Environmental changes affect the assembly of soil bacterial community primarily by mediating stochastic processes

XIMEI ZHANG1,2,†, ERIC R. JOHNSTON3,†, WEI LIU4,5, LINGHAO LI6 and XINGGUO HAN1,6
1State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China, 2School of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA, 3School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA, 4Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, 5Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, 6State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Abstract
Both ‘species fitness difference’-based deterministic processes, such as competitive exclusion and environmental filtering, and ‘species fitness difference’-independent stochastic processes, such as birth/death and dispersal/colonization, can influence the assembly of soil microbial communities. However, how both types of processes are mediated by anthropogenic environmental changes has rarely been explored. Here we report a novel and general pattern that almost all anthropogenic environmental changes that took place in a grassland ecosystem affected soil bacterial community assembly primarily through promoting or restraining stochastic processes. We performed four experiments mimicking 16 types of environmental changes and separated the compositional variation of soil bacterial communities caused by each environmental change into deterministic and stochastic components, with a recently developed method. Briefly, because the difference between control and treatment communities is primarily caused by deterministic processes, the deterministic change was quantified as (mean compositional variation between treatment and control) – (mean compositional variation within control). The difference among replicate treatment communities is primarily caused by stochastic processes, so the stochastic change was estimated as (mean compositional variation within treatment) – (mean compositional variation within control). The absolute of the stochastic change was greater than that of the deterministic change across almost all environmental changes, which was robust for both taxonomic and functional-based criterion. Although the deterministic change may become more important as environmental changes last longer, our findings showed that changes usually occurred through mediating stochastic processes over 5 years, challenging the traditional determinism-dominated view.

Keywords: biodiversity loss, climate change, colonization, dispersal, global change, grassland, metagenome, neutral process, niche, nitrogen deposition

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Introduction
Soil microorganisms account for a large proportion of biodiversity on Earth and play vital roles in the regulation of various ecosystem processes (Torsvik et al., 2002; Falkowski et al., 2008). Meanwhile, they are severely disturbed by multiple anthropogenic environmental changes, such as climate warming, nitrogen deposition, and the loss of biodiversity (Vitousek et al., 1997; IPCC, 2007). Thus, a central aim of current microbial ecology research is to investigate the mechanisms by which environmental changes affect the assembly of soil microbial communities (Hanson et al., 2012; Nemergut et al., 2013; Zhou et al., 2014). Traditionally, the biodiversity and composition of a local microbial community are thought to be influenced predominantly through deterministic processes, following Baas-Becking’s idea (1934) ‘everything is everywhere, but, the environment selects’. In other words, a certain environmental condition will select a given combination of microbial species; as the environmental conditions change, some species will be favored and others will be selected against, and community composition will shift deterministically. However, recent studies have found that deterministic processes could account for only part of the community compositional variation, leaving a large portion of it unexplained (Ramette & Tiedje, 2007; Ge et al., 2008). Therefore, stochastic processes (e.g., random birth, death, dispersal, and colonization),
which are important in shaping assemblages of higher organisms (Hubbell, 1979, 2001; Barber & Marquis, 2011), have been recently identified as another important driver for microbial community composition (Telford et al., 2006; Woodcock et al., 2007; Peay et al., 2010; Maignien et al., 2014). Both deterministic and stochastic processes were governing the spatial distribution of microbial communities concurrently, and different processes were found to be dominant in different cases (Dumbrell et al., 2010; Caruso et al., 2011; Stegen et al., 2012; Wang et al., 2013; Peay & Bruns, 2014; Taylor et al., 2014; Zhou et al., 2014). Therefore, anthropogenic environmental changes, such as nitrogen fertilizer application and warming caused by greenhouse gases, may affect soil microbial assembly through modulating both deterministic and stochastic processes. In other words, as both processes were already driving the assembly of soil microbial community in undisturbed ecosystems, they may be promoted or restrained by environmental changes. For example, while the increase in ammonium availability at low nitrogen addition rate promoted the stochastic assembly of ammonia-oxidizing archaeal community in a temperate steppe ecosystem, soil acidification at high nitrogen addition rate restrained their stochastic assembly (Zhang et al., 2011). However, few studies have investigated the promotion or restraint of stochastic assembly, relative to deterministic shifts, induced by environmental changes.

