)
TN	0.366 (0.008)	0.156 (0.099)
TP	0.209 (0.037)	0.159 (0.116)
TK	-0.104 (0.761)	NA ^a
AN	0.348 (0.022)	NA
AP	0.194 (0.046)	0.084 (0.264)
AK	-0.013 (0.529)	0.051 (0.318)
pH	-0.088 (0.703)	0.103 (0.225)
Rice cultivation years	0.344 (0.024)	0.160 (0.082)

Note: The data outside and inside the parenthesis are r and p values respectively.

^aTK and AN data in CX are not available.

TABLE 4 The linear relationships between relative contribution of each ecological assembly process and rice cultivation years for Yingtan (YT) and Cixi (CX)

	Variable selection	Homogeneous selection	Deterministic	Dispersal limitation	Homogenizing dispersal	Stochastic	Undominated process
YT	r 0.873	NA	0.873	0.438	-0.815	-0.864	0.068
	p 0.127		0.127	0.562	0.185	0.136	0.932
CX	r 0.836	NA	0.836	0.079	-0.955	-0.982	0.786
	p 0.019		0.019	0.866	0.001	0.000	0.036

selection ($r = 0.836$, $p = 0.019$). Significant decreases in stochastic processes ($r = -0.982$, $p = 0.000$) and homogenizing dispersal in particular ($r = -0.955$, $p = 0.001$) were also observed with rice cultivation years at CX. There were no significant correlations between assembly processes and successional year at YT, possibly due to low sample size ($n = 4$ [15, 25, 30 and 100 years], relative the $n = 7$ [50, 100, 300, 500, 700, 1,000 and 2,000 years] at CX). However, the overarching patterns of ecological processes at YT were consistent with CX (Table 2).

3.4 | Bacterial biomarkers in each successional stage

To identify biomarkers discriminating bacterial communities along rice cultivation year, we used RF machine learning to determine bacterial taxa (order level) representative of each successional stage. The top 15 most important families were chosen as the respective biomarker taxa for YT (Figure 4A) and CX (Figure 4B) respectively. Biomarker species were largely classified into several orders of Thermomicrobia, Betaproteobacteria, Gammaproteobacteria, Legionellales, Desulfuromonadales and Nitrosomonadales, Acidobacteria-Gp5, -GP11, -GP1, -GP7 and -GP22, Gaiellales, Cytophagales, Candidatus Saccharibacteria, Firmicutes and Nitrospirae. Heatmaps in Figure 4 further presented the relative abundances of those biomarker species identified by RF in five stages. It was found that phyla Proteobacteria (i.e. Caulobacterales order in CX) in particular played more important roles for paddy soils in later successional stages at both sites.

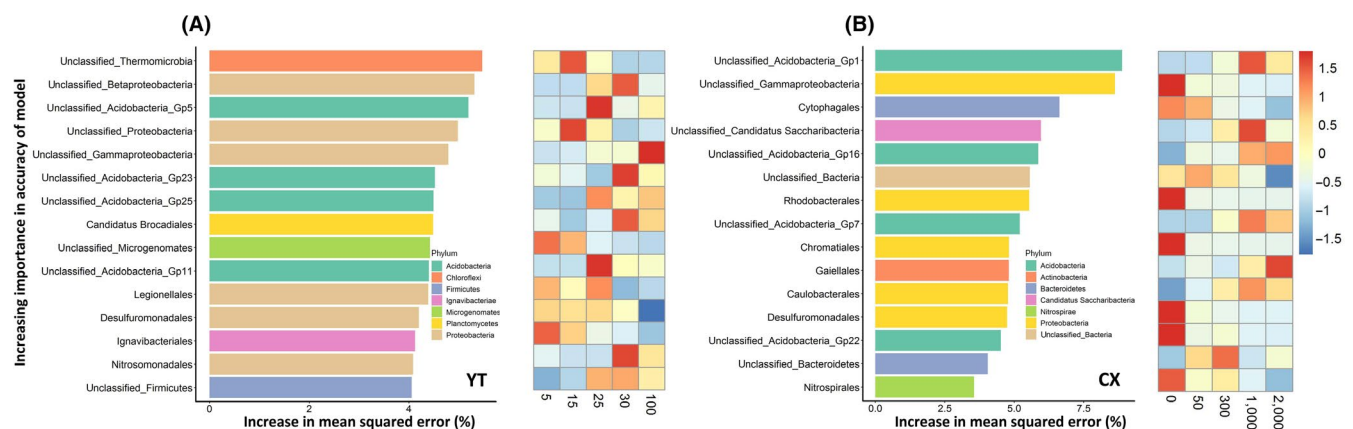


FIGURE 4 Bacterial taxonomic biomarkers within rice cultivation years. Each top 15 biomarker taxa were ranked in descending order of the importance to the accuracy of the models in Yingtan (YT; A) and Cixi (CX; B) respectively. Heatmap showed the variations in the relative abundances of these top 15 predictive biomarker taxa among rice cultivation years

