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Two key features influencing community assembly processes at regional scale: Initial state and degree of change in environmental conditions

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Abstract

Belowground microbial communities strongly influence ecosystem function such that predicting function may rely on understanding ecological processes that assemble communities. Uncertainty remains, however, in what governs the relative contributions of different ecological processes. To help fill this knowledge gap, we test the general hypothesis that both initial state and degree of change in environmental conditions govern the relative contributions of different ecological assembly processes. To do so, we leveraged regional-scale nutrient and organic matter addition experiments and used soil organic matter (SOM) as a proxy of integrated soil environmental conditions. Consistent with our hypothesis, we found that both the initial amount of SOM and the degree of change in SOM—in response to nutrient addition—influenced the relative contributions of different ecological assembly processes. These influences were most clearly observed at the regional scale, suggesting potential scale dependence. More specifically, nutrient additions homogenized bacterial community composition due to enhanced influences of homogenizing dispersal when SOM content was initially high. In contrast, nutrient additions led to divergence in community composition due to variable selection when initial SOM was low and/or when SOM increased significantly in response to nutrient additions. Our findings indicate important connections among initial conditions, degree of change in environmental variables and microbial community assembly processes that may influence ecosystem processes. These conceptual inferences highlight a need to strengthen connections between ecological theory and biogeochemical modelling.

KEYWORDS
community assembly, deterministic, microbial ecology, null models, soil bacterial community, soil organic matter, stochastic

1 | INTRODUCTION

A central goal of microbial ecology is to understand the assembly rules of microbial communities and to predict compositional variation (Emerson & Gillespie, 2008; Hanson, Fuhrman, Horner-Devine, & Martiny, 2012). Research has overwhelmingly documented that there are changes in microbial community composition through space and time in all ecosystems, for example forests (Ferrenberg et al., 2013; Fierer & Jackson, 2006), grasslands (Barnard, Osborne,

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& Firestone, 2013), wetlands (Bodelier et al., 2013) and salt marshes (Dini-Andreote et al., 2014). Beyond observations of composition and diversity patterns, research has shifted to identifying underlying mechanisms. Ecological assembly processes shape biotic communities and are thought to be imperative in coupling microbial community composition with the ecosystem functions they provided (Ferrenberg et al., 2013; Graham & Stegen, 2017; Graham et al., 2016, 2017; Wang et al., 2013). For example, a shift towards a greater contribution of deterministic assembly processes has been associated with elevated microbial respiration (Graham & Stegen, 2017; Stegen et al., 2016a).

Related to the mechanisms of microbial community assembly, attempts have been made to partition the relative contributions of current environmental factors (or contemporary disturbances) and historical contingencies (Martiny et al., 2006) as well as stochastic versus deterministic processes (Chase & Myers, 2011; Zhou & Ning, 2017). Subsequently, a conceptual framework has been developed to disentangle the contributions of four ecological processes governing community assembly. They are homogeneous selection, variable selection, homogenizing dispersal, dispersal limitation (coupled with drift) and a condition for which no single process dominates community assembly (referred to as “undominated”; Figure 1; Stegen, Lin, Fredrickson, & Konopka, 2015; Vellend, 2010). Homogeneous selection leads community composition to be convergent due to a consistent selective environment, whereas variable selection causes divergence in community composition due to differences in selective environments. Homogenizing dispersal leads to compositional similarity due to high rates of dispersal between communities, whereas dispersal limitation results in dissimilarity in community composition due to a low rate of dispersal enabling ecological drift to occur. All ecological communities are governed by some combination of these assembly processes. Quantifying their relative contributions in a consistent manner across systems therefore provides an opportunity to understand factors that govern the processes themselves. In time, this will enable theoretical constructs that are general and transferable across systems.

Working towards general and transferable understanding is particularly important given that previous studies presented inconsistent views on the assembly of microbial communities. For example, some experimental studies indicate that spatial homogenization of microbial composition results from a reduced influence of deterministic selection and increased influence of stochastic dispersal (Barberan & Casamayor, 2010; Hewson, Steele, Capone, & Fuhrman, 2006). On the contrary, several other studies found that spatial homogenization is coupled with increased influences of deterministic assembly processes (Deng et al., 2016; Liang, Zhang, Zhou, & Li, 2015; Zhou et al., 2014). These inconsistent outcomes can be explained by a variety of experimental dependent reasons, such as differences in sampling approaches, data resolution, spatial scales and target microbial taxa (Hanson et al., 2012; Martiny, Eisen, Penn, Allison, & Horner-Devine, 2011; Zhou & Ning, 2017). However, based on the conceptual framework of Vellend (2010), both homogeneous selection and homogenizing dispersal can lead to microbial compositional homogeneity, yet they are associated with deterministic and stochastic processes, respectively. Thus, disentangling contributions of ecological processes is necessary to uncover the underlying rules of microbial community assembly.

A key challenge in community ecology is revealing factors that lead to the dominance of different microbial community assembly processes across different environmental contexts. In parallel, it is important to advance towards a generalizable understanding of community assembly processes and, in turn, a more mechanistic understanding of microbial ecology. Previous works indicate that deterministic assembly processes corresponded to low soil nutrient condition, while stochastic processes increase with higher nutrient condition (Chase, 2010; Feng et al., 2017; Liu et al., 2015b; Zhou et al., 2014). In addition, it has been observed that small changes in environmental variables can lead to stochastic assembly, whereas large changes result in deterministic assembly (Dini-Andreote, Stegen, van Elsas, & Salles, 2015; Stegen et al., 2018; Tripathi et al., 2018; Zhang, Johnston, Liu, Li, & Han, 2016). These outcomes indicate that the absolute state of environmental conditions and the degree of change in those conditions both influence the relative contributions of different ecological assembly processes. Here, our primary aim was to evaluate this general hypothesis within soil microbial communities.