Contrary to the traditional deterministic-dominating viewpoint, here we hypothesize that anthropogenic environmental changes affect soil microbial assembly primarily through promoting/restraining stochastic rather than deterministic processes, for three reasons. First, although many soil microbial species are traditionally viewed as very resistant and hardy (e.g., their populations/communities can survive long periods of stress such as lack of water), their individuals are essentially primitive and vulnerable [especially for the single-cell bacteria and archaea (Kabnick & Peattie, 1991)], such that many environmental changes such as extreme weather often have immediate and severe impact on many individuals of all species (independent of species fitness difference) and lead to their rapid stochastic mortality. This, for example, could occur when environmental changes lead to significant reductions in bacterial community size (Sheik et al., 2011). Second, some environmental changes will alter the soil physicochemical conditions quickly, which may create some new resource/space and thus promote microbial stochastic dispersal/colonization to make use of them (independent of species fitness difference). In contrast, some other changes in soil physicochemical conditions may decrease the resource/space (e.g., increased soil water content decreases void soil space (Zhou et al., 2002) and thus restrain the stochastic dispersal/colonization processes. Finally, while these stochastic processes may be affected quickly after the environmental change (e.g., microbial passive dispersal/colonization driven by the wind), the deterministic processes (e.g., interspecies competition after colonization of the new resource/space, and environmental filtering) caused by the changes in soil physicochemical characteristics will take a much longer time to have an effect on the soil microbial communities. Because these deterministic processes affect community assembly dependent on the difference in species fitness, which was often very small (Hubbell, 2001), it will take many generations for them to have a significant influence. For example, Ferrenberg et al. (2013) have found that a forest wildfire first promoted the stochastic assembly of soil bacterial species and later led to the deterministic community assembly because of the changes in soil chemical properties. In addition, microorganisms grow at only ~1% of their potential under natural settings, such as 1–2 generations per year in bulk soil (Grey & Williams, 1971), which would lead to the result that the promotion/restraint of stochastic processes is more important than the mediation of deterministic processes for many years.

Here, we attempted to ascertain whether changes in stochasticity or deterministic shifts were the primary response of soil bacterial communities subject to anthropogenic environmental changes. We conducted four multi-factorial experiments in a steppe ecosystem in the Inner Mongolia of China, which is representative of much of the Eurasian steppe region floristically and ecologically (Li et al., 1988). There were a total of 16 treatments in the four experiments, mimicking almost all types of anthropogenic environmental changes that occurred in this ecosystem. Specifically, the treatments included the removal of one, two, or three plant functional groups (PFGs), mowing (M), nitrogen addition (N), phosphorus addition (P), warming (T), and several of these conditions in combination (Data S1; Table S1 and S2). The composition of soil bacterial communities was investigated with 454 pyrosequencing targeting part of the 16S rRNA gene. We adopted a recently developed calculation method to directly quantify the deterministic and stochastic components of the treatment effects (Zhang et al., 2011). In particular, we estimated the changes in stochastic processes, relative to deterministic changes, for soil microbial communities undergoing each environmental perturbation, and also assessed the overall relative importance of the two components, independently, across all the 16 environmental changes. To exclude the influence of randomness and biases...
induced by the PCR amplification process on our results, we also assessed the deterministic and stochastic change by repeating the analysis, using 16S rRNA fragments derived from shotgun-metagenomic datasets. To validate the reliability of the direct-calculation method, we further analyzed the data with a null model method (Chase, 2010) and attempted to find linkage between results derived from either method. Different from the direct-calculation method comparing the treatment communities with the control communities and thus separating the deterministic and stochastic components of only the treatment effect (Zhang et al., 2011), the null model method compared the observed communities to the stochastically assembled communities, and thus assessed the deterministic effect caused by both current experimental treatment and past historical factors (such as the environmental changes occurred before the experimental treatment). In addition, to identify whether the dominant process for each condition was consistent for both taxonomic and functional composition, we further analyzed the gene content data from shotgun metagenomic sequencing.

