Soil Biology and Biochemistry 151 (2020) 108023

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Century long fertilization reduces stochasticity controlling grassland microbial community succession

Yuting Liang [a,*], Daliang Ning [b], Zhenmei Lu [b,c], Na Zhang [a], Lauren Hale [b], Liyou Wu [b], Ian M. Clark [d], Steve P. McGrath [d], Jonathan Storkey [d], Penny R. Hirsch [d], Bo Sun [a], Jizhong Zhou [b,**]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China
[b] Institute for Environmental Genomics, Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, 73019, USA
[c] College of Life Sciences, Zhejiang University, Hangzhou, 310058, China
[d] Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

ARTICLE INFO

Keywords:
Time-decay relationship
Community assembly
C N cycling
Stochastic processes
Long-term fertilization

ABSTRACT

Determining the drivers underlying ecological succession is essential for predicting ecosystem functioning in response to human-induced environmental changes. Although various studies have examined the impacts of nitrogen (N) addition on plant and microbial community diversity, structure and activities, it remains unknown how long-term anthropogenic fertilization affects the ecological succession of microbial functional guilds and its underlying community assembly mechanisms. Here, using archived soils, we examined more than a century's succession in soil microbial functional communities (from 1870 to 2008) from the Park Grass Experiment at Rothamsted Experimental Station, the longest running ecological experiment in the world. Long-term fertilization was found to significantly alter soil functional community structure and led to increasingly convergent succession of soil microbial communities. The importance of stochastic assembly varied greatly in regulating the succession of different microbial guilds. Fertilization had large to medium effects on reducing ecological stochasticity for microbial guilds involved in carbon (C) fixation and degradation, nitrogen (N) fixation and mineralization, and denitrification. This century long-term study elucidated the differing influences of assembly mechanisms on soil microbial functional communities involved in C and N cycling, which has important implications for understanding and predicting the microbial mediated ecological consequences of human-induced environmental changes.

1. Introduction

Ecological succession, the change in community composition and structure over time, is one of the oldest and most intensively studied concepts in animal and plant ecology (Clements, 1916; Cowles, 1899; Michaud et al., 2015; Odum, 1969). Determining temporal succession and its underlying mechanisms are fundamental objectives of ecological research and critical for predicting ecosystem functioning in response to anthropogenic activities. Although microbial communities constitute a large portion of the Earth's biosphere and are important in maintaining various biogeochemical processes that mediate ecosystem functions, the temporal succession and assembly mechanisms of microbial communities are less understood. Recently, using DNA fingerprinting methods, various temporal dynamic patterns were reported in microbial communities from various habitats, such as species-time relationship (STR) (Falk et al., 2013; Jiao et al., 2017; Kim et al., 2013; Pla-Rabes et al., 2011; Redford and Fierer, 2009; van der Gast et al., 2008) and time decay relationship (TDR) (Guo et al., 2018; Liang et al., 2015; Shade et al., 2013; Xiong et al., 2015). Microbial community may present different temporal turnover compared with larger organisms due to the unique biology of microorganisms, including their massive population sizes, high dispersal rates, rapid asexual reproduction, and resistance to extinction (Shade et al., 2013). However, the mechanisms driving such temporal successional patterns remain controversial. For instance, microbial succession was found to be governed primarily by stochastic processes (e.g., dispersal and drift) (Ofiteru et al., 2010; Tripathi et al.,

* Corresponding author.
** Corresponding author.
E-mail addresses: ytliang@issas.ac.cn (Y. Liang), jzhou@ou.edu (J. Zhou).

https://doi.org/10.1016/j.soilbio.2020.108023
Received 29 June 2020; Received in revised form 22 September 2020; Accepted 28 September 2020
Available online 28 September 2020
0038-0717/

2018; Zhou et al., 2014). In contrast, microbial succession was reported to be controlled by deterministic abiotic filtering, such as temperature (Campisano et al., 2017; Lin et al., 2017), pH (Tripathi et al., 2018), resource type (van der Gast et al., 2008; Zhalnina et al., 2018), nutrient availability (Darcy et al., 2018; Knelman et al., 2014), and/or biotic interactions (Bardgett et al., 2005; Dini-Andreote et al., 2016). Autogenic succession and deterministic recovery following disturbance in soil bacterial communities was also reported recently (Jurburg et al., 2017).

A grand challenge in contemporary microbial ecology studies is to link the functional succession of microbial communities to environmental change (Dini-Andreote et al., 2016; Knelman et al., 2014). Most studies are based on taxonomic and/or phylogenetic diversity but not on functional diversity. It is believed that functional traits could have more critical impacts on community assembly (Fukami, 2015), and the assembly mechanisms can vary significantly among different microbial functional groups (Caruso et al., 2011). However, microbial succession has rarely been studied based on functional trait compositions (Gutknecht et al., 2012; Zhou et al., 2014), particularly in response to human-induced environmental changes, such as N addition. Through intensive agriculture, humans have greatly accelerated global N deposition over past centuries (Reay et al., 2008; Stevens et al., 2004). Increasing N addition through the application of fertilizers or atmospheric deposition greatly enhances global plant productivity but also dramatically impacts the above- and below-ground biodiversity in terrestrial ecosystems (Stevens et al., 2004, 2018; Zhang et al., 2018). Excessive N input has multiple effects on soil microbial community composition, diversity, activity and function (Geisseler and Scow, 2014; Ramirez et al., 2010; Rousk et al., 2011). Long-term fertilization is expected to have dramatic and persistent impacts on soil characteristics and microbial community structure (Hallin et al., 2009; Liu et al., 2015; Rousk et al., 2011; Zhou et al., 2015a). For instance, an altered soil C content, C:N ratio, and pH were found to be major factors contributing to variations in microbial communities under fertilization (Xun et al., 2016; Zhong et al., 2010; Zhou et al., 2017), suggesting the importance of deterministic processes (e.g. abiotic filtering effects of environmental selection). In contrast, examining both deterministic and stochastic changes revealed that the effect of N fertilizer was largely exhibited through stochastic processes rather than deterministic ones (Zhang et al., 2011, 2016), indicating that complicated assembly mechanisms underlie fertilization effects. A meta-analysis based on 107 datasets from 64 long-term experiments (ranging from 5 to 130 years in length, average 37 years) worldwide revealed that mineral fertilizer application led to a 15% increase in microbial biomass relative to unfertilized soils (Geisseler and Scow, 2014). However, it remains unknown how long-term anthropogenic fertilization affects the ecological succession of microbial functional guilds and its underlying mechanisms, which is critical for predicting the consequences of increasing anthropogenically derived N addition on terrestrial ecosystems.