To examine combined influences of the absolute state of environmental conditions and degree of change in those conditions on microbial community assembly, we leveraged nutrient and organic matter addition experiment at six sites across the Chinese subtropical zone (Supporting information Figure S1 and Table S1). These experimental sites naturally varied in soil chemical properties and also varied in their responses to nutrient additions, thereby providing an ideal opportunity to test our hypothesis. Recent investigation at these sites

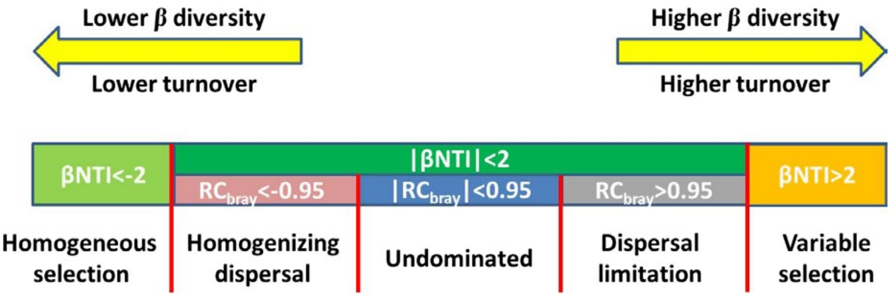

FIGURE 1 The five categories of processes influencing community assembly posited by Stegen et al. (2015). βNTI: between-community nearest taxon index; RC_{bray}: Bray–Curtis-based Raup–Crick

TABLE 1 Description of the six field experimental sites

Sampling sites	Wangcheng	Changshu	Yingtan	Jinxian	Huizhou	Guilin
Province	Hunan	Jiangsu	Jiangxi	Jiangxi	Guangdong	Guangxi
Longitude	112°80′	120°42′	116°55′	116°10′	114°40′	109°40′
Latitude	28°37′	31°33′	28°15′	28°21′	23°09′	26°23′
Altitude (m)	100	3	20	137	37	150
Climate	SM	SM	SM	SM	SM	SM
MAP (mm)	1370	1321	1795	1549	2200	1950
MAT (°C)	17	16.6	17.6	17.7	22.0	19.3

Notes. MAP: mean annual precipitation; MAT: mean annual temperature; SM: subtropical monsoon.

demonstrated that nutrient additions led to taxonomic homogenization of soil bacterial community composition at the regional scale (Chen et al., 2017). In this study, we evaluated how environmental state and change in environmental state affected assembly processes governing microbial communities. The experimental design further enabled evaluation of scale dependence in observed relationships, whereby we first studied the regional scale and then evaluated the degree to which inferences were maintained at smaller scales. The outcomes highlight a need to go beyond documenting patterns by strengthening the theoretical linkages between ecological assembly processes, historical environmental conditions and changes in environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental sites and soil sampling

Soil samples were collected from six agro-ecological experimental sites across subtropical China (Supporting information Figure S1). Description of the six experimental sites is supplied in Table 1, and more details were described in Chen et al. (2017). The maximum straight-line distance between experimental sites was ~1,000 km. Each experimental site contained a plot experiment with nutrient and organic matter additions. Among them, we selected three treatments in the current study: control (without nutrient addition), NPK (mineral NPK addition) and OMN (organic matter addition + NPK). The designs of the three treatments are listed in Supporting information Table S1. The level of nutrient addition was selected to align with local fertilization practices near the experimental site. Three replicate plots of each treatment were established randomly at each site, with each plot area of ca. 20 m^2. A total of 54 samples were collected with three replicate plots of three treatments at six sites. Soil samples were collected after the harvest of paddy rice (for double cropping rice, after the late cropping). Each soil sample was based on combining 10 cores taken at a depth of 0–10 cm using a 30-mm-diameter gouge auger in one plot. Ten cores were mixed in the field and then taken to the laboratory and sieved in <2 days. For each fresh soil sample, around 10 g was stored at −40°C for DNA extraction, and the other subsample was air-dried, sieved and used for analysis of chemical properties.

2.2 | Analysis of chemical properties

Soil chemical properties were analysed according to the protocols of Lu (1999). Soil organic matter (SOM) was determined by potassium dichromate oxidization and back titration of excess potassium dichromate using an ammonium ferrous sulphate solution. Soil pH was determined from soil–water suspensions (1:2.5 v/v). Soil total N (TN) was determined by Kjeldahl digestion. Soil total P (TP) and K (TK) were first digested by hydrofluoric acid (HF)–perchloric acid ($HClO_4$) and then determined by molybdenum blue colorimetry and flame photometry, respectively. Available N (AN) in the soil was alkaline-hydrolysed and produced NH_3 was diffused and determined by acid–base neutralization titration. Available P (AP) in the soil was extracted by sodium bicarbonate and determined using the molybdenum blue method. Available K (AK) in the soil was extracted by ammonium acetate and determined by flame photometry. The detailed information of soil chemical properties is tabulated in Supporting information Table S2. The variation in general chemical properties among sites and treatments is presented by principal component analyses (Supporting information Figure S2).

2.3 | DNA extraction

Genomic DNA was extracted from 0.5 g soil by using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA). The extracted DNA was dissolved in 50 μl TE buffer, quantified by spectrophotometer and quality-evaluated by gel electrophoresis. After that, extracted DNA was evaluated by NanoDrop ND-2000 (Thermo Fisher, USA) and stored at −20°C until further usage.

2.4 | PCR and high-throughput sequencing of bacterial 16S rRNA genes

PCR amplification was conducted for bacteria with primer set 519F/907R. The oligonucleotides of 5-bp bar-coded were fused to the forward primer. PCR was carried out in 50-μl reaction mixture, containing deoxynucleoside triphosphate at a concentration of 1.25 μM, 2 μl (15 μM) forward and reverse primers, 2 μM of Taq DNA polymerase (TaKaRa, Japan), and each reaction mixture received 1 μl (50 ng) of genomic community DNA as a template.

TABLE 2 Mantel tests of soil variables against all βNTI across six sites

	r	*p*
TN	0.3328	0.001
AN	0.3315	0.001
SOM	0.3332	0.001
AP	0.0431	0.272
AK	0.0819	0.100
TK	0.1566	0.003
TP	0.2213	0.002
pH	0.2421	0.002

PCRs were performed according to the following program: 94°C for 5 min, 30 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s), and a final extension at 72°C for 10 min. Reaction products for each soil sample were pooled and purified using the QIAquick PCR Purification Kit (Qiagen), and quantified using NanoDrop ND-2000 (Thermo Scientific, USA).