Materials and methods

Experimental design

To investigate the effects of various environmental changes on the steppe ecosystem, we conducted four long-term experiments in Inner Mongolia, China. The details have been described before (Zhang et al., 2013) and were provided in the Data S1 (Table S1 and S2), and we provide only a brief summary here. In the first experiment, the effect of PFG diversity was investigated. To accomplish this, we identified three dominant PFGs and constructed three PFG diversity levels (0–2) different from the control (3; which contained all three PFGs, undisturbed), with a total of eight combinations of PFG composition and five replicates for each combination. In the second experiment, the effects of M, N addition, P addition, and their combinations were investigated, with a total of eight treatments and four replicates for each treatment. In the third experiment, the effects of M, N addition, W, and their combinations were investigated, with a total of eight treatments and four replicates for each treatment. In the fourth experiment, the effects of W and T and their combinations were evaluated using a total of four treatments and six replicates for each treatment. The second, third, and fourth experiments partly overlapped, and there were a total of 106 samples for all the four experiments (Zhang et al., 2013). All these treatments (environmental changes) were continuously applied every year since 2005 until the time of sampling, 2010. Soil sampling, physicochemical analysis, and bacterial community composition analysis have been described before (Zhang et al., 2013), and a brief summary was provided in the Data S2 and Table S3–S6.

Calculation of the deterministic change, the stochastic change, and their relative importance

The deterministic and stochastic changes caused by each treatment were calculated with a recently developed method (Zhang et al., 2011). Because there was no significant influence of spatial distance on the bacterial compositional variation among all plots in each experiment (Mantel tests: the first experiment, \( r = 0.026, P = 0.363 \); the second, \( r = -0.032, P = 0.339 \); the third, \( r = 0.021, P = 0.308 \); the fourth, \( r = 0.025, P = 0.248 \)), we assume that there was no systematic variation in the spatial heterogeneity of these communities before the treatments were applied. In other words, these plots were within a local homogeneous area (the distance between any two plots was <330 meter) with same environmental conditions (e.g., climate and soil). Therefore, the compositional variation between plots is caused by the deterministic processes of experimental treatments and the stochastic processes of birth/death, dispersal/colonization, etc. The compositional variation between the control plots is not caused by experimental treatments, and the mean compositional variation between each pair of control plots can be taken as the reference point. Although the compositional variation between the control and treatment plots is caused by both deterministic and stochastic processes, the changes of stochastic processes are expected to be nondirectional and those of deterministic processes to be directional. Thus, for each treatment, the deterministic change caused by the treatment can be approximated by \( D = [(\text{mean compositional variation between control and treatment}) - (\text{reference point})] \). For each treatment in every experiment, we used Mantel test (Bonnet & Peer, 2002) to check the influence of the difference in soil physicochemical indices among replicate plots on bacterial compositional variation and found no significant effects for almost all treatments (\( P > 0.05 \); Table S7 and Data S3), suggesting that deterministic processes were not responsible for the compositional variation among replicate plots, and thus, stochastic processes were the primary driver. Therefore, for each treatment, the stochastic change induced by the experimental treatment can be approximated by \( S = [(\text{mean compositional variation within treatment}) - (\text{reference point})] \). The deterministic or stochastic changes might be positive or negative, corresponding to promoting or restraining the compositional variations, respectively; their absolute values represented their magnitudes. Then, for each treatment, we could calculate the relative importance of the stochastic change = \( \frac{S}{D+\mu} \). It follows that the relative importance of the deterministic change = \( \frac{\mu}{D+\mu} \). Accordingly, if the relative importance of the stochastic change was >0.5, it means that stochastic processes were the primarily mediated processes; on the contrary, deterministic processes were the primarily mediated one.