The Park Grass Experiment (PGE) on permanent grassland at the Rothamsted Experimental Station, United Kingdom, is the oldest grassland experiment in the world (Silvertown et al., 2006). Since 1856, when the experiment was initiated, plots have received different combinations and amounts of inorganic N, P, and K fertilizers and lime, while the "Nil" control plots have never received fertilizer. Thus, changes in soil geochemical characteristics and soil biota indicate long-term responses to anthropogenic fertilization. Archived soil samples from the PGE provide a unique opportunity to address the long-term succession and the underlying assembly mechanisms of soil microbial communities, as influenced by fertilization. In this study, we examined archived soil samples from 1870 to 2008 from contrasting plots with inorganic fertilization and the corresponding "Nil" samples as controls. Soil microbial communities were analyzed using GeoChip. We proposed two hypothesis: (i) Century long fertilization reduces the temporal turnover of microbial functional communities involved in carbon and nitrogen cycling; (ii) The importance of stochastic processes controlling community assembly of functional guilds decreased with long-term fertilization.

2. Materials and methods

2.1. Study sites and sample collection

To understand the impacts of long-term inorganic fertilization on soil microbial functional community structure and succession, archived soil samples were taken from Plot 3 and Plot 14/2 of Park Grass, dating back to 1870, 1876, 1906, 1923, 1959, 1976, 1984, 1991, 1998, 2002, 2005 and 2008 for plot 3 and 1870, 1876, 1906, 1923, 1939, 1959, 1984, 1991, 1998, 2002, 2005 and 2008 for plot 14/2. Plot 3 (96.4 m × 12.6 m) had not received fertilizer since 1856 (control). Plot 14/2 (62.8 m × 6.3 m) was fertilized with 96 kg N ha^{-1} y^{-1} as sodium nitrate in spring since 1856 with additional minerals (35 kg P ha^{-1} y^{-1} as triple superphosphate, 225 kg K ha^{-1} y^{-1} as potassium sulfate, 15 kg Na ha^{-1} y^{-1} as sodium sulfate, and 10 kg Mg ha^{-1} y^{-1} as magnesium sulfate, and no lime was applied). Plots 3 and 14/2 are approximately 25 m apart. In total, there are 24 archived soil samples, namely, 12 control samples and 12 fertilized samples. Soils were sampled from 0 to 10 cm in depth around the middle of the plot to avoid the spatial effect, air-dried and archived in glass bottles. The soil pH was approximately 5–6 in both plots, measured in water. Soil C and N were measured by a LECO analyzer, and soils were extracted for Olsen P measurements by colorimetry. The mean annual yields of dry matter were 2.9 t ha^{-1} and 6.7 t ha^{-1} for Plot 3 and Plot 14/2, respectively. Detailed information on the PGE can be found at http://www.rothamsted.ac.uk.

2.2. DNA extraction and GeoChip hybridization

Genomic DNA was extracted from archived soil samples (Zhou et al., 1996). Despite air-drying and long-term storage, high-quality DNA was recovered from the soils archived for more than 150 years (260/280 and 260/230 > 1.8). GeoChip 4.0 was used to dissect the microbial community functional structure. Hybridization, imaging, and data preprocessing were performed as previously described (Tu et al., 2014).*2.3 Time-decay relationships and time-scale dependence of turnover*.

We used time-decay to evaluate the decline of community similarity over time. A log-linear model was fitted between the change in community structure (assessed by pair-wise similarities based on Bray–Curtis distance) and days elapsed (Shade et al., 2013). Time decay relationships (TDRs) is calculated as follows: $\log_{10}(S_s)$ = constant - w $\log_{10}(T)$, where S_s is the pairwise similarity in community composition (1–Bray-Curtis distance), T is the time interval. The slope of the log-linear model is a rate of community change, referring as turnover. For the constant value, it is the intercept of TDRs equation (log scaled), which means the initial pairwise similarity in community composition between control and fertilized samples (when time interval = 1). The intercept of this model is similar to the nugget effect (residual inter-sample variance as distance decreases to zero) in semi-variograms (Nekola and White, 1999). Considering the initial similarity of microbial community, we think the similarity of each taxon as a constant value in terms of them characteristic (i.e., when time interval is close to zero, the w is close to zero). Comparisons of w values between control and fertilized samples and p values were achieved by bootstrapping (999 times), followed by a pairwise t-test.

2.3. Estimating stochasticity of community assembly

Null models are an essential tool for assessing the issue of multiple assembly processes by mimicking the consequences of random processes (Chase and Myers, 2011; Myers et al., 2013; Stegen et al., 2013). The STij values are only calculated using 24 samples in our Rothamsted data. The relative importance of stochastic processes were assessed with the

ST, i.e., the ratio of mean expected similarity in the null model ($\overline{E_{ij}}$) to observed similarity (C_{ij}), when deterministic factors generally drove community structure to be more similar than the null expectation ($C_{ij} \geq \overline{E_{ij}}$) (Zhou et al., 2014). In the present study, *ST* is calculated with some modifications as below to be more general (Guo et al., 2018; Ning et al., 2019).

$$ST_{ij} = \frac{\overline{E_{ij}}}{C_{ij}} \cdot \frac{D_{ij}}{\overline{G_{ij}}} \quad if\ C_{ij} \geq \overline{E_{ij}}$$

$$ST_{ij} = \frac{\overline{G_{ij}}}{D_{ij}} \cdot \frac{C_{ij}}{\overline{E_{ij}}} \quad if\ C_{ij} < \overline{E_{ij}}$$

where C_{ij} is the observed similarity between samples *i* and *j*, D_{ij} is the dissimilarity, and $D_{ij} = 1\text{-}C_{ij}$. E_{ij} is an expected similarity between null samples *i* and *j*, while G_{ij} is an expected dissimilarity in the null model. Thus, ST_{ij} ranges from 0 to 1, where the minimum value is zero when extremely deterministic assembly drives communities completely the same or totally different ($C_{ij} = 0$ or $D_{ij} = 0$), and the maximum value is one when completely stochastic assembly makes communities the same as null expectation ($C_{ij} = \overline{E_{ij}}$ and $D_{ij} = \overline{G_{ij}}$). Considering that relative abundances carry information useful for understanding ecological processes (Anderson et al., 2011; Stegen et al., 2013), the similarity/dissimilarity was measured by the abundance-weighted Bray-Curtis index. The null communities are generated by randomizing the observed community structure 1000 times based on a null model algorithm described previously (Stegen et al., 2013). Since we focus on mechanisms underlying temporal succession rather than spatial turnover, the null hypothesis is stochastic succession over time, i.e., the detection or non-detection of an individual of a certain species at a time point is due to stochastic processes (e.g., neutral dispersal from a metacommunity, stochastic birth/death, and random diversification) without any determinism (e.g., environmental filtering, competition, mutualism, and evolution under natural selection). Accordingly, all samples from the same plot at different time points are assumed to be from the same species pool across time in our null model algorithm. During randomization, the richness (observed gene numbers) and total gene abundance in each sample are fixed as observed, while the occurrence probability of each gene is proportional to its observed occurrence frequency, and the abundance of each gene is based on random draws of individuals with probability proportional to its relative abundance in the regional species pool. Then, $\overline{E_{ij}}$ is calculated as the average null expected similarity between samples *i* and *j* and $\overline{G_{ij}}$ as the average null expected dissimilarity. ST_{ij} was calculated between every two time points for each plot. Then, the differences in ST_{ij} values between the control and fertilized plots were tested with a paired *t*-test. The relationship between the pairwise ST_{ij} and the time interval in each plot was fit to a linear model, and the significance of each model and the slope difference between control and fertilized plots were tested in the same way as described for the time-decay model.