High-throughput sequencing was performed with Illumina MiSeq sequencing platform (Illumina Inc., CA, USA). The bar-coded PCR products from all samples were normalized in equimolar amounts before sequencing. After sequencing was completed, 16S rRNA gene data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline for data sets (Caporaso et al., 2010, http://qiime.source.org). Sequences with a quality score below 25 and the length fewer than 200 bp were trimmed and then assigned to soil samples based on unique barcodes. In total, we obtained 743,785 sequences of bacterial 16S rRNA gene, and between 5,370 and 21,403 sequences per sample. Sequences were binned into operational taxonomic units (OTUs) using a 97% identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence. Taxonomy was then assigned to OTUs with reference to a subset of the SILVA 119 database (http://www.arb-silva.de/download/archive/qiime/). OTU representative sequences were aligned using PYNAST (Caporaso et al., 2010). A phylogenetic tree was then constructed using FASTTREE (Price, Dehal, & Arkin, 2009). All samples were then rarefied to 5,370 sequences per sample to evaluate beta diversity of bacterial phylotypes, which allowed us to compare general diversity patterns among treatments, even though it is highly unlikely that we surveyed the full extent of diversity in each community (Shaw et al., 2008).

2.5 | Phylogenetic analysis

To evaluate the phylogenetic signal across a range of phylogenetic distances, we used Mantel correlograms with 999 randomizations for significance tests (Diniz-Filho, Terribile, da Cruz, & Vieira, 2010; Warren, Glor, & Turelli, 2008) with the function "mantel.correlog" in the R package VEGAN v2.0-2 (http://vegan.r-forge.r-project.org). We partitioned phylogenetic distances into 50 classes (i.e., evolutionary time steps), and within each distance class, we tested the correlation coefficient relating between-OTU phylogenetic distances to between-OTU niche distances (Stegen et al., 2013; Wang et al., 2013). For any pair of OTUs, their between-OTU phylogenetic distance was quantified as the total phylogenetic branch length between them. A niche for each OTU was estimated for each environmental variable as in Stegen, Lin, Konopka, and Fredrickson (2012). Resulting niche estimates for all environmental variables were used to calculate multivariate Euclidean niche distances among all OTUs. Prior to calculating Euclidean distances, niche estimates for each environmental variable were normalized to be z-scores (i.e., mean of 0 and standard deviation of 1). The Euclidean distance was then calculated from the OTU-specific niche estimates associated with each environmental variable. That is, each OTU had a niche estimate for each environmental variable and those values were used to calculate a multivariate Euclidean distance between any pair of OTUs. For the Mantel correlogram analysis, 50 phylogenetic distance classes can provide enough resolution across short phylogenetic distances to evaluate whether between-OTU phylogenetic distances could be used to make ecological inferences. Using a smaller number of classes may be possible, but may not provide enough resolution across short phylogenetic distances.

We found that significant positive correlations between OTU niche differences and phylogenetic distances were strongest at short phylogenetic distances (approximately 0%–33% of the maximum phylogenetic distance; Supporting information Figure S3). This indicated that OTU environmental preferences were phylogenetically conserved across relatively short phylogenetic distances. In turn, we calculated the between-community mean nearest taxon distance (βMNTD; Fine & Kembel, 2011) and β-nearest taxon index (βNTI; Stegen et al., 2012) to quantify phylogenetic turnover between communities. These metrics emphasize relatively short phylogenetic distances, which is appropriate given strong phylogenetic signal across short phylogenetic distances (Stegen et al., 2012).

βMNTD quantifies the phylogenetic distance between each species in one community (*k*) and its closest relative in a second community (*m*):

$$\beta\mathrm{MNTD} = 0.5\left[\sum_{i_k=1}^{n_k} f_{i_k} \min\left(\Delta_{i_k j_m}\right) + \sum_{i_m=1}^{n_m} f_{i_m} \min\left(\Delta_{i_m j_k}\right)\right],$$

where f_{i_k} is the relative abundance of species *i* in community *k*, n_k is the number of species in *k*, and min ($\Delta i_k j_m$) is the minimum phylogenetic distance between species *i* in community *k* and all species *j* in community *m*. βMNTD was calculated using the R function "comdistnt" (abundance.weighted = TRUE; package "picante"). βNTI was estimated as the number of standard deviations that the observed βMNTD is from the mean of the null distribution of βMNTD generated by 1,000 randomizations of the "phylogeny.pool" null model: ($\beta\mathrm{NTI} = (\beta\mathrm{MNTD}_{\mathrm{Observed}}$-mean $(\beta\mathrm{MNTD}_{\mathrm{Null}}))/$ sd $(\beta\mathrm{MNTD}_{\mathrm{Null}})$). βNTI values <−2 or >+2 indicate that observed βMNTD is more than two standard deviations away from the mean of the null βMNTD distribution (which is the stochastic expectation).

As such, βNTI values that are <−2 or >+2 indicate there is a statistically significant divergence between observed and expected βMNTD, which is interpreted as less than or greater than expected phylogenetic turnover, respectively (Stegen et al., 2013).

2.6 | Calculation of estimated ecological processes

To infer the relative influences of homogenizing dispersal and dispersal limitation, we combined the outcome of βNTI analyses with a second null model referred to as Bray–Curtis-based Raup–Crick (RC_{bray}), as in Stegen et al. (2013). RC_{bray} was estimated for each pair of communities. To do so, null versions of the two communities were assembled probabilistically from the species pool. OTU richness was maintained at observed levels because variation in richness will influence expected levels of Bray–Curtis under stochastic community assembly. In addition, the probability of a given OTU being selected was proportional to its occurrence across all samples. For selected OTUs, the probability of accumulating sequences (interpreted here as individuals) within that OTU (for the probabilistically assembled communities) was proportional to the total abundance of that OTU across all samples. Individuals were sampled into selected OTUs with replacement until reaching the rarefication depth of 5,370 sequences (interpreted here as individuals). Following probabilistic assembly, Bray–Curtis was calculated to generate a null value. This process was repeated 999 times for each community pair to generate a distribution of null Bray–Curtis values. 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. We refer readers to Stegen et al. (2013) and Chase, Kraft, Smith, Vellend, and Inouye (2011) for the detailed principle and calculation (see also https://github.com/stegen/Stegen_etal_ISME_2013). The relative contributions of variable and homogeneous selection were estimated as the percentage of pairwise βNTI values that fell above +2 and below −2, respectively. Then, from the percentage of pairwise comparisons with $|\beta NTI| < 2$ but $RC_{bray} < -0.95$ or $> +0.95$, we inferred the relative contributions of homogenizing dispersal or dispersal limitation processes, respectively. Finally, in the case of $|\beta NTI| < -2$ and $|RC_{bray}| < 0.95$, no single process drives shifts in community composition (Figure 1).