For the first experiment, we calculated the deterministic change, the stochastic change, and their relative importance for seven treatments (Table S3). For the relative importance of the stochastic change, we further calculated the global mean of treatments in which one PFG was removed (three treatments) or two PFGs were removed (three treatments). The relative importance of the stochastic change for three cases (removal of one, two, or three PFGs) was then used in the subsequent analysis. For the second experiment (M, N, P and
their combinations), we calculated these values for seven cases, including M, N, P, M+N, M+P, N+P, and M+N+P (Table S4). Similarly, for the third experiment (M, N, W, and their combinations) and fourth experiment (W, T, and their combination), we calculated these values for seven and three cases, respectively (Table S5 and S6). For the same cases in the second, third, and fourth experiments, we selected the result in the case from the former rather than the latter experiment for the subsequent analysis, because the three experiments were partly overlapped and the same-treatment plots of the latter were only a subpart of the former according to the experimental design (Data S1) (Zhang et al., 2013). For example, for the case of ‘N’, we selected the result from the second rather than the third experiment (Table S4 and S5). Therefore, there were a total of 13 different cases in the latter three experiments, and 16 different cases in all the four experiments (Fig. 1). The method of one-sample T Test was used to test whether the relative importance of the stochastic change was significantly different from 0.5 across the entire study design (involving all 16 conditions). Linear regression was used to establish the relationship between the deterministic change and the relative importance of the stochastic change.

**Accounting for the influence of under-sampling**

Soil bacterial communities are too diverse to adequately sample all species present with only 3478 amplicon sequences (Data S2). Therefore, under-sampling of diversity may potentially contribute to stochastic processes. To account for the effect of under-sampling on the relative importance of the stochastic change, four different approaches were employed. First, all amplicon sequences were clustered into operational taxonomic units (OTUs) at the 97% threshold as well as at 95% and 90% thresholds. As the thresholds decreases, the potential total number of OTU decreases, and thus, the effect of under-sampling from 3478 sequences should decrease accordingly (Horner-Devine et al., 2004; Zhang et al., 2011). Second, for each experiment, the weighted UniFrac distance between every pair of plots was also calculated, which accounted for phylogenetic relatedness between sequences as well as the number of times a sequence was observed, and thus, is not affected as much by under-sampling (Lozupone et al., 2006, 2007). Third, under-sampling should have a larger effect on the relatively rarer OTUs which were sampled in only part of the plots; thus, the analysis was also performed using only the relatively abundant OTUs present in all plots. Finally, the PCR procedure in the aforementioned 16S rDNA gene sequencing process may have influenced the relative importance of the stochastic change due to uncontrolled PCR biases and experimental noise (Acinas et al., 2005). To eliminate this possibility, all 106 samples were also sequenced with a PCR-independent shotgun metagenomic approach (Mackelprang et al., 2011; Fierer et al., 2013). Briefly, DNA extracts were processed according to the description of the Illumina Paired-End Prep kit protocol. DNA was sheared mechanically and size-selected to ~180-bp and gel purified. Sequencing was performed in an Illuminia Hiseq 2000 platform located at Shanghai Majorbio.

![Fig. 1](image-url) The relative importance of the stochastic change under 16 environmental changes at three bacterial OTU definition levels. The value for ‘Removal of one PFG’ is the mean of three treatments (‘PR+PF’, ‘PB+PF’, and ‘PB+PR’ in Fig. S1); the value for ‘Removal of two PFGs’ is the mean of another three treatments (‘PF’, ‘PR’, and ‘PB’); the treatment of ‘Removal of three PFGs’ is the treatment of ‘No PFGs’. M, N, P, W, and T represent mowing, N addition, P addition, watering, and warming, respectively; ‘+’ means their concur-
Bio-pharm Technology Co., Ltd. 2.18 ± 0.08 (mean ± SE) Giga base pairs of DNA sequences were acquired for each sample. 16S rRNA gene encoding metagenomic reads were assigned against the 16S rDNA gene full-length sequences in the Greengenes database (May 2013 release), which were subsequently clustered into OTUs at the 97% threshold, using UCLUST closed-reference OTU picking in Qiime (Caporaso et al., 2010). To exclude the influence of unequal sampling, the relatively rarer OTUs with <1/892 relative abundance (there were 892 reads assigned to OTUs in the smallest sample) in each sample were removed for the calculation of the Bray–Curtis distance. Linear regression was used to establish the relationship between the relative importance of the stochastic change from all OTUs at the 97% threshold of 454 amplicon sequencing and that from each of the aforementioned four methods.

