Spatial patterns of microbial nitrogen genes along precipitation gradient in different temperate grasslands of Inner Mongolia, China

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Abstract

Background: Despite the importance of microorganisms in soil nitrogen (N) cycling, studies on spatial patterns of microbial N genes in the temperate grassland are still lacking, whose productivity is limited by N. Here, we investigated microbial N genes from 60 temperate grassland sites across 1 161 km in Inner Mongolia, China.

Results: All N gene abundances tended to decrease from northeast to southwest, consistent with precipitation change but contrary to temperature trend. Most N gene abundances increased with rising precipitation when < 321 or 403 mm, but remained stable after breaking points, indicating non-linear saturation curves dominated response patterns. Moreover, decay relationships were discovered for N gene community over geographic distance, whose effect was direct in temperate desert steppe but indirect via environmental heterogeneity in temperate meadow and typical steppe. Representativeness of geographic distance on historical-contingency was dominant only in temperate meadow (81.2%). N gene community similarity decay was mainly attributed to plant community (76.98%), with wider range, in typical steppe; while contemporary-disturbance was the attributor more important in temperate meadow (29.41%).

Conclusions: Overall, we discovered non-linear patterns of N genes along precipitation gradient, and quantified attributions of geographic distance, plant community, historical-contingency and contemporary-disturbance to N gene community similarity decay, clearly ecosystem-dependent.

1. Background

Nitrogen (N) is a general limiting factor for plant productivity in most terrestrial ecosystems [1] and closely linked to soil nutrient status, soil acidification, greenhouse gas emission and water eutrophication [2–4]. Soil N cycling processes, including N fixation, nitrification and denitrification, were mainly driven by microorganisms through releasing corresponding enzymes encoded by their functional genes [5–8]. Thus, the spatial patter of microbial functional genes involved in N cycling in soils or their shifts along natural environmental gradients may have important implications for corresponding N processes and ecosystem functions or services they govern.

However, spatial patterns of microbial N functional genes have been investigated by only a few studies, e.g. N gene community measured by real-time quantitative PCR (qPCR)at a landscape scale in Burgundy of France [9], by GeoChip in five oil-contaminated fields in China (660-2 030 km apart) [10], and diazotroph communities measured by qPCR and MiSeq sequencing at a regional scale on the Tibetan Plateau, China [11]. Various factors were discovered to influence spatial patterns of microbial N genes, including geographic [10], topographic and hydrological properties [12], as well as soil geochemical parameters [10, 11], e.g. pH [9, 10] and oil pollution [10]. However, the underlying mechanisms shaping these spatial patterns remain elusive.

Importance of contemporary environmental conditions has been discovered previously in shaping the microbial N gene diversity [13–15], though not at large scales for their spatial patterns. However, responses of various microbial functional genes to contemporary environmental conditions were observed to be distinct and ecosystem-dependent [16–22]. These phenomena were likely due to interactions among environmental factors [23, 24], complex microbial response patterns to environmental changes, and the range of critical environmental factors, which were less explored.

Other than contemporary environmental conditions, whether historical-contingencies work on microbial N genes is still unclear, which have been proved to control distribution patterns of large organisms [25–27] and taxonomic or phylogenetic compositions of microbial communities [28, 29]. In fact, the boundary between contemporary-disturbance and history-contingency is kind of vague [30]. Geographic location [31], soil types [32, 33] and long-term climate [34] have been adopted for history contingencies previously in microbial studies, but their representativeness have never been tested.

Distance-decay relationship has been recognized for microbial communities from taxonomic or phylogenetic aspect in different habitats, e.g. tropical forest soils [35], oceans [36] and salt marshes [37]. Though no consensus has been reached, distance-decay relationship for microbial communities was proposed to be controlled by forces of environmental selection/heterogeneity and dispersal limitation [27, 28, 38, 39], coupled with drift and mutation [40]. So far, distance-decay relationship studies focusing on microbial function genes are still limited [41–43].

Grassland is one of the most important and widely distributed ecosystem types in terrestrial land, critical for global nitrogen cycling [44, 45]. In China, 21% of grassland distributed in Inner Mongolia under the temperate continental climate [46], generally restricted by nitrogen for its productivity [28, 47]. However, studies on spatial pattern of soil microbial functional genes involved in N cycling in temperate grassland in Inner Mongolia are still lacking.

In this study, we investigated microbial N functional genes (i.e. ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), *nxr*, *nifH*, *narG*, *nirS*, *nirK* and *nosZ*) by qPCR collected from 60 temperate grassland sites across 1 161 km along the natural precipitation (162 mm-507 mm, from southwest to northeast) and temperature (0.09℃-9.54℃, from northeast to southwest) gradients in Inner Mongolia, China. Our objectives were to investigate 1) spatial distribution patterns of various microbial N gene in the temperate grassland along natural precipitation and temperature gradients; 2). distance decay relationship for N gene community and underlying mechanisms shaping the spatial distribution patterns of N genes.

2. Results

2.1 Microbial N functional genes *Vs* natural precipitation and temperature gradients

Copy numbers of all measured N genes differed significantly among ecosystem types (Fig. 1), including temperate meadow, typical steppe and temperate desert steppe. Almost all these genes had the highest gene copy numbers in temperate meadow and the lowest values in temperate desert steppe, except AOA. The copy number of AOA did not differ significantly between temperate meadow and typical steppe, though both were significantly higher than in temperate desert steppe.