2.7 | Statistical analysis

To unravel the environmental variables influencing phylogenetic turnover of community composition, Mantel tests were conducted to test the statistical significance between βNTI of bacterial communities and soil chemical variables across six sites, across any five sites and across any four sites, using R software (the VEGAN package, version 3.1.2). These analyses showed that soil organic matter (SOM) had the strongest correlation with βNTI. SOM strongly co-varied with other environmental variables, however, such that we interpreted SOM as an integrated proxy of soil environmental conditions.

To evaluate patterns at the regional scale, we estimated assembly processes within treatments and between treatments across six sites as well as those across any five sites and across any four sites. Data within the three treatments (control, NPK and OMN) were used to investigate the relationship between assembly processes and the absolute state of environmental conditions. Differences in SOM content among treatments were evaluated with one-way ANOVA followed by post hoc Tukey's HSD tests. Comparisons between treatments (control vs. NPK, control vs. OMN and NPK vs. OMN) were used to investigate microbial assembly in response to change in environmental conditions. Mantel tests were used to evaluate relationships between βNTI and pairwise changes in environmental conditions between treatments. A comparison of Mantel tests associated with different environmental variables was used to identify environmental variables most likely to influence ecological assembly processes. For completeness, relationships with all environmental variables were evaluated, but SOM is the focus of our conceptual interpretations due to commonly having the strongest relationships with assembly processes and due to being an integrated proxy.

To evaluate the degree to which regional-scale observations and inferences were maintained at smaller scales, we used the same statistical methods to estimate assembly processes and the relationship between βNTI and changes in environmental conditions for fifteen site-by-site comparisons and six within-site comparisons.

3 | RESULTS

3.1 | Changes in environmental conditions

Mantel tests indicated that all environmental variables, except AP and AK, were important for ecological assembly processes at the regional scale (i.e., across all six sites; Table 2). However, SOM, TN and AN had the strongest associations with assembly processes. These results were maintained following sensitivity analysis for which any five sites (Supporting information Table S3) or any four sites (Supporting information Table S4) were used to conduct Mantel tests. Because of the high correlations between SOM, TN and AN as well as between SOM and the first PCA axis associated with soil chemical properties among sites and treatments (Supporting information Table S5), SOM was chosen as an integrated proxy in the downstream analyses and conceptual interpretations. In turn, we focused below on SOM content and changes in SOM content.

Across the six sites, mean SOM content and variation (shown as standard deviation) in SOM content increased in the order of control (25.1 ± 7.84 g/kg) < NPK (30.5 ± 8.63 g/kg) < OMN (33.8 ± 9.79 g/kg; ANOVA, $p < 0.05$). Meantime, these data indicate that control versus OMN had the largest degree of change in SOM, NPK versus OMN had the smallest degree of change, and the degree of change in SOM between control and NPK was in the middle.

TABLE 3 The mapping of five ecological processes influencing community turnover within and between treatments across six, any five, and any four sites

	Ecological process	Variable selection (%)	Homogeneous selection (%)	Dispersal limitation (%)	Homogenizing dispersal (%)	Undominated process (%)
Across six sites	Within treatment					
	Control	45.8	18.3	20.9	9.8	5.2
	NPK	30.1	16.3	12.4	23.5	17.6
	OMN	18.3	22.9	19.6	28.1	11.1
	Between treatments					
	Control versus NPK	41.7	15.7	17.0	15.1	10.5
	Control versus OMN	41.4	16.7	12.3	17.9	11.7
	NPK versus OMN	25.3	22.5	12.0	25.0	15.1
Across five sites	Within treatment					
	Control[a]	51.9	12.9	22.9	6.2	7.1
	NPK	37.6	9.5	32.9	4.8	12.4
	OMN	40.0	8.1	21.9	9.0	21.9
	Between treatments					
	Control versus NPK	58.6	6.2	22.9	6.7	6.2
	Control versus OMN	61.0	7.1	16.7	6.2	10.5
	NPK versus OMN	33.8	13.3	28.6	9.5	13.8
Across four sites	Within treatment					
	Control	57.6	9.1	16.7	9.1	7.6
	NPK	43.9	7.6	27.3	7.6	10.6
	OMN	45.5	6.1	16.7	12.1	19.7
	Between treatments					
	Control versus NPK	66.7	6.1	15.2	9.1	4.5
	Control versus OMN	63.6	6.1	13.6	7.6	9.1
	NPK versus OMN	37.9	9.1	22.7	13.6	13.6

[a] The assembly processes across any five and any four sites are median values of six and fifteen nonindependent outcomes.

3.2 | Ecological assembly processes associated with environmental conditions

The relative contributions of ecological processes differed among the three treatments across the six sites (Table 3). The control was associated with a greater influence of variable selection (45.8%) than NPK (30.1%) and OMN (18.3%). In contrast, the greater contributions from homogenizing dispersal and undominated condition were observed for NPK (23.5% and 17.6%) and OMN (28.1% and 11.1%) than for the control (9.8% and 5.2%). In addition, NPK had the lowest contributions from homogeneous selection and dispersal limitation (Table 3).

The contributions of deterministic (i.e., variable selection plus homogeneous selection) and differentiating processes (including variable selection plus dispersal limitation) significantly decreased in treatments OMN (41.2% and 37.9%) and NPK (46.4% and 42.5%), compared to control (64.1% and 66.7%; Table 4). On the contrary, the contributions of stochastic (i.e., homogenizing dispersal plus dispersal limitation) and homogenizing processes (including homogeneous selection and homogenizing dispersal) were significantly higher in OMN (47.7% and 51.0%) and in NPK (36.0% and 39.8%) than in the control (30.7% and 28.1%).