Furthermore, ST_{ij} was also separately calculated for each of the major C and N cycling functional gene groups, considering that ecological stochasticity and its response to fertilization could be different in different guilds. Subcommunities were extracted for each gene group, including the nitrification group, the denitrification group, the labile C degradation group, etc. Then, the ST_{ij} of each gene group was calculated using the same method described above. To evaluate the effect of fertilization on ecological stochasticity, Cohen's *d* (paired per time point) (Cohen, 1988) was calculated between the ST_{ij} values in the control and fertilized plots for each gene group. The standardized effects are large, medium, small or negligible when $|d| > 0.8$, $0.5 < |d| < 0.8$, $0.2 < |d| < 0.5$, or $|d| < 0.2$, respectively, and *d* values can be positive or negative to reflect increases or decreases in the standardized effect size of fertilization on the stochasticity ratio.

2.4. Other statistical analyses

Detrended correspondence analysis (DCA) were used to determine the overall pattern of microbial functional communities. The microbial temporal patterns under fertilized and control plots were also determined by non-metric multidimensional scaling ordination based on the Bray–Curtis dissimilarity. Three different non-parametric multivariate statistical tests (analysis of similarity (ANOSIM), nonparametric multivariate analysis of variance (Adonis), and multi-response permutation procedure (MRPP)) were used to test the differences in soil microbial communities under fertilization and control treatments. The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups and is calculated as follows:

$$R = \frac{r_b - r_w}{\frac{1}{4}[n(n-1)]}$$

where r_b is the mean rank of between-group dissimilarities, r_w is the mean rank of within-group dissimilarities, and n is the total number. According to the ranks of the dissimilarities, statistic *R* ranges from −1 to +1. Ecological communities rarely achieve an R value < 0. Thus, $R \approx 0$ indicates no difference among groups, and $R > 0$ indicates that groups differ in community composition.

To fit the w and time duration, linear and other nonlinear models in Table S1 were implemented by using the functions "lm" and "nls" in the R package "stats". Since coefficient of determination (R^2) can be inadequate for nonlinear models (Spiess and Neumeyer, 2010), model fitting was compared by standard error of the estimate (SEE) (Weisberg, 2015), accuracy (χ_a) and precision (ρ) coefficients derived from concordance correlation coefficient (Lin et al., 2002), in addition to R^2. The significance of each model was calculated using a permutational test in which the time-scale values were permutated 2000 times, and the observed SEE was compared with those of permutated data to calculate the *p* value.

3. Results

3.1. Overall pattern of microbial functional communities

An average of 25,842 and 29,541 functional genes were detected in the control and fertilized soils, respectively (Fig. S1). The number of genes overlapping between pairwise samples significantly decreased with time in both control ($p = 0.048$) and fertilized soils ($p < 0.0001$). Detrended correspondence analysis (DCA) was performed to visualize the overall patterns of microbial functional communities (Fig. 1a). The control and fertilized samples were clearly separated, indicating dissimilar microbial functional communities between control and long-term inorganic fertilized plots. The microbial communities from fertilized soils were more tightly clustered than those from control soil. Additionally, the dispersion of samples around the group centroid (Houseman et al., 2008) was quantified. Fertilization led to microbial community convergence ($p < 0.01$) (Fig. 1b). Moreover, three complementary nonparametric multivariate statistical tests (Adonis, ANOSIM and MRPP) further revealed that the overall microbial functional structures were significantly different ($p < 0.01$) between the fertilized and control plots (Table 1). These results indicated that inorganic fertilization significantly altered the functional composition and structure of soil microbial communities.

3.2. Microbial temporal turnover under long-term fertilization

Microbial community succession patterns were estimated by linear regression between double log-transformed community similarity (1 – Bray-Curtis distance) and time intervals (Fig. 2a). As expected, significant TDRs were observed for both control (slope = 0.028, $p = 0.005$) and fertilized soils (slope = 0.018, $p = 0.001$). Long-term fertilization

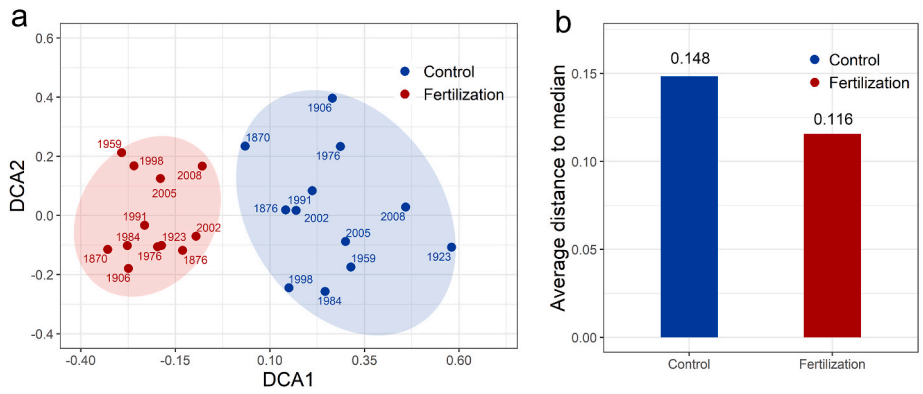

Fig. 1. Effects of century-long fertilization on the distributions of soil microbial communities. (a) Overall pattern of microbial functional communities from 1870 to 2008 by Detrended Correspondence Analysis (DCA). Numbers beside the dots indicate sampling years. (b) Dispersion of soil microbial communities in control and fertilized samples.

Table 1
Significance tests of the effects of inorganic fertilization on the microbial functional community structure across century long-term with three different statistical approaches.

Data sets	ANOSIM		Adonis		MRPP	
	R	*p*	F	*p*	δ	*p*
All functional genes	0.641	0.001	9.397	0.001	0.197	0.001
C cycling	0.615	0.001	8.639	0.001	0.199	0.001
N cycling	0.658	0.001	9.687	0.001	0.195	0.001
Phosphorus	0.703	0.001	10.582	0.001	0.212	0.001
Sulfur	0.670	0.001	9.630	0.001	0.214	0.001

All three tests are non-parametric multivariate analyses based on Bray–Curtis dissimilarities among samples, including analysis of similarity (ANOSIM), the permutational multivariate analysis of variance (Adonis), and multiple response permutation procedure (MRPP).

significantly decreased microbial temporal turnover rates as determined by a pairwise *t*-test based on bootstrapping ($p < 0.0001$), which indicated greater community similarity in fertilized soils than in control soil. The effect of DNA degradation with storage time was normalized by applying the same molar quantity of DNA to each GeoChip array (Wu et al., 2006), regardless of extraction yield. Whether or not DNA recovery was considered, temporal turnover decreased under long-term fertilization (Fig. S2).