These N genes had consistent spatial patterns across different ecosystems, tending to decrease from northeast to southwest (Fig. 1) in the temperate grassland of Inner Mongolia, China. Intriguingly, mean annual precipitation (MAP) either averaging for 36 years, 5 years or 1 year also tended to decrease from northeast to southwest in Inner Mongolia, China (Additional file 2: Fig S1), while mean annual temperature (MAT) either averaging for 36 years, 5 years or 1 year tended to decrease from southwest to northeast.

Piecewise regression was adopted to identify breaking points automatically for these N genes over MAP-5 y and MAT-5 y, if existed (Fig. 2). Before a common breaking point of 321 mm for MAP-5 y, copy numbers of AOB, *nirS* and *narG* increased with higher MAP, while they did not change significantly after this breaking point. Similarly, copy numbers of AOA, *nxr* and *nirK* increased with higher MAP before 403 mm, but did not change after this breaking point. The breaking point for *nifH*, 472 mm, was quite close to the upper value of MAP in all sampling sites. Thus, copy number of *nifH* increased with higher MAP almost within the whole MAP range of all sampling sites. Differently, the copy number of *nosZ* increased with MAP even 2.26 times faster after its breaking point of 367 mm than before it.

Based on MAT-5 y, there were no breaking points for AOB, *nxr*, and *nosZ* as their gene copy numbers decreased with higher MAT within the whole MAT range of all sampling sites. The breaking point for *nifH*, 0.29℃, was quite close to the lowest value of MAT in all sampling sites, and thus had almost no breaking point as well. After breaking points of 1.70 and 3.79℃ for *nirK* and AOA, respectively, their copy number decreased significantly with higher MAT, while they did not change before these points. However, copy numbers of *nirS* and *narG* decreased significantly with higher MAT before 5.44℃, but increased after it. Interestingly, the MAT range in temperate meadow was almost lower than 1.70℃, while that in temperate desert steppe was almost higher than 5.44℃.

2.2. Microbial N functional genes *Vs* Soil and Plant variables

Other than climate factors, Multiple linear regression (MLR) results showed that soil total organic carbon (TOC)attributed highest proportions of the variance in nitrification genes (20.35%, 27.38% and 26.81% for AOA, AOB and *nxr*, respectively), and plant richness attributed proportions of the variance in some denitrification genes (20.42% and 19.32% for *nirS* and nosZ, respectively) (see details in Additional file 1 and Additional file 3: Table S1-S4).

As shown in Fig S2 (Additional file 2), the breaking point of TOC for AOA, *nxr* and *nirK* was 49.23 g kg^{-1}, quite close to the upper value of TOC in all sampling sites, while the breaking point for AOB and *narG* was

3.68 g kg^{-1}, quite close to the lowest value. Thus, copy numbers of AOA, *nxr*, *nirK*, AOB and *narG* increased with higher TOC almost within the whole TOC range of this study. Differently, copy numbers of *nosZ* or *nirS* and *nifH* increased significantly with TOC before the breaking point of 29.68 and 34.24 g kg^{-1}, respectively, but did not change after breaking points.

After plant richness reached 17, copy numbers of *nifH* increased with higher plant richness, while they did not change significantly before 17. Copy numbers of *nosZ* increased with higher plant richness when < 12, while it did not change significantly after this breaking point. Copy number of AOB increased with higher plant richness almost within the whole plant richness range of this study. On the contrary, AOA, *nxr*, *nirS* and *nirK* were not correlated with plant richness within its whole range of this study. The breaking point for *narG*, 24, was quite close to the upper value of plant richness in all sampling sites. Thus, there was almost no significant correlation between *narG* and plant richness within the whole plant richness range as well.

By Pearson correlation test, N gene copy numbers were significantly correlated with most environmental factors (see details in Additional file 1 and Additional file 3: Table S5). Moreover, relationship between N gene copy numbers and environmental variables varied dramatically by ecosystem types (see details in Additional file 1 and Additional file 2: Fig S3).

2.3 Decay relationship between the microbial N gene community similarity and geographic distance

Significant distance-decay relationships were observed between the whole microbial N gene community similarity based on Bray-Curtis index and the geographic distance (km) ($p < 0.001$). Such relationship was applied to all investigated ecosystems, including temperate meadow, typical steppe and temperate desert steppe (Fig. 3A and Additional file 2: Fig S4 A1-A2), though with different slopes. The distance-decay slopes of the whole N gene, nitrification gene and denitrification gene communities were all in order of temperate meadow > temperate desert steppe > typical steppe. The geographic distance explained 17.58%, 17.00% and 37.5% variance in the whole N gene, nitrification gene and denitrification gene similarities, respectively, in the temperate desert steppe, higher than other two ecosystems.

Interestingly, in temperate meadow, environmental distance based on long-term properties explained 81.2% of the variance in geographic distance, while short-term environmental distance only explained 30.6%. In typical steppe, long-term environmental distance explained 51.8% of the variance in geographic distance, as twice as the short-term environmental distance did. Though the long-term environmental distance explained more than half (58.1%) of the variance in the geographic distance, the short-term environmental distance also explained a considerable proportion 44.2% in temperate desert steppe. It is worthwhile noting that the proportions of explained variance only < 1% by the long-term or short-term environmental distance across different ecosystems when put all three ecosystem types together, indicating the relationship was ecosystem-dependent (Additional file 2: Fig S5).