Linking the result from the direct-calculation method with that from a null model method

β diversity (such as the Jaccard’s dissimilarity), which represents the compositional variation between communities, is often used to infer the possible mechanisms (such as the roles of deterministic and stochastical processes) of community assembly. However, the difference in the β diversity indexes may be caused by the difference in the ecological processes as well as α and γ diversity. To exclude the influence of the other two diversity components, Chase (2010) has developed a null model method. Because this method was dependent on species presence/absence data and was sensitive to the noise from rare species, the OTUs with low abundance (<2/3478 relative abundance) were removed in each sample (Ferrenberg et al., 2013). Here we analyzed whether the observed communities were different from the stochastically assembled communities for each treatment, following the steps of Chase (2010) and Zhou et al. (2014). The following calculations were made: First, observed OTU richness in each replicate plot (e.g., \( \gamma_1 \) and \( \gamma_2 \) for plot 1 and 2, respectively) and the number of shared OTUs (SS\(_{obs} \)) between two plots; second, the total number of OTUs detected in the ‘OTU pool’ (γ diversity) from all replicate plots, and the proportion of the plots occupied by each OTU; third, the distribution of the expected shared OTUs from null model (SS\(_{exp} \)) by randomly drawing \( \gamma_1 \) and \( \gamma_2 \) OTUs from the OTU pool, and the probability of an OTU to be drawn is proportional to its among-plot occupancy. The SS\(_{exp} \) and the expected Jaccard’s similarity (J\(_{exp} \)) are obtained for each drawing, and the average Jaccard’s similarity (J\(_{exp} \)) and its SD are estimated based on 10 000 drawings (\( \sigma_{exp} \)). For each treatment, permutational analysis of multivariate dispersions (PERMDISP) was used to test whether the observed and expected Jaccard’s similarities were significantly different (Anderson, 2004). The magnitude of deterministic processes on community structure was further quantified with the index of SES (standard effect size): SES = \( (J_{obs} - J_{exp}) / \sigma_{exp} \) (Kraft et al., 2011; Zhou et al., 2014).

Because the SES value measures the deterministic effect caused by both current experimental treatment and past historical factors (for the control, the SES value measures the deterministic effect caused by past historical factors alone), the difference in the SES values between treatment and control can represent the deterministic effect caused by current treatment alone. Therefore, linear regression was used to establish the relationship between the absolute of the difference in the SES value between treatment and control (from the null model method) and the absolute of the deterministic change (from the direct-calculation method).

The relative importance of the stochastic change for functional composition

The shotgun metagenomic data contained information of community-wide microbial functional genes, which would be used to explore the influences of these environmental changes on various functional genes and the feedback of microbial communities to these environmental changes in the future. Here we only aimed to identify the primarily mediated processes for the functional composition. Briefly, the merged reads (>170 bp) were aligned to the KEGG database (Kanehisa & Goto, 2000), and the relative abundance of different functions was calculated. We focused on functions at two levels, including pathway and KO gene. For each pair of samples, the Bray–Curtis distance was calculated to represent the functional compositional variation (Bray & Curtis, 1957). The relative importance of the stochastic change under each of the 16 environmental changes was calculated with the experimentally based method (Zhang et al., 2011).

Results

The relative importance of the stochastic change across 16 environmental changes

The effects of experimental treatments on soil physico-chemical properties and bacterial communities have been reported previously, and a brief summary was provided in the Data S4 and Table S3–S6. The relative importance of the stochastic change was larger than 0.5 under 11 environmental changes, with the largest values at two watering treatments (W, W+T; Fig. 1). It was smaller than 0.5 under only five N-addition treatments (N, M+N, N+P, M+N+P, and M+N+W), but was still larger than 0.5 under the combination of N addition with watering (N+W). Considering changes in deterministic and stochastic processes holistically—as a wide array of relevant environmental changes influencing microbial community structure—the relative importance of the stochastic change was significantly larger than 0.5 across all the 16 environmental changes (\( P < 0.05 \); see the insect in Fig. 1), with a mean ± SE (standard error) of 0.588 ± 0.040. In addition, the relative importance of the stochastic change showed significant negative linear relationship with the deterministic change (Fig. 2).
The influence of under-sampling on the relative importance of the stochastic change