The slopes of the TDRs for various functional (C, N, P and S cycling) and phylogenetic (archaea, fungi and bacteria) groups were estimated to obtain additional insights into the temporal changes of different functional and phylogenetic groups in response to fertilization (Fig. 2b, Table S1). Significant TDRs were found for most functional groups in both control and fertilized soils, with nitrification groups being an exception. Denitrification groups exhibited the greatest difference of *w* between fertilized and control samples. In addition, long-term fertilization decreased *w* for all phylogenetic groups. The greatest differences were found in fungi ($p < 0.0001$), followed by gram-positive bacteria.

A meta-analysis was conducted to further understand the long-term microbial succession pattern over time periods (Fig. 3a, Table S2). Microbial temporal turnovers varied considerably, ranging from 0.001 to 0.31, in different habitats and across different time scales (days to more than a century). All the published TDR slopes and those from our study were tested as a function of their time scales, and the rational function model showed a better fit than other models (Table S3). We noted that although several models were significant, they are not well fitted with low R^2 (0.16–0.18) and the variability of *w* during shorter period were quite high. So we can only infer that the microbial succession rate is potentially time scale-dependent. We propose a two-phase conceptual schematic diagram to indicate the potential tendency of microbial

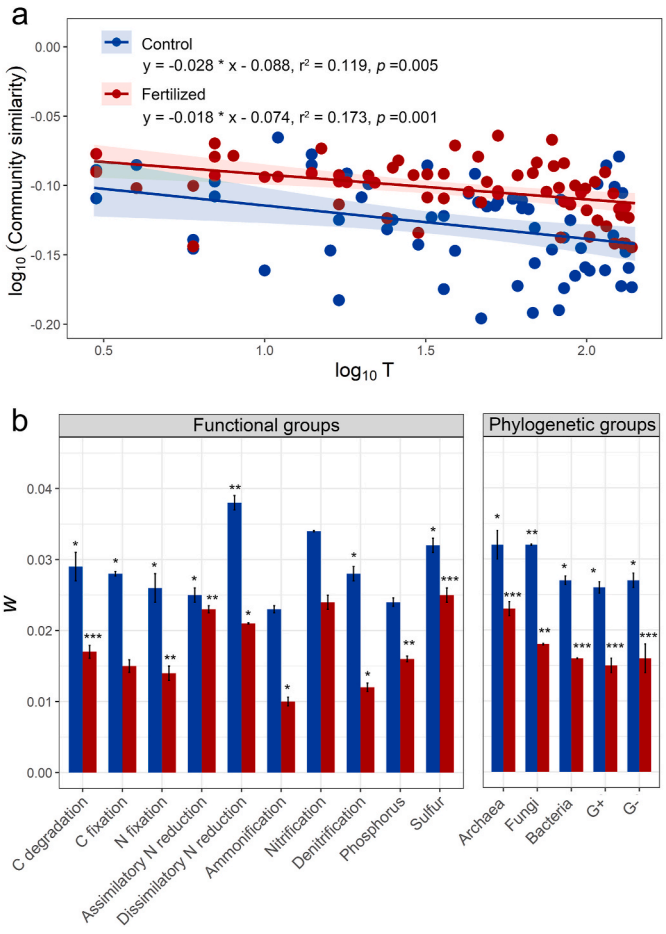

Fig. 2. Time-decay relationships (TDR) of soil microbial communities across more than a century. (a) Linear regression between community similarity (*Ss*) and logarithmic time difference (T) of fertilized (red) and control samples (blue), respectively. (b) Temporal turnover (slope of the linear regression) of functional and phylogenetic groups. *<0.05, **<0.01, ***<0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

succession pattern over time periods (Fig. 3b). Based on a rational function, When $b_1 \ll T_s \ll b_2$, probably over periods from weeks to years, *w* becomes more or less constant (Phase I). After this, *w* should decrease as the time scale becomes very large, and hence with a long tail phase where *w* consistently decreases along time and gradually becomes close

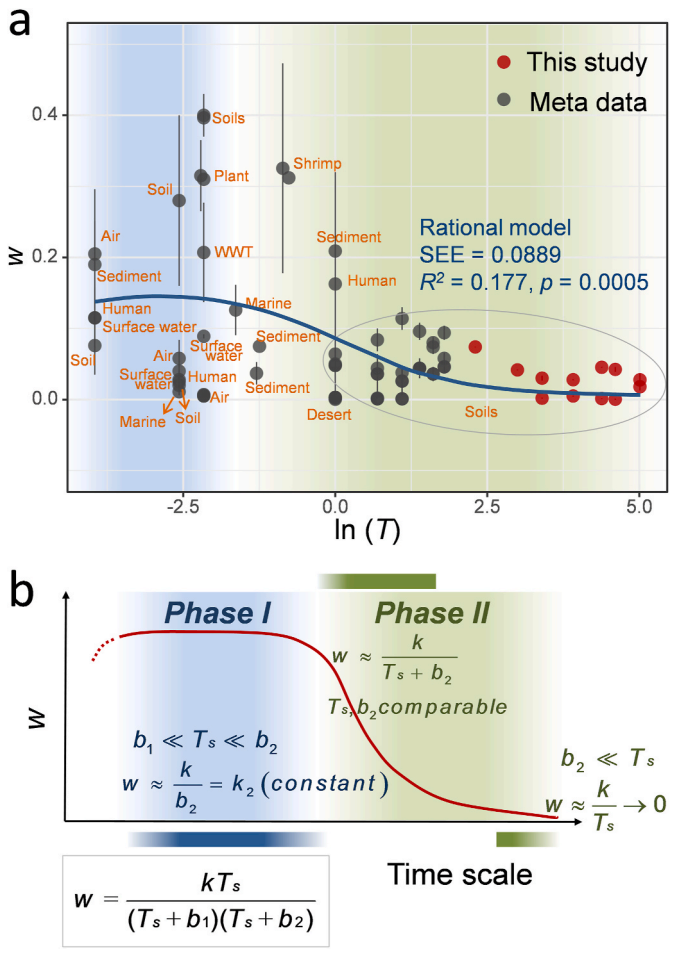

Fig. 3. Microbial temporal turnover (w) across time scales. (a) A meta-analysis combined with this was conducted to understand the microbial temporal turnover at different time scales. The red line represents the best-fitted rational function model. SEE, standard error of the estimate. Succession data of microbial communities were obtained from 76 datasets in published studies (Supplementary Table S2) and this study. The habitats include air, marine, leaf, surface water, wastewater treatment and soil habitats. (b) Conceptual schematic diagram to indicate the potential two-phase pattern of microbial temporal turnover. The trend can be described by a rational function model that has different approximate formulas at different time scales (phases). T_s, time scale, in year; k, b_1, b_2, k_1, k_2, constant coefficients. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to zero (Phase II).