2.4 Decay relationship between the microbial N gene community similarity and environmental distances

Due to dominant roles of deterministic processes discovered by Normalized stochasticity ratio (NST) (see details in Additional file 1 and Additional file 2: Fig S6) in N gene community assembly of investigated ecosystems, the relationship between the microbial N gene community similarity and environmental distances were explored. The whole microbial N gene community similarity decreased significantly with the environmental distance based on all measured variables (see details in Additional file 1 and Additional file 2: Fig S7), short-term or long-term variables ($p < 0.001$). For either long-term or short-term environmental distance, the turnover rate of the whole N gene or nitrification gene community similarity was highest in the temperate desert steppe, followed by the temperate meadow and lowest in the typical steppe, while the turnover rate of denitrification gene community was in the order of temperate meadow > temperate desert steppe > typical steppe (Additional file 2: Fig S4).

It is notable that long-term and short-term multiple environmental distances only explained < 0.1% and 0.8% of the variance in the N gene community similarity across ecosystems, respectively. However, for each individual ecosystem, the proportion of the explained variance in the N gene community similarity reached ranges of 4.9–17.4% and 3.6–24.1% by long-term and short-term environmental distances, respectively. These phenomena indicated the relationship was ecosystem-dependent.

To investigate drivers for significant decay relationship between the N gene community similarity and long-term or short-term environmental distances, partial Mantel test was adopted (see details in Additional file 1 and Additional file 3: Table S6).

Attributions of geographic distance, short-term and long-term environmental distances, and plant community dissimilarity to the N gene community similarity were investigated (Fig. 4 and Additional file 3: Table S7). In the temperate meadow, plant community dissimilarity and short-term environmental distance were important for the N gene community similarity, with attribution of 34.70% and 29.41%, respectively. In typical steppe, plant dissimilarity was the dominant interpreting variable, attributing 76.98%, while long-term environmental distance was also significant but only attributed 13.34%. In the temperate desert steppe, the highest-interpreting variable was the long-term environmental distance (35.96%), followed by geographic distance (33.76%).

2.5 Regulatory pathways of the N gene community similarity

Structural equation model (SEM) (Fig. 5) was performed to investigate the direct and indirect effect of geographic distance, plant community dissimilarity, short-term environmental distance, long-term environmental distance on N gene community similarity. Geographic distance only had direct effect on the N gene community similarity in temperate desert steppe, but its effects was indirect via short-term and long-term environmental distance and plant community dissimilarity in temperate meadow and

typical steppe. In contrast, plant community dissimilarity directly drove the N gene community similarity in all three ecosystems. Moreover, long-term environmental distance directly affected the N gene community similarity in typical steppe and temperate desert steppe, but indirectly affected it through influencing plant dissimilarity in temperate meadow. Short-term environmental distance directly drove the N gene community similarity in temperate meadow, but its effects was indirectly via plant community dissimilarity in typical steppe and temperate desert steppe. In sum, SEM (Fig. 5) model explained 14.9%-31.1% of the variation in functional N gene community similarity.

Notable, geographic distance was highly correlated with long-term environmental distance, with the coefficients of -0.901 and − 0.720 in temperate meadow and typical steppe, respectively. Though geographic distance was correlated with long-term environmental distance with the coefficient of -0.760 in temperate desert steppe, its relationship with short-term environmental distance was also comparable (-0.662).

Standardized total effects derived from the SEM (Fig. 5D) revealed that the N gene community similarity in temperate meadow was mainly driven by short-term environmental distance, geographic distance, and plant community dissimilarity. In typical steppe, N gene community similarity was mainly driven by plant dissimilarity. N gene community similarity in temperate desert steppe was mainly driven by geographic distance and short-term environmental distance.

3. Discussion

Across 1 661 km in temperature grassland of Inner Mongolia, China, copy numbers of N functional genes from soil microbial communities tended to decrease from northeast to southwest, similar to the changing trend of precipitation. Consistently, spatial patterns of biological communities including microbial functional genes have been observed to be shaped by climatic factors (e.g. precipitation or temperature) by numerous studies at regional scales [16, 18, 48–51]. However, most previous studies presented linear relationships between microbial diversity [52, 53] or functional gene abundances [16] and precipitation. In fact, the precise response curves of biological communities to abiotic factors could be more complex but seldom linear if ranges of abiotic factors are wide enough [54, 55]. In this regional scale study, we discovered that the saturation curve dominated the response patterns of most N genes and identified breaking points automatically along the natural precipitation gradient ranged from 215 to 501 mm. Copy numbers of most genes (i.e. AOB, *nirS, narG*, AOA, *nxr* and *nirK*) increased with the precipitation when it was lower than 321 or 403 mm, but did not change after these breaking points, indicating the precipitation effect became saturated after breaking points on microorganism containing these genes.

Differently, the copy number of *nifH* increased with precipitation almost along the full range of natural precipitation gradient of this study. Consistently, *nifH* was observed to increase continuously on Qinghai-Tibet Plateau along a natural precipitation gradient ranged from 62 to 614 mm [11]. The accelerated increasing trend of *nosZ* copy number after its breaking point of 367 mm likely implied a stimulating

effect of intensified anaerobic condition [56]. As far as we know, this is the first study to recognize these diverse patterns of various N functional genes along the natural precipitation gradient.