4 | DISCUSSION

4.1 | Overarching stochasticity underlies paddy agroecosystem sustainability

Deterministic and stochastic assembly processes occur simultaneously along successional chronosequences to structure microbial communities (Chase & Myers, 2011; Ofiteru et al., 2010), but the balance of assembly processes through time and its influence on agroecosystem sustainability is poorly understood. Previous investigations found a predominate role for determinism in upland agroecosystems that was associated with intensive anthropogenic activities, such as wildfires, repeated fertilization, plowing and tillage (Knelman, Schmidt, Garayburu-Caruso, Kumar, & Graham, 2019; Prach & Walker, 2011). These repeated external disturbances are thought to select for specific species capable of tolerating such perturbations (Fan et al., 2017; Feng, Adams, et al., 2018; Feng et al., 2017; Ferrenberg et al., 2013; Sengupta et al., 2019). In contrast to upland ecosystems, we found that turnover of microbial communities in paddy agroecosystems was primarily dominated by stochasticity (Figure 3; Table 2).

We propose that specific habitat characteristics of rice paddies have dramatic impacts on community assembly. For example, hydrologic connectivity is a major factor in structuring most ecosystems, including both fluidic (e.g. groundwater, rivers, ocean and lakes) and low or non-fluidic ecosystems (e.g. soils and sediments; Langenheder & Lindstrom, 2019). Water-saturated conditions increase the ability for micro-organisms to migrate through pore space, which should therefore facilitate dispersal (Ebrahimi & Or, 2014). Additionally, flooding can temper extremes in environmental changes that typically impose deterministic assembly processes. Stegen et al. (2012) originally demonstrated that stochastic processes contribute proportionally more to community assembly when environmental stresses were low. Recent work of Tripathi et al. (2018) and Sengupta et al. (2019) has supported this assumption: stochasticity was dominant under mild environmental conditions, whereas determinism contributed a greater proportion of assembly processes under extreme conditions. Flooding can also promote soil fertility in rice paddies and lead to higher resource bioavailability that releases micro-organisms from environmental stresses (Chen, Zhang, & Effland, 2011; Feng, Chen, et al., 2018; Feng et al., 2017). Collectively, these ecosystem features are likely to enhance stochastic processes relative to determinism. Indeed, consistent outcomes have been found for various aquatic environments, such as groundwater (Zhou et al., 2014), bioreactor (Zhou et al., 2013) and even the blood of pikas (Li, Li, & Qu, 2018), highlighting the importance of stochasticity in assembly processes in aquatic environments.

In this respect, micro-organisms in stochastically assembled communities should contain more diversified traits than deterministically structured communities (Graham et al., 2017). Here, we identified diverse biomarker species (i.e. Acidobacteria, Actinobacteria, Bacteroidetes, Candidatus Saccharibacteria, Firmicutes and Proteobacteria) at both YT (Figure 4A) and CX (Figure 4B) that may support multifunctionality in microbial communities driven by stochastic processes.

4.2 | Determinism increases with rice cultivation years and soil fertility

Although stochasticity dominated ecological assembly processes in this study, the contribution of determinism to assembly, and variable selection in particular, increased linearly along rice cultivation years at CX, with a similar trend at YT (Tables 2 and 4). *ses.MNTD* values also indicated an increasing phylogenetic structuring with SOC content and successional year for both YT (Figure 2A) and CX (Figure 2B), showing increasingly non-random microbial communities with elevated SOC and longer rice cultivation years. Paddy agroecosystems become progressively compartmentalized with longer cultivation duration with different physiochemical conditions (e.g. oxic surface soil, anoxic bulk soil and rhizosphere; Liesack, Schnell, & Revsbech, 2000; Renny & Supriyanto, 2018). In particular, limitation of external electron acceptors could form different ecological niches, leading microbial functional groups to co-occur, such as nitrate-, iron- and sulphate-reducing species and methanogenic species, consistent with variable selection.

Therefore, microbial communities may also be increasingly structured by compartmentalized ecological niches as the duration of rice cultivation increases. SOC also tends to accumulate with progressive rice cultivation, and SOC content promotes macroaggregates formation and stability in paddy soils (Tang et al., 2018; Zhang, Sun, Liu, & Xue, 2018; Zou et al., 2015). The abundance and stability of soil aggregates are positively correlated with soil fertility because the cohesion of aggregates is promoted mainly by organic polymer binding agents such as mycelium and excreted proteins of micro-organisms (Zhang et al., 2018). Because of physical disconnection between aggregates, each aggregate may also represent a different ecological niche for microbial colonization (Trivedi et al., 2015). We therefore hypothesize that micro-organisms are largely contained in macroaggregates between which dispersal processes are constrained, and local environmental conditions promote variable selection.