3.3. Ecological stochasticity under long-term fertilization

Null model-based quantification of ecological stochasticity was conducted to test the hypothesis that long-term inorganic fertilization alters the relative importance of stochastic and deterministic processes in driving community succession (Fig. 4). Community assembly was relatively more stochastic in the control plot (73% stochasticity ratio (ST) > 0.5) than in the fertilized plots (42% ST > 0.5). The temporal succession changed from relatively deterministic (3-year ST = 0.35–0.45 for control and fertilization) to more stochastic (138-year ST = 0.82 for control and 0.68 for fertilization), suggesting that stochastic processes became increasingly important with increasing lengths of community succession. Moreover, fertilization significantly slowed down the increase rate of stochastic processes, resulting in an ST-time slope of nearly half that of the control (slope = 0.132 for control and 0.070 for fertilization, bootstrapping test $P < 0.0001$).

We further compared the effects of fertilization on the ecological stochasticity of different soil functional guilds involved in C and N cycling. As shown in the conceptual diagram (Fig. 5, Table S4), nutrient amendment directly increased soil N availability and indirectly increased soil labile and recalcitrant C pools by enhancing aboveground biomass. Simultaneously, century-long fertilization showed medium-to large-effect-size (Cohen's d = −0.603~ −1.459) decreases in ecological stochasticity in the succession of functional guilds involved in C fixation and degradation; N mineralization, fixation and reduction; and denitrification, coupled with significantly ($p < 0.0001$) decreased TDR slopes. Only *hao* genes were detected from the nitrification group, and the direct addition of sodium nitrate as fertilizer only slightly (Cohen's d = −0.398) decreased the stochasticity of succession in this group, corresponding to a nonsignificant change in the TDR slope.

4. Discussion

Assembly mechanisms shaping community structure have recently been of a great interest (Chase, 2010; Ellwood et al., 2009; Kraft et al., 2011; Zhou and Ning, 2017). It is important to understand the drivers controlling ecological succession in response to environmental perturbations, especially on long-term time scales (Chang and Hill-eRisLambers, 2016; Ferrenberg et al., 2013). Stochasticity has been shown to decrease through succession (Dini-Andreotea et al., 2015) and Tripathi et al. (2018) showed that change in assembly processes during succession was not due to time per se, but rather to changes in pH. Here, our study indicated that the succession of microbial functional structure was increasingly driven by stochastic processes as time proceeded. This result is in accordance with the increasing importance of stochastic succession previously observed in taxonomic/phylogenetic turnovers of microbial communities in resource-rich or low-stress environments (Ofiteru et al., 2010; Tripathi et al., 2018; Zhou et al., 2014). This pattern of increasing stochasticity might be due to the cumulative effects of neutral dispersal and random drift over time and/or decrease in cumulative deterministic linkages under unidirectional selection across a long time scale (Nemergut et al., 2013). Furthermore, we found that fertilization reduced the stochasticity of functional structure turnover across the century. The deterministic/stochastic balance is fundamentally tied to the spatial scale of sampling. For instance, the soil samples analyzed here contain hundreds to thousands of individual micro-environments on which communities could deterministically assemble, yet manifest as being stochastic when aggregated. Fertilization may serve to homogenize these micro-sites with regard to nutrient availability, and this may be why we observed more apparent determinism in this experimental treatment.

Our results further indicated that variations in TDRs of different microbial functional groups were strongly associated with a reduction in stochastic assembly. Inorganic fertilization may exert different selective effects on the regional species pool and specific species (Francioli et al., 2016) through changes in soil C and N stoichiometric characteristics (Jian et al., 2016). Nitrate fertilization increased the concentration of a specific substrate for denitrifiers and stimulated plant growth and thus increased specific C and organic N plant inputs (Huang et al., 2019; Liang et al., 2015a). Hence, large effects derived from the decrease in stochastic assembly were observed for functional groups involved in C degradation and fixation, N fixation, and denitrification. Correspondingly, of the genes involved in N cycling, nitrification genes were the least impacted by the changing importance of deterministic factors. This behavior may be because the nitrifiers are less abundant and more specialized than other taxa involved in N cycling. There are relatively few ammonia oxidizing bacteria (and archaea) and nitrite oxidizers, resulting in limited potential for stochastic or deterministic divergence. Second, fertilization could also indirectly enhance the selection of host-associated microbial communities through changing plant diversity and biomass (Yuan et al., 2016). Considerable species loss following N enrichment is observed across terrestrial herbaceous ecosystems (Clark

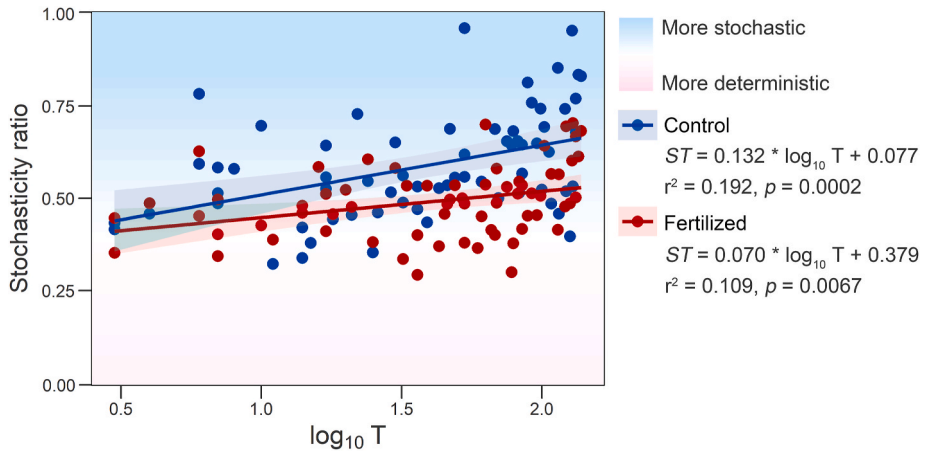

Fig. 4. Effect of fertilization on community assembly stochasticity and its temporal change based on null model analysis. The stochasticity ratio was calculated from each pairwise comparison between time points in each plot. The STij values are only calculated using 24 samples in our Rothamsted data.