The changing trend of N functional genes were contrary to that of temperature. Inconsistently, number studies found that microbial functional gene abundances increased with higher temperature [51, 57, 58]. Such discrepancy implied that intensified water loss or deficiency underlying the temperature increase in our studying sites may surpass the stimulating effect of warming itself and limit abundances of these functional genes (e.g. AOB, *nxr*, *nosZ* and *nifH*) in temperate grassland, which was generally deficient in water [59]. Consistently, Aridity Index (AI) representing the degree of drought was positively correlated with MAT significantly in this study, and abundances of all measured N genes decreased significantly with the increase of AI after their breaking points (see details in Additional file 1 and Additional file 2: Fig S2). Moreover, the copy numbers of *nirK* and AOA did not change before their breaking points of 1.70 and 3.79℃ for MAT, respectively, indicating microorganism containing these genes may be less responsive to the lower arid under lower temperature in our sampling sites. Higher resistance of *nirK* and AOA was also observed in the polar regions and dryland ecosystems [51, 60]. Furthermore, increased copy numbers of *nirS* and *narG* after a breaking point of 5.44℃ indicated stimulating effect of temperature itself may become dominant under such condition.

Significant geographic distance-decay relationships were discovered between microbial N gene community similarity and geographic distance, indicating dispersal limitation and history mattered [28, 33, 37]. This is the first study to explore such relationship for microbial N genes, as far as we know, though distance decay relationships have been found for a comprehensive set of microbial functional genes in GeoChip studies [10, 41–43]. Interestingly, SEM analysis showed that geographic distance only had direct effect on the N gene community similarity in the temperate desert steppe, likely implying the importance of dispersal limitation and relatively less environmental heterogeneity in such ecosystem. Similarly, a previous study found that, geographical distance was the only reason for explaining the similarity of bacterial community in the desert [61], even harsh than the temperate desert steppe. However, its indirect effects via plant community dissimilarity, short-term and long-term environmental distances were observed in temperate meadow and temperate desert steppe, implying that environmental heterogeneity may be more responsible for geographic distance-decay relationships in these ecosystems. Consistent with previous discoveries, geographic distance-decay relationship could be attributed to environmental heterogeneity depending on their niche preferences [36, 61, 62].

Geographic factor or distance used to represent history contingencies in previous studies [16, 30, 33], though the underlying logic or its representativeness on history contingencies was never clarified or proved. Our results presented a clear evidence for the first time that the representativeness of geographic distance on history contingencies was dependent on ecosystem types. In temperate meadow, geographic distance was dominantly explained by history contingencies characterized by long-term environmental distance, which were highly correlated with geographic distance with a R^2 of 0.81 by Pearson or a coefficient of -0.90 by SEM. In typical grassland, the representativeness of geographic distance on history contingencies was also high, as their correlation with a R^2 of 51.8% by Pearson or a coefficient of -0.72 by

SEM, as double as the interpretation power of short-term environmental distance. However, in the temperate desert steppe where geographic distance had a direct effect on the N gene community similarity, the interpretation of long-term environmental distance for geographical distance is still > 50%, but comparable to that of short-term environmental distance, revealing less representativeness of geographic distance on history contingencies in the temperate desert steppe.

SEM and attribution analysis based on MLR showed that environmental distance based on short-term variables representing contemporary factors had the greater influence on N gene similarity in temperate meadow. Microbial community composition shifts were previously observed to result from gradual or sudden changes in abiotic and biological factors such as soil moisture, temperature, weather, season and plant species in a relatively short period of time in forest, farming-pastoral ecotone and wetland ecosystems [63–66]. In the typical steppe, the N gene community similarity was dominantly affected by plant community dissimilarity, while other factors only attributed < 15%. The plant coverage range was widest in the typical steppe with higher plant richness (Additional file 1 and Additional file 2: Fig S8), indicating that plant community might be more dynamics in typical steppe, likely responsible for its dominant role in N gene community similarity. Consistently, plant community shifts in typical steppe also exhibited a much stronger explanatory power for explaining the regional variation of ecosystem respiration than that in temperate meadow and temperate desert steppe [59]. In temperate desert steppe, N gene community similarity was attributed to long-term environmental distance and geographic distance. Temperate desert steppe had higher AI and lower soil resources like soil organic carbon (SOC)and total N (TN), creating a harsh environment for microbial communities [67]. Thus, soil microorganisms that can survive in the temperate desert steppe may have developed higher tolerance for short-term environmental disturbances in such harsh environment and adapted to the long-term environmental dynamics.

Other than deterministic processes (e.g. abiotic and biotic factors) that have been studied for a long period of time [68, 69], neutral mutation and random genetic drift theory asserts that stochastic processes (e.g. birth, death, immigration, and limited dispersal) are responsible for shaping the microbial community structure [70]. However, in this study, NST revealed that deterministic processes were predominant for N gene community in the temperate grassland. Significant decay relationships between the N gene community similarity and environmental distances, either long-term or short-term, observed in this study were consistent with many previous microbial studies [37, 71–73], though not focusing on microbial N genes. As revealed by Partial Mantel, such distance-decay relationships based on long-term environmental distance was driven by TOC, TN and climatic factors, e.g. precipitation or temperature while such relationship based on short-term environmental distance was driven by available nitrogen and phosphorus, AI-m, plant richness and so on. The importance of these environmental variables was consistent with many previous studies [26, 74–76].