Similar patterns were observed for any five sites or any four sites (Tables 3, 4 and Supporting information Table S6). For

TABLE 4 The mapping of deterministic, stochastic, homogenizing and differentiating processes influencing community turnover within and between treatments across six, any five and any four sites

	Ecological processes	Deterministic (%)	Stochastic (%)	Homogenizing (%)	Differentiating (%)
Across six sites	Within treatment				
	Control	64.1	30.7	28.1	66.7
	NPK	46.4	36.0	39.8	42.5
	OMN	41.2	47.7	51	37.9
	Between treatments				
	Control versus NPK	57.4	32.1	30.8	58.7
	Control versus OMN	58.1	30.2	34.6	53.7
	NPK versus OMN	47.8	37.0	47.5	37.3
Across five sites	Within treatment				
	Control[a]	62.9	29.0	19.0	75.2
	NPK	45.2	38.1	14.8	72.9
	OMN	48.6	31.0	16.2	61.0
	Between treatments				
	Control versus NPK	64.8	30.0	12.9	81.4
	Control versus OMN	67.6	22.9	12.9	75.7
	NPK versus OMN	48.6	37.6	22.9	60.5
Across four sites	Within treatment				
	Control	65.2	27.3	18.2	74.2
	NPK	53.0	34.8	15.2	71.2
	OMN	53.0	28.8	18.2	60.6
	Between treatments				
	Control versus NPK	71.2	24.2	13.6	81.8
	Control versus OMN	68.2	22.7	13.6	77.3
	NPK versus OMN	45.5	39.4	24.2	62.1

Notes. Deterministic = Variable selection + Homogeneous selection.
Stochastic = Dispersal limitation + Homogenizing dispersal.
Homogenizing = Homogeneous selection + Homogenizing dispersal.
Differentiating = Variable selection + Dispersal limitation.
[a] The assembly processes across any five and any four sites are median values of six and fifteen nonindependent outcomes.

example, the influence of variable selection was higher in control (median 51.9% of any five sites and median 57.6% of any four sites) than in NPK (37.6% and 43.9%) and OMN (40.0% and 45.5%; Table 3). Meanwhile, the control had greater contributions of deterministic processes (median 62.9% for any five sites and median 65.2% for any four sites) than NPK (45.2% and 53.0%, for any five or four sites, respectively) and OMN (48.6% and 53.0%, for any five or four sites, respectively; Table 4). The same changing pattern was observed on differentiating processes (the control [75.2% and 74.1%] vs. NPK [72.9% and 71.2%] and OMN [61.0% and 60.6%]).

3.3 | Changes in ecological assembly processes in response to changes in environmental conditions

The ecological processes influencing community assembly between treatment pairs across six sites are also summarized in Tables 3 and 4. A significantly higher contribution of variable selection (41.7% and 41.4%) as well as significantly lower contributions of homogeneous selection (15.7% and 16.7%), homogenizing dispersal (15.1% and 17.9%) and the undominated condition (10.5% and 11.7%) was observed for control versus NPK and control versus OMN, in comparison with NPK versus OMN (25.3%, 22.5%, 25.0% and 15.1%;

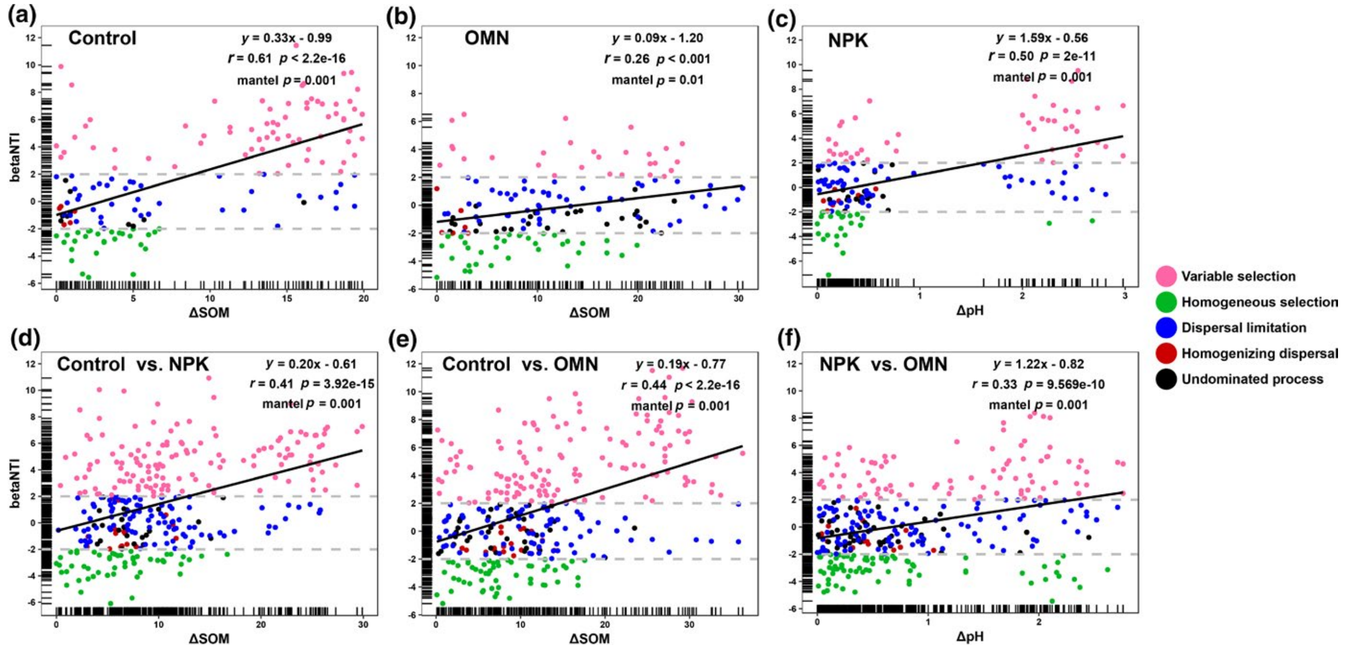

FIGURE 2 Linear regressions between β-nearest taxon index (βNTI) and changes in SOM content for control (a), OMN (b), control versus NPK (d) and control versus OMN (e) as well as between βNTI and changes in pH for NPK (c) and NPK versus OMN (f) across six sites. Linear models (shown as black lines) and associated correlation coefficients are provided on each panel. Horizontal dashed lines indicate the βNTI significance thresholds of +2 and −2

Table 3). Additionally, control versus NPK had the highest contribution of dispersal limitation.