We adopted four different methods to examine the influence of under-sampling on the results (see the detailed logic in the Methods). As the OTU threshold decreased from 97% to 95% and 90%, the relative importance of the stochastic change across all the 16 environmental changes kept almost unchanged (0.588 ± 0.040, 0.587 ± 0.032 and 0.596 ± 0.036, respectively) and was always significantly larger than 0.5 (P < 0.05; see the insect in Fig. 1).

The relative importance of the stochastic change based on the weighted UniFrac distance was positively correlated with that based on the Bray–Curtis distance from OTUs (R² = 0.537; Fig. 3a), with a mean ± SE across all the 16 environmental changes of 0.640 ± 0.077.

The relative importance of the stochastic change based on the Bray–Curtis distance from only common OTUs was positively correlated with that from all OTUs (R² = 0.487; Fig. 3b), with a mean ± SE across all the 16 environmental changes of 0.584 ± 0.042.

The relative importance of the stochastic change based on the Bray–Curtis distance from OTUs of shotgun metagenomic data was positively correlated with that from OTUs of 454 amplicon sequencing (R² = 0.613; Fig. 3c), with a mean ± SE of 0.616 ± 0.028 across all 16 environmental changes.

Linking the result from the direct-calculation method with that from the null model method

We analyzed whether the community similarities between replicate plots were significantly different from those between stochastically assembled communities, with a null model method. For each control and treatment in the four experiments, the observed Jaccard’s similarities were significantly smaller than the expected similarities (PERMDISP: P < 0.05; Table S8), indicating that the deterministic processes were operating. In every experiment, the difference in the SES value between each treatment and the control was small (compared to the absolute of the SES value in the control; Table S8), meaning that these environmental changes had small influences on the magnitude of deterministic vs. stochastic processes. The absolute of the difference in the SES value between treatment and control showed significant positive linear relationship with the absolute of the deterministic change from the direction-method (Fig. 4), successfully linking the results of the two methods.

The relative importance of the stochastic change for functional composition

At both functional levels (pathway and KO gene), the relative importance of the stochastic change across all the 16 environmental changes was larger than 0.5 statistically (P < 0.05; see the inside insect in Fig. 5). Actually, it was <0.5 under only three environmental
changes, including removal of one, two, and three PFGs.

**Discussion**

Briefly, we mimicked many impactful anthropogenic environmental changes that occurred in this steppe ecosystem, and directly quantified the deterministic change, the stochastic change and their relative importance in driving soil bacterial assembly caused by each environmental change. These environmental changes promoted or restrained the deterministic and stochastic changes, and different environmental changes had different influences (Data S5; Fig. S1). More importantly, the relative importance of the stochastic change was significantly larger than 0.5 across all these environmental changes ($P < 0.05$; Fig. 1). Considering each condition independently, it was larger than 0.5 under eleven environmental changes, including the loss of PFGs, mowing, P addition, watering, warming, and some of their combinations. It was smaller than 0.5 under only five N-addition treatments (Fig. 1). Here we argue that these N-addition treatments may have also caused the relative importance of the stochastic change to be >0.5 in the earlier years, similar to the other eleven environmental changes. It has been found that in a six-year experiment in the same steppe ecosystem, as N-addition rate increased from 0–5.25 to 10.5–28 g N/m$^2$ yr, the relative importance of the stochastic change for soil bacterial community assembly changed from >0.5 to <0.5 (Zhang et al., 2011). Because N addition has cumulative effect (Duprée et al., 2010), the result at high N-addition rate in earlier years (e.g., the first and second year) should be similar to that at low N-addition rate in later years (e.g., the sixth year). In other words, the result suggested that the relative importance of the stochastic change at high N-addition rate in earlier

![Graph](image)

**Fig. 4** Linking the result from the direct-calculation method with that from the null model method.

![Graph](image)