Under deterministic selection, taxa succeed or fail in a given ecosystem based on how well their functional traits align with environmental conditions (Horner-Devine & Bohannan, 2006; Webb, 2000), which is speculated to maximize ecosystem functioning (Graham, Crump, et al., 2016; Jangid et al., 2013). We found that Proteobacteria, fast-growing *r*-strategists that thrive in copiotrophic environments (Fierer, Bradford, & Jackson, 2007), were an indicator taxon for late rice cultivation years with high soil fertility in both paddy soils (Figure 4). In related work, the Caulobacteriales order of Proteobacteria was the keystone order for degradation of exogenous organic matter and SOC accumulation in paddy soils (Zhan et al., 2018). In addition, species affiliated with Candidatus Saccharibacteria are ubiquitous in a range of anoxic environments, and play important roles in degradation of recalcitrant organic matter that favours SOC accumulation (Opdahl, Gonda, & St-Pierre, 2018; Takenaka, Aoi, Ozaki, Ohashi, & Kindaichi, 2018). Indeed, both Li et al. (2017) and Liu (2016) found that the activities of the enzymes involved into carbon cycling increased with longer rice

cultivation duration. Furthermore, we also found that the multifunctionality index for soil fertility, integrating several enzymes related to carbon, nitrogen, phosphorous and sulphur cycling, increased with higher SOC content ($r = 0.679$, $p < 0.05$ for YT and $r = 0.754$, $p < 0.05$ for CX) and with the longer rice cultivation years ($r = 0.982$, $p < 0.05$ for YT and $r = 0.917$, $p < 0.05$ for CX; Figure 1). Collectively, these enzymes represent the strength of microbial processes that drive soil fertility. We therefore suggest that SOC accumulation with increasing rice cultivation history filters for microbial taxa specializing in processes that bolster soil fertility in paddy soils. In other words, determinism selects for biogeochemical traits in microbial communities that support soil fertility as SOC accumulates with longer rice cultivation.

4.3 | Implications of assembly processes underlying agroecosystem sustainability

Understanding how specific environmental factors relate to assembly processes that drive microbial community composition and associated ecosystem function is critical to managing

agroecosystem sustainably. In this study, SOC was the best predictor of community assembly processes (Table 3 and Figure S3). SOC is commonly used as an integrated evaluation index for soil fertility (Lehmann & Kleber, 2015) and is intertwined with soil microbial community structure and function (Chen et al., 2014; Trivedi, Anderson, & Singh, 2013). For example, bacterial community assembly processes and composition shift in response to increases in SOC due to organic manure application in many agroecosystems (Feng et al., 2015, 2017). The importance of SOC on microbial assembly processes and agricultural sustainability had been observed in previous investigations of upland and paddy soils (Dini-Andreote et al., 2015; Feng, Chen, et al., 2018), and our work suggests that this relationship could be a general feature of all agroecosystems.

In addition, we show long-term trends in the balance of stochastic and deterministic assembly processes, and their relationships with soil fertility that are important and overlooked factors in maintaining agroecosystem sustainability. Our work culminates in a new conceptual model of the relationships between assembly processes, microbial community structure and agroecosystem sustainability as depicted in Figure 5. We propose that overarching