The contributions of deterministic and differentiating processes were also observed to be significantly greater in control versus NPK (57.4% and 58.7%) and control versus OMN (58.1% and 53.7%), in comparison with those in NPK versus OMN (47.8% and 37.3%; Table 4). On the contrary, the contributions of stochastic and homogenizing processes imposed greater influences on NPK versus OMN (37.0% and 47.5%) than on control versus OMN (30.2% and 34.6%) and control versus NPK (32.1% and 30.8%).

The assembly processes across any five sites or any four sites showed similar patterns (Tables 3, 4 and Supporting information Table S6). Relative to control, NPK versus OMN had lower influences of variable selection, deterministic and differentiating processes, but higher influences of homogeneous selection, dispersal limitation, homogenizing dispersal, undominated condition, stochastic and homogenizing processes.

Mantel tests indicated that change in SOM greatly influenced assembly processes within control ($r = 0.61$, $p = 0.001$) and OMN ($r = 0.26$, $p = 0.01$) treatments, as well as between control versus NPK ($r = 0.41$, $p = 0.001$) and between control versus OMN ($r = 0.44$, $p = 0.001$) across six sites (Figure 2a, b, d and e and Supporting information Table S7). For within NPK ($r = 0.5$, $p = 0.001$) and between NPK versus OMN ($r = 0.33$, $p = 0.001$), change in pH was the best environmental predictor of βNTI (Figures 2c, f and Supporting information Table S7). The Mantel tests of the within-treatment and the between-treatment comparisons across any five and four sites showed similar patterns of assembly processes influenced by changes in SOM and pH (Supporting information Tables S8, S9).

3.4 | Ecological assembly processes governing community turnover at smaller spatial scales

To evaluate the influences of decreasing the scale of analysis and size of the regional species pool on our conceptual inferences, we further conducted site-by-site analyses that simultaneously included within- and between-treatment comparisons to maximize sampling efforts (Supporting information Figure S4). The influences of deterministic processes increased from the WC versus CS pair (36.6%) to the HZ versus GL pair (79.1%; Supporting information Figure S5). The statistical analyses indicated that the ecological assembly processes between ten pairs (out of 15 total pairs) were mainly associated with SOM (and other variables SOM was strongly correlated with; Supporting information Table S10 and Figure 3). Assembly processes in the other five pairs were most strongly associated with pH (i.e., CS vs. WC, CS vs. JX and CS vs. HZ) and total K (i.e., CS vs. GL and JX vs. WC), respectively (Supporting information Table S10).

4 | DISCUSSION

4.1 | High resource content leads to homogenizing dispersal

SOM is an integrated proxy of environmental conditions associated with soil fertility and biogeochemistry (Lehmann & Kleber, 2015). Here, we found that SOM was highly correlated with other variables associated with resources used by bacterial communities (e.g., TN, AN, TP, AP and AK). In turn, we interpret SOM as an integrated proxy of environmental conditions and more specifically of soil resource

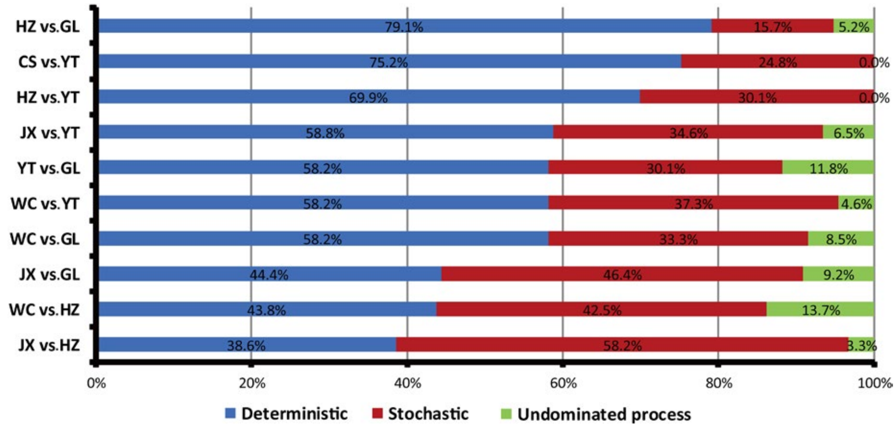

FIGURE 3 Contributions of deterministic and stochastic processes governing bacterial community turnover between ten pairs of sites, which were influenced by SOM

content. We found that increases in SOM (and thus resource content) were associated with decreases in variable selection and increases in homogenizing dispersal and the undominated condition (Table 3). These results are consistent with previous work showing spatial homogenization of microbial composition in response to increases in SOM content at the regional scale (i.e., a flattened distance–decay relationship (DDR) between compositional similarity and spatial distance; Chen et al., 2017). It can be inferred from the model posited by Hanson et al. (2012) that variable selection often increases the strength of the DDR, whereas high rates of dispersal weaken the DDR. Thus, our results provide support to this theoretical construct related to factors shaping regional-scale microbial community composition.

As SOM and other resources strongly influence microbial community composition and their ecophysiology (Feng et al., 2015; Trivedi, Anderson, & Singh, 2013; Trivedi et al., 2016), several mechanisms are likely associated with changes in microbial assembly processes in response to increased resource content due to nutrient addition. First, increased resources may release microbes from resource limitation, as evidenced by increased microbial biomass (Chu et al., 2007). This situation may allow for greater colonization, less exclusion and (in turn) a decreased influence of variable selection, consistent with our experimental observations (Table 3). In contrast, more taxa would be excluded by environmental selection, which was more significant in the control treatment, which had the highest influence of variable selection. Second, nutrient and organic matter additions introduced more resource and energy to soil microbes, which can improve the ability of microorganisms to disperse (Yang & van Elsas, 2018). Furthermore, the greatest contribution of homogenizing dispersal was observed for the OMN treatment (Table 3), which had the highest resource content. Alternatively, a greater contribution from homogenizing dispersal may have resulted from repeated application of microbe-rich organic material. This organic matter may have been a source for a consistent set of immigrating taxa, thereby homogenizing community composition via dispersal. If true, this predicts that the addition of inorganic nutrients would not lead to elevated influences of homogenizing dispersal. The reason is that inorganic nutrient addition would not have provided repeated immigration of a consistent set of microbial taxa. Consistent with this prediction, the influence of homogenizing dispersal for NPK was less than that of OMN (Table 3).