**Fig. 5** The relative importance of the stochastic change under 16 environmental changes at two function definition levels. See the meaning of all treatments in the legends of Fig. 1. The relative importance of the stochastic change across all 16 treatments is significantly larger than 0.5 (One-Sample T Test: pathway, $t = 3.206$, $P = 0.006$; KO gene, $t = 3.262$, $P = 0.005$). See mean ± SE (standard error) of all the 16 treatments in the insect.

years was also >0.5, and it changed into <0.5 in only the later years. In the current study, the N-addition treatments were at a high rate (10 g N/m²/yr) in a later year (the fifth year), so the relative importance of the stochastic change has already changed from >0.5 to <0.5. In addition, we found a pattern that the relative importance of the stochastic change showed negative linear relationship with the deterministic change (Fig. 2), which also implied that for N-addition treatments at the earlier years when the deterministic change was smaller, the relative importance of the stochastic change should be much larger. Overall, our results demonstrated the importance of stochastic changes relative to deterministic changes after 5–6 years’ continuous environmental perturbation.

The importance of stochastic vs. deterministic change might be caused by the under-sampling of bacterial diversity and/or the novel direct-calculation method, besides the ecological stochastic processes. Therefore, we have adopted four different methods to test the influence of under-sampling. Each approach consistently showed that under-sampling had little influence on the relative importance of the stochastic change (see the detailed logic in the Methods; Figs 1 and 3). In addition, there is no reason for us to expect that there is different deterministic vs. stochastic importance for the sampled and un-sampled species, because here we calculated deterministic/stochastic changes basing on the relative abundance of OTUs rather than their presence/absence.

To test the direct-calculation method, we applied it to a previous study and found that the result was consistent with the previous conclusion, which demonstrated the reliability of this method (Data S6). In addition, the negative linear relationship between the deterministic change and the relative importance of the stochastic change shown in Fig. 2 is consistent with the traditional expectation, demonstrating the effectiveness of the method once again.

To validate the direct-calculation method, we also analyzed the data with a null model method, which has been widely used in macro-ecological researches (Chase, 2010). Although the observed Jaccard’s similarities were significantly smaller than the expected similarities for stochastically assembled communities (P < 0.05; Table S8), which suggested that the deterministic processes have played some role in structuring soil bacterial communities, the possible effect of stochastic processes, such as birth, death, and dispersal/colonization in such cases cannot be excluded (Hubbell, 2001; Ramette & Tiedje, 2007; Woodcock et al., 2007). In particular, environmental filtering was not the acting process, because it would cause the observed similarities between replicate communities to be larger than the expected similarities between stochastically assembled communities, being contrast with the actual pattern (Table S8). Therefore, the observed similarities being smaller than the expected similarities should be due to certain OTUs first becoming dominant in different plots through stochastic processes such as dispersal/colonization, which then prevented some other OTUs from becoming dominant through competitive exclusion (deterministic process following a stochastic process). Verifying the underlying mechanism would require temporal (time series) data. Meanwhile, the small difference in the SES value between each treatment and the control (relative to the absolute of the SES value in the control; Table S8) implied that the experimental treatments had only a weak influence on the effect of deterministic processes. Because the deterministic effect for the control communities was caused by historical factors before the experimental treatment, including the common properties (such as low oxygen content) of soil environment and past environmental changes (such as the vegetation changes). For the treatment communities, the deterministic effect was also caused by current experimental treatments, besides those for the control communities. Thus, the small SES difference suggests that the effect of current experimental treatments is very small when compared to the effect of past historical factors. Notwithstanding, the absolute of the difference in the SES value between treatment and control (from the null model method) showed significant positive linear relationship with the absolute of the deterministic change (from the direct-calculation method; Fig. 4), which demonstrated that the two methods are consistent in the logic.