4. Conclusion

In summary, microbial N genes from 60 temperate grassland sites across 1 161 km along a natural precipitation gradient were investigated in Inner Mongolia, China. Abundances of most N genes (i.e. AOB, *nirS, narG*, AOA, *nxr* and *nirK*) increased with the rising precipitation when < 321 or 403 mm, but remained stable after these breaking points, indicating non-linear saturation curves dominated response patterns of most N genes to precipitation, which were seldom linear except *nifH*. However, the changing trend of N functional genes were contrary to that of temperature, implying that intensified water deficiency underlying the temperature rise surpassed the stimulating effect of warming itself in the temperate grassland. Moreover, decay relationships were discovered for microbial N gene community similarity over geographic distance, whose effect was only direct in temperate desert steppe but indirect via environmental heterogeneity in temperate meadow and typical steppe as revealed by SEM. The representativeness of geographic distance on historical-contingencies depends on ecosystem types as it was dominant in temperate meadow (81.2%), but not in typical steppe and temperate desert steppe though still high (51.8–58.1%). N gene community similarity decay was dominantly attributed to plant community dissimilarity (76.98%) in typical steppe, while contemporary disturbance was the important in attributor temperate meadow (29.41%) but not in temperate desert steppe. Overall, our study firstly discovered distinct patterns of various N genes, mostly nonlinear, along the natural precipitation gradient. The attributions of geographic distance, plant community dissimilarity, short-term and long-term environmental distance to N gene community decay were quantified, which were ecosystem-dependent, with important implications for underlying mechanisms shaping spatial patterns of microbial N genes.

5. Materials And Methods

5.1 Site description

The sampling sites (107.27°-122.28°E, 38.22°-50.20°N) are located in the temperate grassland of Inner Mongolia, China (Fig. 6). The average elevation is over 1 000 m. It belongs semiarid continental climate, with a short plant-growing period from May to September. The average annual temperature is 0.3℃ and the annual precipitation is 343 mm [77]. From east to west, ecosystem types include the meadow steppe in the east of Inner Mongolia, the typical steppe in the middle of Inner Mongolia and the desert steppe in the middle and west of Inner Mongolia.

5.2 Sample collection

Soil samples were collected in September, 2015 from 60 sites in Inner Mongolia, China (Fig. 6). Sampling points were randomly selected along the highway at intervals of 50–100 km. In each site, three soil cores were randomly collected within a 1 m^2 quadrat frame by a standard soil corer with a diameter of 5 cm, from 0–5 cm and 5–20 cm soil layers, and then composited into one sample for each soil layer. Collected soil samples were stored in the − 20℃ mobile refrigerator in the field. After being transported to the laboratory, soil samples were stored at -80℃ for molecular analysis, and 4℃ for physical and chemistry analyses. For all N gene copy numbers, no significant difference between the upper (0–5 cm) and sub

(5–20 cm) soil layers was observed, thus data from both layers in each site was combined for the following analyses.

Aboveground plant samples were collected separately by plant species in each quadrat, and put in paper envelopes. In the laboratory, plant samples of each species was dried at 65℃ and weighted as aboveground biomass (AGB) when the constant weight was reached. After soil samples were sieved by 2 mm and dried at 65℃, root samples were separated and weighted as belowground biomass (BGB).

5.3 DNA extraction and N gene quantification

DNA was extracted from 0.25 g of soil using the MoBio PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), following the kit instructions. DNA quality and quantity were assessed using the Nanodrop 2000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA).

Microbial functional genes involved in key processes of N cycle were quantified by qPCR, including ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), *nxr* encoding nitrite oxidoreductase, *nifH* encoding nitrogenase, *narG* encoding nitrate reductase, *nirS* and *nirK* encoding nitrite reductase and *nosZ* encoding nitrous oxide reductase (see primers in Additional file 3: Table S8) [78–82]. All samples were analyzed three times in parallel. PCR runs were started with an initial denaturation for 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at the annealing temperatures and 40 s or 30 s at 72 °C, and 30 s at 82 °C (see detailed description for each gene in Additional file 3: Table S8). The SYBR fluorescent dye was adopted during qPCR processes. Each 20 μL PCR reaction system included 10 μL of Takara SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan), 0.2 μM of each primer and 12.5 ng DNA template or no template DNA as the negative control. DNA template was diluted to 10 ng μL^{-1}. The qPCR reactions were performed on the Real-Time PCR System (Applied Biosystems 7500, Foster City, CA, USA). The specificity of PCR products was determined by the melting curve.

5.4 Soil physical, chemical properties and climatic variables

Soil moisture content was determined by oven-drying soil samples at 105 °C for 24 hours [83]. Soil pH was measured with a 1:4 soil to water ratio by Delta pH meter (STARTER3100, AUX, Shanghai, China) [84]. TOC and TN were measured by potassium dichromate heating and kjeldahl, respectively. Soil NO_3^- and NH_4^+ contents were determined by potassium sulfate extraction with a 1:4 soil to potassium sulfate solution (0.5 mol L^{-1}) ratio. Soil total phosphorus (TP) and available phosphorus (AP) were determined by Molybdenum antimony spectrophotometry in UV-VIS spectrophotometer (UV2700, 147 SHIMADZU, Shanghai, China).

Mean annual temperature (MAT) averaging for 36 years (MAT-36 y, 1980–2015), 5 years (MAT-5 y, 2011–2015) or 1 year (MAT-y, 2015) was calculated based on data from Resource and Environment Date Cloud Platform (http://www.resdc.cn/data.aspx?DATAID=228), so did mean annual precipitation (MAP) averaging for 36 years (MAP-36 y, 1980–2015), 5 years (MAP-5 y, 2011–2015) or 1 year (MAP-y, 2015).