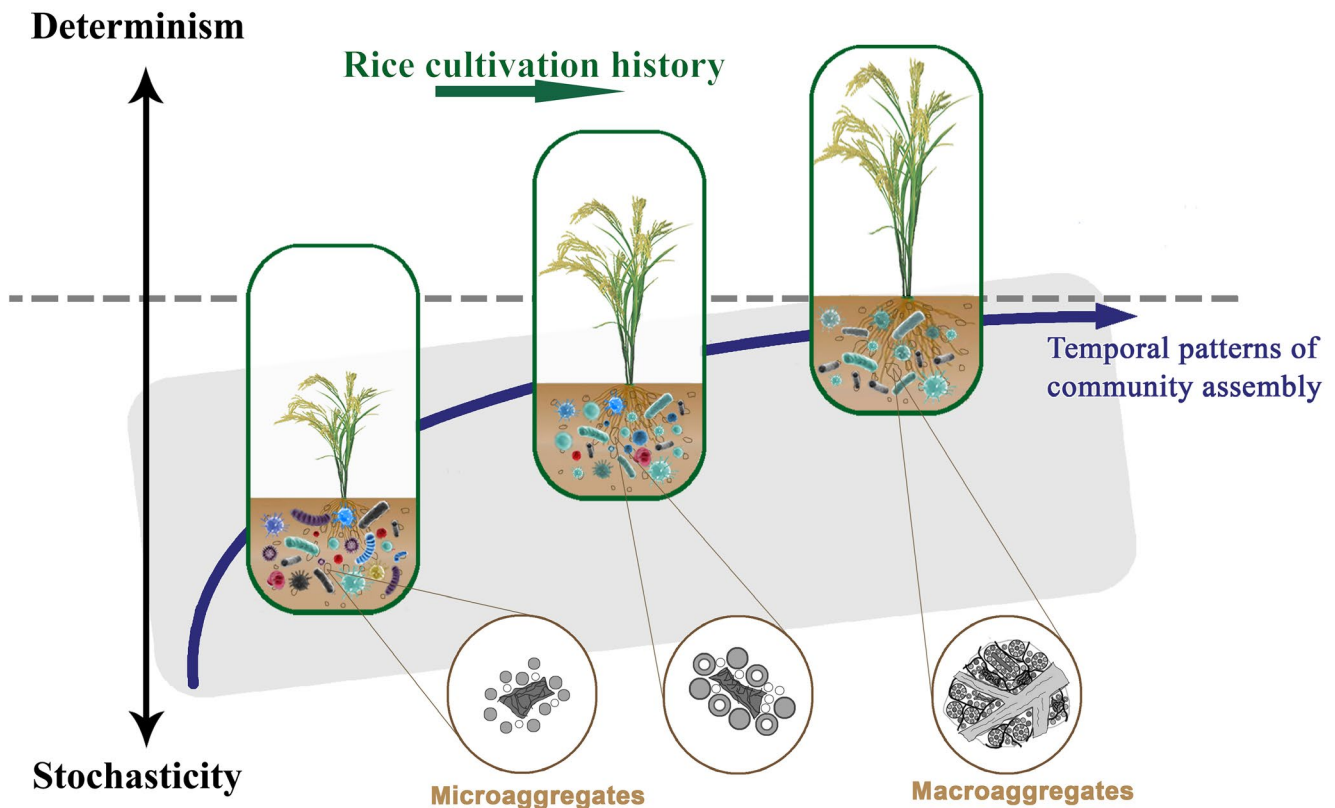


FIGURE 5 Conceptual model of assembly processes of microbial community shifted along rice cultivation years for paddy agroecosystems. In the conceptual model, stochasticity due to frequent flooding condition is an overarching force structuring paddy soil bacterial communities. Along the rice cultivation history, determinism gradually increases due to the changed soil physical and chemical properties under over centuries of agricultural practices and corresponds to increases in soil fertility. The balanced assembly processes preserve a diverse array of traits that can dominate under various circumstances (stochasticity) while allowing for particular traits to be selected for by soil fertility (determinism), thus supporting multifunctionality that underlies the sustainability of paddy agroecosystems

stochastic microbial assembly is supported by soil saturation and underlies paddy agroecosystem sustainability by maintaining microbial diversity and multifunctionality. With increasing rice cultivation duration, changes in soil physical and chemical properties promote soil fertility and increase determinism in microbial communities. In turn, increased determinism enhances the fitness of microbial clades well-adapted to high-SOC and -macroaggregates conditions that feedback into soil fertility. Because there is an upper limit to the number of macroaggregates and the SOC content that can exist in a given system, we hypothesize that increases in determinism asymptote to generate balanced assembly processes in sustainable rice paddy systems. Such a combination of ecological assembly processes generates a phylogenetically diverse and functionally efficient community that is self-sustaining, which in turn underpins the overall sustainability of paddy agroecosystems. In this work, soil microbes were generally highly diverse due to overarching stochasticity, and their ecological functions associated with soil fertility increased with the contribution of determinism to microbial assembly. Similar phenomena in which a dynamic balance of stochastic versus deterministic assembly processes generates sustainable ecosystem functions have been alluded to by other work (Stegen et al., 2016; Zhang, Deng, et al., 2019; Zhou et al., 2014); however, the centurial time-scale of our work allows for a deeper investigation of microbial assembly processes that enables predicting and managing sustainable agroecosystems in the coming centuries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Y.F., X.L. and Z.L. designed the experiment; W.J.L., J.Z., W.T.L. and Y.F. performed the experiments; W.J.L., E.B.G., J.Z., L.Z. and Y.F. analysed the data. All the authors contributed to data interpretation and writing of the manuscript.

DATA AVAILABILITY STATEMENT

The sequences were deposited in NCBI SRA database (accession no. SRP218212 and no. SRP068522).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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