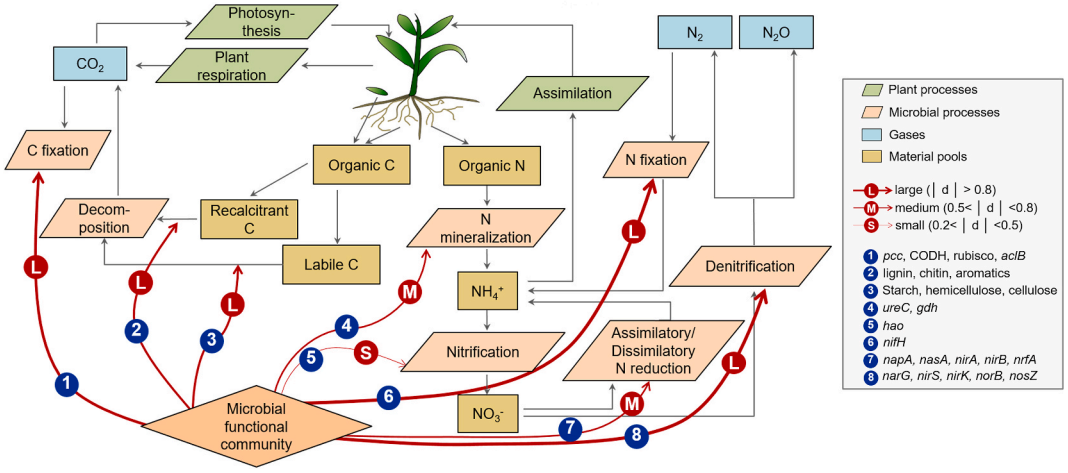

Fig. 5. A conceptual diagram of the impact of long-term fertilization on the assembly of microbial functional communities and its potential impacts on microbial functions and ecological variables. Red circles denote impacts on the underlying ecological stochasticity, based on the standardized effect size of fertilization on the stochasticity ratio of each gene group, all of which decreased (negative Cohen's *d* values) in response to fertilization (Supplementary Table S3). Labels of "L", "M", and "S" in red circles and the widths of the red lines are proportional to absolute values of Cohen's *d* and indicate that the decrease in stochasticity was large ($|d|>0.8$), medium ($0.5<|d|<0.8$), or small ($0.2<|d|<0.5$) in effect size. Black arrows indicate material flows. Material pools are represented by yellow rectangles, gases by blue rectangles, microbial processes by orange parallelograms, and plant processes by green parallelograms. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

et al., 2007). Plant species diversity significantly decreased with long-term fertilization in the PGE (Liang et al., 2015b), which may result in a more specialized habitat and is another important selection process (Robinson et al., 2010; Valyi et al., 2016). This trend could explain why fertilization showed large to medium effects on the guilds related to plant C and organic N transformation and root-associated N fixation.

Moreover, it has been recognized that numerous patterns and processes are dependent on temporal scales (Ladau and Eloe-Fadrosh, 2019). Significant insights into assembly processes on long (>100 year) time scales have been provided (Dini-Andreotea et al., 2015; Tripathi et al., 2018). Different processes may dominate at different temporal scales. For example, some stochastic processes, such as dispersal, are likely to be particularly relevant to understand the temporal variations in β-diversity and succession over a longer period of time (Fierer et al., 2010; Langenheder et al., 2012). Based on the meta data (from days to decades) and this study (decades to a century), there showed a tendency that microbial turnover rate may be temporal scale-dependent only at longer period, and the turnover rate is getting smaller and smaller. In contrast, at shorter period, the turnover rate may be scale-independent, which may be more influenced by habitat, host, treatment etc. However, compared with spatial scale, temporal scale is far less considered in microbial ecology study (Shade et al., 2013). In future research, more attention should be paid to microbial succession on different time scales to solve the long-standing uncertainty of which processes are important in shaping microbial communities (Ladau and Eloe-Fadrosh, 2019).

In addition, the impact of archiving on microbial community DNA includes not only degradation in DNA quantity over time, but also rapid and long-term changes in microbial community composition during air-drying and storage. In this case, the DNA that remains in the air-dried

soil definitely could not fully represent what are in the original soil. There could be an influence of time-since-collection on the measured microbial community. Indeed, we could not assess the magnitude of the storage-induced biases or how these biases may affect the temporal changes of biodiversity. Since archived soil is the only option here, thus we mainly focus on the comparison of the relative changes between treatment and control. Moreover, microbial functional genes can be regarded as functional traits for microbes. For functional gene data, it is still not very clear whether both selection (variable/heterogeneous selection) and abnormal dispersal (limited/homogenizing dispersal) can make dissimilarity/similarity significantly different from null expectation. Thus, in this study, we generally use "stochastic" and "stochasticity" rather than specific name of a certain process. Further studies are required to overcome biases from the above reasons (Zhou et al., 2015b; Zhou and Ning, 2017).

In conclusion, our study demonstrates that intensive N applications resulted in decelerating the temporal turnover of soil functional communities, underpinning the differing influences of assembly mechanisms on century long microbial succession. The importance of stochastic assembly differed for soil functional guilds (C, N, P and S) with century long fertilization. These findings have important implications for predicting the ecological consequences of anthropogenically derived N addition at the century scale. The deterministic factors influencing community assembly in fertilized soils may reflect a community with low functional redundancy and hence possibly high susceptibility to disturbances. Terrestrial microbial communities would be much more convergent with increasing N load. Because N addition reduced stochasticity over time, the communities could converge more quickly to a community state with less stochasticity. This effect should be considered when predicting and protecting ecosystem services of soil microbial communities and informing sustainable agricultural practices. In addition, our study demonstrates the value of archived soils for microbial ecology studies employing metagenomic techniques (Cary and Fierer, 2014). The availability of archived soil samples makes it possible to track long-term (over a century) impacts of fertilization on grassland ecosystems and to better understand the succession of microbial communities in response to long-term anthropogenic activities across more than a century. It should be noted that our estimation of ecological stochasticity was based on null model analysis and thus cannot avoid interference from randomness due to some deterministic processes (e.g., environmental stochasticity), statistical uncertainties, and inherently high variation in molecular methods (Zhou et al., 2011, 2013). Thus, the values should be viewed as statistically approximate and are better used for relative comparison (Zhou and Ning, 2017). However, the long-term average trends, particularly the comparison of average trends between the contrasting treatments, are informative and provide novel and important insights.

Data accessibility

Microbial functional genes of century long-term soil samples by GeoChip data are available at Figshare, https://doi.org/10.6084/m9.figshare.7418504.

Author's contributions

All authors contributed intellectual input and assistance to this study and manuscript preparation. J.Z. developed the original conceptual idea. Y.L., D.N., L.W., I.C., D.N., S.M., J.S., P.H., B.S. and Z.L. contributed reagents and data analysis. L.W., and Z.L. did GeoChip analysis. Y. L., D.N. and J. Z. wrote the paper with help from S.M., P.H., I.C. and J.S.

Declaration of competing interest

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

Acknowledgements

This study was supported by the National Science Foundation of China (41877060, 41530856), the Youth Innovation Promotion Association of Chinese Academy of Sciences (grant 2016284), Scholar Program of the Jiangsu Province (BRA2019333), the UK Biotechnology and Biological Sciences Research Council through an Underwood Fellowship to L.W., the Rothamsted Research's Institute Strategic Programme – Soil to Nutrition (BBS/E/C/000I0310), the Long-Term Experiments National Capability (BBS/E/C/000J0300), and the U.S. National Science Foundation MacroSystems Biology program under contract (NSF EF-1065844).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.108023.

References

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H.V., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C., Swenson, N.G., 2011. Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. Ecology Letters 19–28.

Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology & Evolution 20, 634–641.

Campisano, A., Albanese, D., Yousaf, S., Pancher, M., Donati, C., Pertot, I., 2017. Temperature drives the assembly of endophytic communities' seasonal succession. Environmental Microbiology 19, 3353–3364.

Caruso, T., Chan, Y., Lacap, D.C., Lau, M.C.Y., McKay, C.P., Pointing, S.B., 2011. Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. The ISME Journal 5, 1406–1413.

Cary, S.C., Fierer, N., 2014. The importance of sample archiving in microbial ecology. Nature Reviews Microbiology 12, 789–790.