Measured environmental variables were divided into two groups, including long-term properties (i.e. TOC, TN, TP, MAT-36 y, MAP-36 y, MAT-5 y, MAP-5 y and AI-5 y) generally remaining stable for years, and short-term properties (i.e. AP, pH, SWC, NH_4^+, NO_3^-, MAT-y, MAP-y, AI-m, AI-y, AGB, BGB, plant richness and plant Shannon-Wiener index) that were dynamics within a year or between years.

5.5 Statistic analyses

One-way analysis of variance (ANOVA) was performed to assess the effects of ecosystem types, including temperate meadow, typical steppe and temperate desert steppe, on N genes and measured environmental variables. Pearson correlation was used to explore the relationship between N genes and measured environmental variables. MLR was applied and attributions of different variables (geographic distance, plant community dissimilarity, short-term and long-term environmental distances) to N gene community similarity were calculated in different ecosystems based on the method developed previously [85]. The above statistical analyses were all performed by R software (R Development Core Team, 2018).

SEM was applied to evaluate the direct and indirect effects of geographic distance, plant community dissimilarity, short-term and long-term environmental distances on the N gene community similarity, using the IBM SPSS Amos software (SPSS Inc., Chicago, IL, USA).

Declarations

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Availability of data and materials

Please contact author for data requests.

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Author contributions

YFW, KX and STZ designed the experiment. STZ performed laboratory experiments, evaluated the data, and wrote the manuscript under the instruction from KX. BZ, LT, JFD and JQD performed data analysis, ZP, FW, RXC, QWR, AQX, KW and LFL pre-processed the samples and provided the soil metadata. YFW, RHH, YBH and XYC provided input for experimental design and manuscript writing. All authors improved and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Additional Files

Additional file 1:

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Supplementary Methods, Supplementary Results, Supplementary Discussion and Supplementary References.

Additional file 2:

File format: .docx

Figure S1 Spatial pattern of MAT-36 y, MAT-5 y, MAT-y, MAP-36 y, MAP-5 y, MAP-y, AI-5 y, AI-y, AI-m. **Figure S2** Response patterns of log-transformed N gene copy numbers along AI-5y, TOC and plant richness. Breaking points were automatically identified by piecewise regression. **Figure S3** Log transformed N gene copy numbers along measured environmental variables in temperate meadow, typical steppe, and temperate desert steppe. **Figure S4** Relationships for similarities of nitrification and denitrification gene communities over geographic distance, environmental distances based on all measured environmental variables, long-term environmental variables, short-term environmental variables, or plant community dissimilarity. **Figure S5** Decay relationship for or long-term environmental variables, short-term environmental variables over geographic distance, as well as plant community similarities over long-term environmental distance based on long-term environmental variables or short-term environmental distance based on short-term environmental variables. **Figure S6** Normalized stochasticity ratio (NST) in temperate meadow, typical steppe, temperate desert steppe. **Figure S7** Decay relationship for plant community dissimilarities, environmental distances based on all measured environmental variables over geographic distance, as well as N gene similarity over plant community dissimilarities, environmental distances based on all measured environmental variables. **Figure S8** Changes in measured environmental variables in temperate meadow, typical steppe, temperate desert steppe.

Additional file 3:

File format: .docx

Table S1 Attributors for log-transformed N gene copy number. **Table S2** Attributors for log-transformed N gene copy number in temperate meadow. **Table S3** Attributors for log-transformed N gene copy number in typical steppe. **Table S4** Attributors for log-transformed N gene copy number in temperate desert steppe. **Table S5.** Pearson test for relationships between N cycle genes and measured environmental variables. **Table S6** Partial Mantel test for relationships between each measured environmental variable and N gene community after fixing effect of all other environmental variables in either short-term or long-term catalog in temperate meadow, typical steppe, temperate desert steppe. **Table S7** Attributions of different variables (geographic distance, plant community dissimilarity, short-term and long-term environmental distance) to N gene community similarity in different ecosystems. **Table S8** Primers used for functional genes in this study and the corresponding thermal conditions for qPCR.