Deterministic processes might play more important roles in affecting functions than species (Fukami et al., 2005; Burke et al., 2011). However, the relative importance of the stochastic change for the functional composition was also significantly larger than 0.5 across all the 16 environmental changes (Fig. 5). And it showed no significant linear relationship with the relative importance of the stochastic change for taxonomic composition (P > 0.05). In particular, while the relative importance of the stochastic change for taxonomic composition was <0.5 under most N-addition treatments (Fig. 1) because of its acidification effect on the soil (Data S5), that for functional composition was <0.5 under the removal of one, two or three PFGs (Fig. 5). Soil microbial communities possess a very large proportion of functional genes related to carbon metabolism (Fierer et al., 2012); the removal of PFGs will reduce soil carbon sources and thus have large deterministic effect on microbial functional composition, resulting in the small value of the relative importance of the stochastic change (Fig. 5). Notwithstanding, as the number of removed PFGs increased from one to three, soil carbon sources would decrease more and
thus the relative importance of the stochastic change showed a decrease trend (especially for the pathway level; Fig. S). This pattern implied that in the early years of PFG-removal treatments, soil carbon sources would decrease less, and thus, the relative importance of the stochastic change might be much larger (or even >0.5). Overall, all these environmental changes primarily mediated stochastic rather than deterministic processes. Actually, Lekberg et al. (2012) have found that stochastic processes also played some role in the assembly of arbuscular mycorrhizal fungal communities after a disturbance, implying that this pattern might be general for both bacteria and fungi. Meanwhile, we suggest that as the time since the environmental change passes, the relative importance of the stochastic change will become smaller (or eventually <0.5; Fig. 2), because the deterministic ‘species fitness difference’-based processes due to the changes in the soil physicochemical characteristics would play more important roles (Ferrenberg et al., 2013). However, the relative importance of the stochastic change under most environmental changes was still >0.5 after five-year continual treatments (Fig. 1), showing the importance of stochastic vs. deterministic changes. These findings emphasize the consideration that stochastic processes should have when addressing microbial ecology questions, particularly in studies involving similar environmental perturbations over similar time scales. Meanwhile, many environmental changes happen very quickly in nature, for example, precipitation and temperature vary both daily and yearly, which possibly makes mediation of stochastic processes a dependant life factor in soil microbial assembly. Anthropogenic environmental changes can affect the rate of certain ecosystem processes through direct routes such as altering soil physicochemical factors and microbial physiological activities. In addition, they are also found to have effect through indirectly altering soil microbial composition (Carney et al., 2007); for example, the compositional variation in microbial community under climate warming was found to exacerbate their future response to temperature changes (Karhu et al., 2014; García-Palacios et al., 2015). The dominance of stochastic changes relative to deterministic changes in microbial composition under environmental changes means that the compositional variation was unpredictable in most cases, and thus, both the structure of microbial community (the indirect factor) and the soil physicochemical indices (the direct factor) should be integrated to predict the ecosystem functioning (Ferrenberg et al., 2013).

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Data accessibility

The pyrosequence reads were deposited in the Sequence Reads Archive database of the National Center for Biotechnology (accession no. SRA057669), and the shot-gun metagenomic data have been deposited in MGRAST with the project name of ‘Multifactorial Environmental Changes in Inner Mongolia of China’.

References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1. Supporting Experimental Design S1.**

**Data S2. Supporting Method S1.**

**Data S3. Supporting Text S1.**

**Data S4. Supporting Result S1.**

**Data S5. Supporting Text S2.**

**Data S6. Supporting Text S3.**

**Data S7. Supporting References.**

**Fig. S1.** The deterministic change, the stochastic change and the relative importance of the stochastic change caused by each treatment.

**Table S1.** Plant functional groups and their properties.

**Table S2.** Design of the first experimental part.

**Table S3.** The effect of treatments on soil physicochemical and bacterial indices in the first experiment.

**Table S4.** The effect of treatments on soil physicochemical and bacterial indices in the second experiment.

**Table S5.** The effect of treatments on soil physicochemical and bacterial indices in the third experiment.

**Table S6.** The effect of treatments on soil physicochemical and bacterial indices in the fourth experiment.

**Table S7.** The effect of within-treatment environmental heterogeneity on bacterial community dissimilarity.

**Table S8.** The mean of observed Jaccard’s similarity, expected Jaccard’s similarity and SES for every treatment in each experiment.