Chang, C., HilleRisLambers, J., 2016. Integrating succession and community assembly perspectives. F1000 Research 5.

Chase, J.M., 2010. Stochastic community assembly causes higher biodiversity in more productive environments. Science 328, 1388–1391.

Chase, J.M., Myers, J.A., 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical Transactions of the Royal Society B: Biological Sciences 366, 2351–2363.

Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10, 596–607.

Clements, F.E., 1916. Plant Succession: an Analysis of the Development of Vegetation. Carnegie Institute of Washington, Washington, DC.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, second ed. Lawrence Erlbaum Associates, Hillsdale, NJ.

Cowles, H.C., 1899. The ecological relations of the vegetation on the sand dunes of Lake Michigan. Botanical Gazette 27, 95–117, 167-202, 281-308, 361-391.

Darcy, J.L., Schmidt, S.K., Knelman, J.E., Cleveland, C.C., Castle, S.C., Nemergut, D.R., 2018. Phosphorus, not nitrogen, limits plants and microbial primary producers following glacial retreat. Science Advances 4, q942.

Dini-Andreote, F., Pylro, V.S., Baldrian, P., van Elsas, J.D., Salles, J.F., 2016. Ecological succession reveals potential signatures of marine-terrestrial transition in salt marsh fungal communities. The ISME Journal 10, 1984–1997.

Dini-Andreotea, F., Stegenb, J.C., van Elsasa, J.D., Sallesa, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proceedings of the National Academy of Sciences of the United States of America 112, E1326–E1332.

Ellwood, M.D.F., Manica, A., Foster, W.A., 2009. Stochastic and deterministic processes jointly structure tropical arthropod communities. Ecology Letters 12, 277–284.

Falk, M.W., Seshan, H., Dosoretz, C., Wuertz, S., 2013. Partial bioaugmentation to remove 3-chloroaniline slows bacterial species turnover rate in bioreactors. Water Research 47, 7109–7119.

Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Melbourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. The ISME Journal 7, 1102–1111.

Fierer, N., Nemergut, D., Knight, R., Craine, J.M., 2010. Changes through time: integrating microorganisms into the study of succession. Research in Microbiology 161, 635–642.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of