Figures

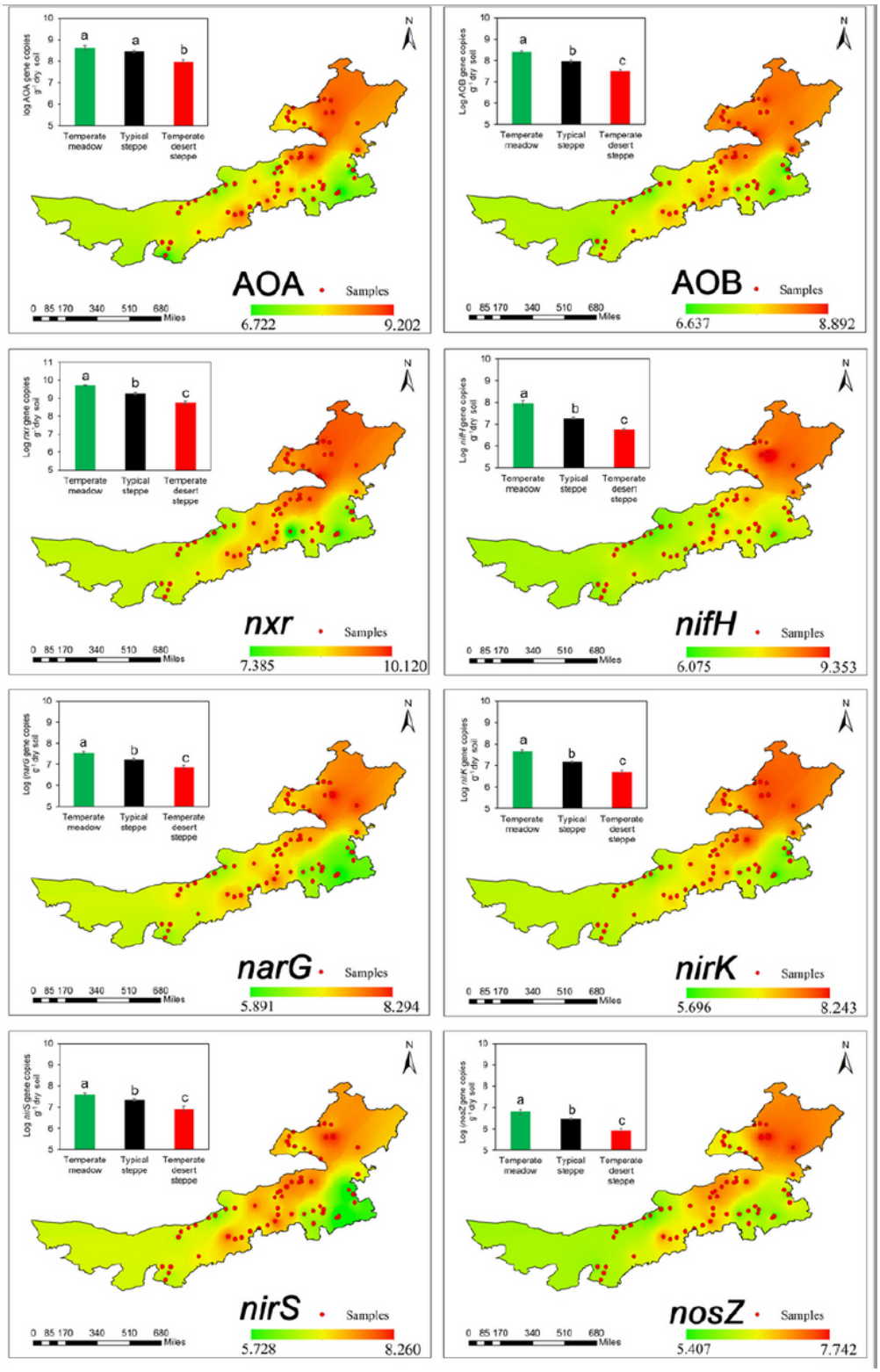

Figure 1

Spatial pattern of log-transformed nitrogen gene copy numbers in the temperate grassland of Inner Mongolia, China. N genes included AOA and AOB, nxr for nitrification, nifH for nitrogen fixation, as well as narG, nosZ, nirK and nirS for denitrification. Red points represented sampling sites, other values in the map were interpolated based on the measured gene copy numbers by ArcGIS. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion

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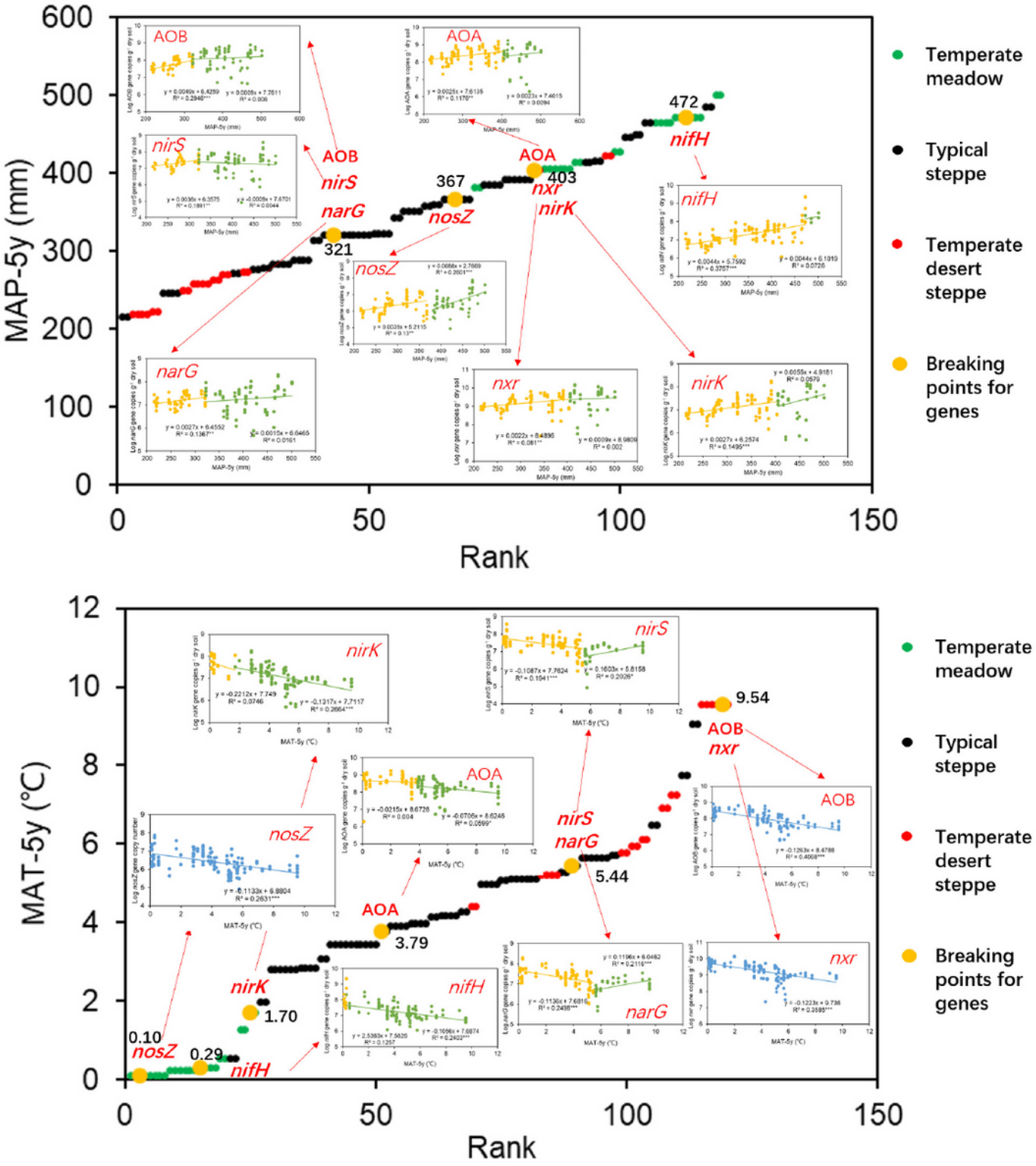