Similar patterns of ecological assembly processes in response to high SOM content also appears in upland agricultural soils (Feng et al., 2017; Jiang et al., 2016), grassland (Sayer et al., 2013) and surface water (Hewson et al., 2006; Zhou et al., 2014), suggesting that the outcomes observed here may be generalizable across ecosystems. Furthermore, the increased contents of phosphorus and nitrogen built up the stochastic processes on microbial assembly processes, similar to those observed here (Feng et al., 2017; Liu et al., 2015b). Consistency across studies and systems suggests that there may be generalizable rules linking the absolute state of the resource environment to ecological assembly processes.

4.2 | Large changes in resource content impose variable selection

Comparing the effects of nutrient addition on assembly processes between treatments to processes within treatments provides insight into the effects of changes in resource content. In contrast to within-treatment effects, nutrient addition resulted in variable selection driving community differentiation between control and both nutrient addition treatments, with a weaker influence of variable selection between the two nutrient addition treatments (Table 3). Furthermore, βNTI increased significantly with large changes in SOM content when comparing control to OMN or NPK treatments (Figures 2d, e). This suggests that variable selection between control and nutrient addition treatments was due to nutrient addition causing increases in resource content. We speculate that a large increase in resource content can stimulate specific microorganisms and impose a strong directional change in composition. This can be observed at the plot scale, whereby previous work found that in sandy loam soils, *Bacillus* species flourished in response to organic manure amendment (Feng et al., 2015). This influence of resource content on assembly processes aligns with other previous work showing that changes in resource content alter community assembly processes (Dini-Andreote et al., 2015; Liu et al., 2015b; Stegen et al., 2016a; Zhang et al., 2016).

Although changes in SOM content (or resource content, more generally) deterministically drove community turnover between control and NPK or OMN, change in soil pH appeared to have a stronger influence on turnover between NPK and OMN and within NPK (Figure 2c, f). The reason is likely related to the absolute state of and relative changes in resource content. NPK and OMN had higher SOM contents and smaller changes in SOM content than within the control. In this case, changes in resource content may have been too small to deterministically drive bacterial community composition apart. Meantime, NPK (0.93 of standard deviation) and NPK versus OMN (0.22 of ΔpH) had relatively large changes in soil pH, comparing to OMN (0.71) and control versus OMN (0.18). In these cases, pH became the dominant driver of deterministic community assembly most likely due to the relatively large changes in pH. This emphasizes the strong influence environmental extent has on the identity of environmental variables that drive variation in community composition (Stegen et al., 2016b). Furthermore, it is well known that for upland soils, long-term chemical fertilizer application can decrease soil pH (Ding et al., 2016; Liu et al., 2015a), and organic matter amendments shift soil pH towards neutral (Lin et al., 2012). Consistent with these previous studies, OMN resulted in relatively neutral soil pH compared to lower pH in NPK, except for the Changshu and Yingtan sites (Supporting information Table S2). These differences in the magnitude of change in pH, combined with elevated resource content in both OMN and NPK, likely resulted in pH having the strongest influence over divergence in community composition. Indeed, the similar influences of pH on microbial assembly processes had been reported previously (Shi et al., 2018; Tripathi et al., 2018), and Stegen et al. (2016b) have shown how inferred environmental drivers depend on environmental extent. Similarities between our results and these previous studies suggest that it may be generally true that soil pH is a driving environmental variable when resource content is consistently high and/or the range of resource content is small, while resource content is a key driving variable when its range (across communities) is large.

4.3 | Resource content and change in resource content jointly govern assembly processes

Our results suggested that resources, in terms of both absolute content and the degree of change in content, strongly influenced microbial assembly processes. Thus, we synthesized our results into a conceptual model as depicted in Figure 4. With increasing resource content, the contribution of variable selection decreases while stochastic processes increase. On the other hand, increasingly large changes in resource content deterministically drive community turnover by increased variable selection. Collectively, our results lead to a two-part interpretation. (a) If resource content is low, even small increases in resource content lead to differences in selective pressures that are large enough to deterministically drive shifts in community composition. For example, the strength of variable selection was highest within the control treatment (Table 3), with the lowest SOM content and the smallest changes in SOM, relative to those of NPK and OMN. (b) If resource content is initially relatively high, any further increases open ecological niches and allow for a greater influence of homogenizing dispersal. As evidence, the large range of—but consistently high—SOM content in OMN treatments was associated with the greatest contribution from homogenizing

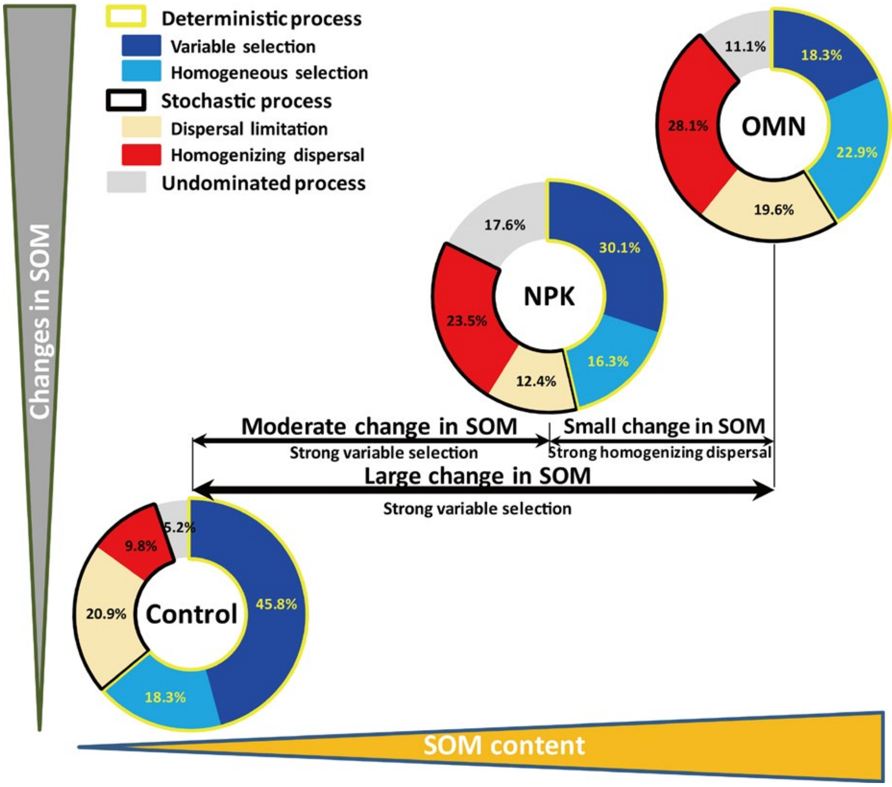

FIGURE 4 Overview of the two-part conceptual model linking ecological processes to the convergence and divergence of bacterial community composition, within and between experimental treatments. In this conceptual model, when SOM content is low, both small (i.e., within control treatments) and large (i.e., between control and OMN or NPK) changes in SOM content lead to variable selection that deterministically drives shifts in bacterial community composition. In contrast, relatively high SOM content (i.e., within NPK and OMN) opens ecological niches and allows for a greater influence of stochasticity via homogenizing dispersal