agriculturally relevant microbes are driven by long-term fertilization strategies. Frontiers in Microbiology 7.
Fukami, T., 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. In: Futuyma, D.J. (Ed.), Annual Review of Ecology Evolution and Systematics. Annual Reviews, Palo Alto, pp. 1–23.
Geisseler, D., Scow, K.M., 2014. Long-term effects of mineral fertilizers on soil microorganisms - a review. Soil Biology and Biochemistry 75, 54–63.
Guo, X., Feng, J., Shi, Z., Zhou, X., Yuan, M., Tao, X., Hale, L., Yuan, T., Wang, J., Qin, Y., Zhou, A., Fu, Y., Wu, L., He, Z., Van Nostrand, J.D., Ning, D., Liu, X., Luo, Y., Tiedje, J.M., Yang, Y., Zhou, J., 2018. Climate warming leads to divergent succession of grassland microbial communities. Nature Climate Change 8, 813–818.
Gutknecht, J.L.M., Field, C.B., Balser, T.C., 2012. Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. Global Change Biology 18, 2256–2269.
Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. The ISME Journal 3, 597–605.
Houseman, G.R., Mittelbach, G.G., Reynolds, H.L., Gross, K.L., 2008. Perturbations alter community convergence, divergence, and formation of multiple community states. Ecology 89, 2172–2180.
Huang, R., McGrath, S.P., Hirsch, P.R., Clark, I.M., Storkey, J., Wu, L., Zhou, J., Liang, Y., 2019. Plant–microbe networks in soil are weakened by century-long use of inorganic fertilizers. Microbial Biotechnology 12, 1464–1475.
Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biology and Biochemistry 101, 32–43.
Jiao, S., Luo, Y., Lu, M., Xiao, X., Lin, Y., Chen, W., Wei, G., 2017. Distinct succession patterns of abundant and rare bacteria in temporal microcosms with pollutants. Environmental Pollution 225, 497–505.
Jurburg, S.D., Nunes, I., Stegen, J.C., Le Roux, X., Priemé, A., Sørensen, S.J., Salles, J.F., 2017. Autogenic succession and deterministic recovery following disturbance in soil bacterial communities. Scientific Reports 7.
Kim, T., Jeong, J., Wells, G.F., Park, H., 2013. General and rare bacterial taxa demonstrating different temporal dynamic patterns in an activated sludge bioreactor. Applied Microbiology and Biotechnology 97, 1755–1765.
Knelman, J.E., Schmidt, S.K., Lynch, R.C., Darcy, J.L., Castle, S.C., Cleveland, C.C., Nemergut, D.R., 2014. Nutrient addition dramatically accelerates microbial community succession. PloS One 9.
Kraft, N.J.B., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M., Boyle, B., Anderson, M.J., Cornell, H.V., Davies, K.F., Freestone, A.L., Inouye, B.D., Harrison, S.P., Myers, J.A., 2011. Disentangling the drivers of beta diversity along latitudinal and elevational gradients. Science 333, 1755–1758.
Ladau, J., Eloe-Fadrosh, E.A., 2019. Spatial, temporal, and phylogenetic scales of microbial ecology. Trends in Microbiology 27, 662–669.
Langenheder, S., Berga, M., Ostman, O., Szekely, A.J., 2012. Temporal variation of beta-diversity and assembly mechanisms in a bacterial metacommunity. The ISME Journal 6, 1107–1114.
Liang, Y., Jiang, Y., Wang, F., Wen, C., Deng, Y., Xue, K., Qin, Y., Yang, Y., Wu, L., Zhou, J., Sun, B., 2015. Long-term soil transplant simulating climate change with latitude significantly alters microbial temporal turnover. The ISME Journal 9, 2561–2572.
Liang, Y., Wu, L., Clark, I.M., Xue, K., Yang, Y., Van Nostrand, J.D., Deng, Y., He, Z., McGrath, S., Storkey, J., Hirsch, P.R., Sun, B., Zhou, J., 2015a. Over 150 years long-term fertilization alters spatial scaling of microbial biodiversity. mBio 6, e215–e240.
Liang, Y., Wu, L., Clark, I.M., Xue, K., Yang, Y., Van Nostrand, J.D., Deng, Y., He, Z., McGrath, S., Storkey, J., Hirsch, P.R., Sun, B., Zhou, J., 2015b. Over 150 years long-term fertilization alters spatial scaling of microbial biodiversity. mBio 6, e215–e240.
Lin, L., Hedayat, A.S., Sinha, B., Yang, M., 2002. Statistical methods in assessing agreement: models, issues, and tools. Journal of the American Statistical Association 257.
Lin, Q., De Vrieze, J., Li, C., Li, J., Li, J., Yao, M., Hedenec, P., Li, H., Li, T., Rui, J., Frouz, J., Li, X., 2017. Temperature regulates deterministic processes and the succession of microbial interactions in anaerobic digestion process. Water Research 123, 134–143.
Liu, W., Wang, Q., Wang, B., Wang, X., Franks, A.E., Teng, Y., Li, Z., Luo, Y., 2015. Changes in the abundance and structure of bacterial communities under long-term fertilization treatments in a peanut monocropping system. Plant and Soil 395, 415–427.
Michaud, J., Schoenly, K.G., Moreau, G., 2015. Rewriting ecological succession history: did carrion ecologists get there first? Quarterly Review of Biology 90, 45–66.
Myers, J.A., Chase, J.M., Jimenez, I., Jorgensen, P.M., Araujo-Murakami, A., Paniagua-Zambrana, N., Seidel, R., 2013. Beta-diversity in temperate and tropical forests reflects dissimilar mechanisms of community assembly. Ecology Letters 16, 151–157.
Nekola, J.C., White, P.S., 1999. The distance decay of similarity in biogeography and ecology. Journal of Biogeography 26, 867–878.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77, 342–356.
Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. Proceedings of the National Academy of Sciences of the United States of America 116, 16892–16898.
Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270.
Ofiteru, I.D., Lunn, M., Curtis, T.P., Wells, G.F., Criddle, C.S., Francis, C.A., Sloan, W.T., 2010. Combined niche and neutral effects in a microbial wastewater treatment community. Proceedings of the National Academy of Sciences of the United States of America 107, 15345–15350.
Pla-Rabes, S., Flower, R.J., Shilland, E.M., Kreiser, A.M., 2011. Assessing microbial diversity using recent lake sediments and estimations of spatio-temporal diversity. Journal of Biogeography 38, 2033–2040.
Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463–3470.
Reay, D.S., Dentener, F., Smith, P., Grace, J., Feely, R.A., 2008. Global nitrogen deposition and carbon sinks. Nature Geoscience 1, 430–437.
Redford, A.J., Fierer, N., 2009. Bacterial succession on the leaf surface: a novel system for studying successional dynamics. Microbial Ecology 58, 189–198.
Robinson, C.J., Bohannan, B.J.M., Young, V.B., 2010. From structure to function: the ecology of host-associated microbial communities. Microbiology and Molecular Biology Reviews 74, 453–476.
Rousk, J., Brookes, P.C., Baath, E., 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. FEMS Microbiology Ecology 76, 89–99.
Shade, A., Caporaso, J.G., Handelsman, J., Knight, R., Fierer, N., 2013. A meta-analysis of changes in bacterial and archaeal communities with time. The ISME Journal 7, 1493–1506.
Spiess, A.N., Neumeyer, N., 2010. An evaluation of R2 as an inadequate measure for nonlinear models in pharmacological and biochemical research: a Monte Carlo approach. BMC Pharmacology and Toxicology 6.
Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. The ISME Journal 7, 2069–2079.
Stevens, C.J., David, T.I., Storkey, J., 2018. Atmospheric nitrogen deposition in terrestrial ecosystems: its impact on plant communities and consequences across trophic levels. Functional Ecology 32, 1757–1769.
Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303, 1876–1879.
Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. The ISME Journal 12, 1072–1083.
Tu, Q., Yu, H., He, Z., Deng, Y., Wu, L., Van Nostrand, J.D., Zhou, A., Voordeckers, J., Lee, Y., Qin, Y., Hemme, C.L., Shi, Z., Xue, K., Yuan, T., Wang, A., Zhou, J., 2014. GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. Molecular Ecology Resources 14, 914–928.
Valyi, K., Mardhiah, U., Rillig, M.C., Hempel, S., 2016. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. The ISME Journal 10, 2341–2351.
van der Gast, C.J., Ager, D., Lilley, A.K., 2008. Temporal scaling of bacterial taxa is influenced by both stochastic and deterministic ecological factors. Environmental Microbiology 10, 1411–1418.
Weisberg, S., 2015. Applied Linear Regression, third ed. John Wiley & Sons, NJ.
Wu, L., Liu, X., Schadt, C.W., Zhou, J., 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Applied and Environmental Microbiology 72, 4931–4941.
Xiong, J., Chen, H., Hu, C., Ye, X., Kong, D., Zhang, D., 2015. Evidence of bacterioplankton community adaptation in response to long-term mariculture disturbance. Scientific Reports 5, 15274.
Xun, W., Zhao, J., Xue, C., Zhang, G., Ran, W., Wang, B., Shen, Q., Zhang, R., 2016. Significant alteration of soil bacterial communities and organic carbon decomposition by different long-term fertilization management conditions of extremely low-productivity arable soil in South China. Environmental Microbiology 18, 1907–1917.
Yuan, X., Knelman, J.E., Gasarch, E., Wang, D., Nemergut, D.R., Seastedt, T.R., 2016. Plant community and soil chemistry responses to long-term nitrogen inputs drive changes in alpine bacterial communities. Ecology 97, 1543–1554.
Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., Da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loque, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology 3, 470–480.
Zhang, T., Chen, H.Y.H., Ruan, H., 2018. Global negative effects of nitrogen deposition on soil microbes. The ISME Journal 12, 1817–1825.
Zhang, X., Johnston, E.R., Liu, W., Li, L., Han, X., 2016. Environmental changes affect the assembly of soil bacterial community primarily by mediating stochastic processes. Global Change Biology 22, 198–207.
Zhang, X., Liu, W., Bai, Y., Zhang, G., Han, X., 2011. Nitrogen deposition mediates the effects and importance of chance in changing biodiversity. Molecular Ecology 20, 429–438.
Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., Shen, W., 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant and Soil 326, 511–522.
Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D., Yang, Y., He, Z., Wu, L., Stahl, D.A., Hazen, T.C., Tiedje, J.M., Arkin, A.P., 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proceedings of the National Academy of Sciences of the United States of America 111, E836–E845.
Zhou, J., Guan, D., Zhou, B., Zhao, B., Ma, M., Qin, J., Jiang, X., Chen, S., Cao, F., Shen, D., Li, J., 2015a. Influence of 34-years of fertilization on bacterial communities

in an intensively cultivated black soil in northeast China. Soil Biology and Biochemistry 90, 42–51.
Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S.G., Alvarez-Cohen, L., 2015b. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. mBio 6, e2214–e2288.
Zhou, J., Jiang, Y., Deng, Y., Shi, Z., Zhou, B.Y., Xue, K., Wu, L., He, Z., Yang, Y., 2013. Random sampling process leads to overestimation of beta-diversity of microbial communities. mBio 4.
Zhou, J., Ning, D., 2017. Stochastic community assembly- Does it matter in microbial ecology? Microbiology and Molecular Biology Reviews 81, e2–e17.
Zhou, J., Wu, L., Deng, Y., Zhi, X., Jiang, Y., Tu, Q., Xie, J., Van Nostrand, J.D., He, Z., Yang, Y., 2011. Reproducibility and quantitation of amplicon sequencing-based detection. The ISME Journal 5, 1303–1313.
Zhou, J.Z., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Applied and Environmental Microbiology 62, 316–322.
Zhou, Z., Wang, C., Zheng, M., Jiang, L., Luo, Y., 2017. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. Soil Biology and Biochemistry 115, 433–441.