dispersal (Table 3). Although previous investigations had reported both patterns of assembly processes (Dini-Andreote et al., 2015; Feng et al., 2017; Tripathi et al., 2018), this study provides is the first time that they have been integrated within a unified conceptual framework. Such integration of and interaction between the absolute value of environmental variables and the proportional change in those variables add an additional dimension to our conceptualization of linkages between environmental conditions and ecological assembly (as summarized in Figure 4).

Our conceptual model is consistent with a number of previous studies. For example, Dini-Andreote et al. (2015) found that when there were small changes in SOM, Na content governed microbial assembly processes, whereas when there were large changes in SOM, SOM governed assembly processes. In a separate study, near-neutral soil pH led to a greater contribution of stochastic assembly, while a shift in pH towards relatively extreme conditions led to more deterministic assembly (Tripathi et al., 2018). Our results combined with these previous studies indicate that the degree to which deterministic assembly is influenced by a given magnitude of environmental change is dependent on the absolute value of environmental conditions. For example, small changes in resource content will not lead to deterministic shifts in community composition if resource content is high, but the same magnitude of change may lead to strong deterministic shifts if resource content is low. There is a need to further understand and generalize how assembly processes are influenced by interactions between the absolute state of environmental conditions and the change in environmental conditions.

4.4 | Influences of scale on the conceptual model

It is important to recognize that the results of null models are always in reference to the scale at which they are conducted. This provided an opportunity to evaluate assembly process at the within- and between-site scales. This analysis indicated that resource content was not the main driver of assembly processes at the within-site scale (Supporting information Table S11). One possible reason is that within site, the change in resource content was not large enough to deterministically influence microbial assembly. For example, SOM was the key environmental variable only in the GL site, presumably because it had the largest increase in SOM content in response to nutrient additions (66.0% in GL vs. 10.5%–49.5% in the other five sites; Supporting information Table S2). In addition, there were only 36 βNTI values within each site. This level of sampling is likely too low to make any strong assertions. As such, while it is intriguing that there may be differences across scales in the identity of factors imposing deterministic ecological selection, we consider this result preliminary and did not include it in our conceptual model.

In contrast to the within-site analyses, comparing pairs of sites revealed patterns that were mostly consistent with our conceptual model. Generally, the between-site results indicate that high resource content was associated with stochastic processes and large changes in resource content were associated with deterministic processes (Supporting information Figure S4). Specifically, YT had the lowest SOM content (Supporting information Table S2) and the highest contributions of deterministic processes were observed when examining shifts in composition between YT and those sites with high SOM (e.g., YT vs. CS and YT vs. HZ). The greatest influence of stochastic processes—including the highest contribution from homogenizing dispersal—was observed for the JX versus HZ and WC versus HZ comparisons. These sites all had high SOM content and relatively small between-site changes in SOM. It is important to recognize that different site pairs represent different spatial scales due to differences in the geographic distances among pairs (Supporting information Figure S1). Repeated observations across between-site comparisons of an important influence of resource content therefore indicate that our conceptual model provides a valid representation of microbial community assembly across a range of spatial scales. However, there were some between-site comparisons that did not conform to our conceptual model (e.g., HZ vs. GL and JX vs. YT). This suggests that factors beyond resource content are at work, which is not surprising and indicates a need to further explore the multifactor nature of ecological assembly in soil microbial communities.

Additional analyses indicated that our results and inferences were robust to variation in within-site diversity (i.e., α-diversity) and the size of the species pool, which are both important factors to consider in null model analyses (Swenson, Enquist, Pither, Thompson, & Zimmerman, 2006; Ulrich et al., 2017). To help address issues associated with variation in α-diversity, some null models account for α-diversity (Chase et al. (2011)), including the RC_{bray} metric used here. Furthermore, α-diversity was not significantly different among experimental treatments (control, NPK and OMN; Supporting information Table S12), suggesting that variation in α-diversity did not confound our analyses and associated inferences. Related to the species pool, it was found that most bacterial taxa were found across multiple sites (Supporting information Figure S6). This indicates that the bacterial taxonomic composition was relatively similar among sites and suggests that it is reasonable to assume one species pool across the six sites. Additionally, we checked for an influence of changing the species pool, whereby for the between-site analyses, we used the species pool containing only those taxa found in a given pair of sites. This provided an analysis—discussed above—for how sensitive our results were to the size of the species pool, and revealed that our results and conceptual inferences were consistent across species pool sizes.

In conclusion, our manipulative experiment revealed that nutrient addition homogenizes bacterial community composition due to enhanced influences of homogenizing dispersal when mean SOM is elevated, but leads to strong divergence in community composition due to increased influences of variable selection when initial SOM content is low and/or the degree of change in SOM is elevated (Figure 4). Our findings imply important interactions between the mean and variance of environmental variables and ecological assembly processes that, in turn, may influence microbial community and ecosystem processes. This highlights a need to strengthen the connection between ecological theory related to community assembly processes and biogeochemical and ecosystem modelling (Graham & Stegen, 2017).

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DATA ACCESSIBILITY

The sequences were deposited in DDBJ database (Accession no. DRA006218).

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

X.G.L and Z.P.L designed the experiment. Y.Z.F. and R.R.C. performed the experiments. Y.Z.F., R.R.C., J.C.S., Z.Y.G. and J.W.Z. analysed the data. Y.Z.F., R.R.C. and J.C.S. wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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