Figure 2

Response patterns of log-transformed N gene copy numbers along precipitation (MAP-5 y) and temperature (MAT-5 y). Breaking points were automatically identified by piecewise regression.

Significance levels are represented as follows: *P < 0.05, **P < 0.01 and ***P < 0.001.

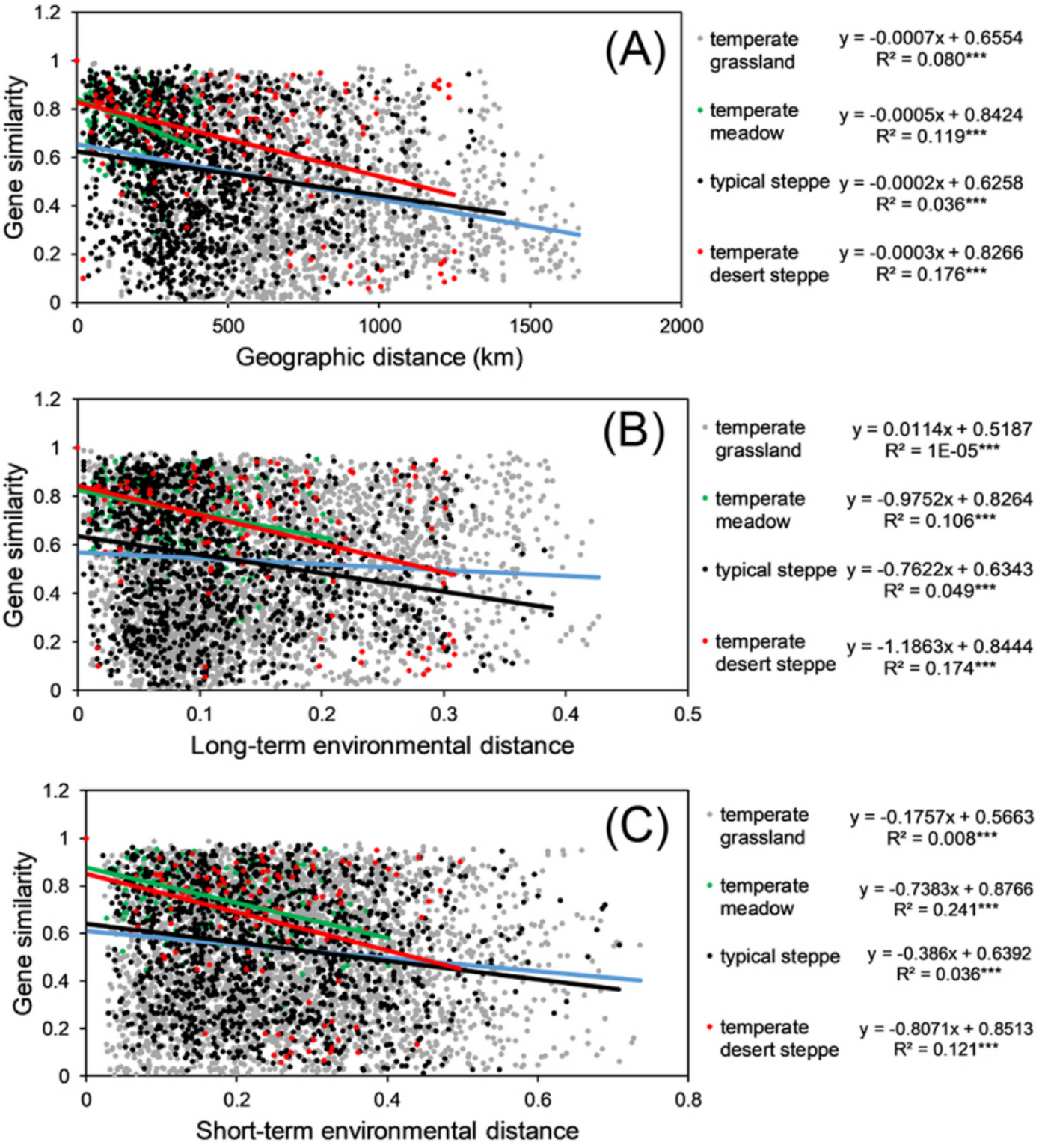

Figure 3

Relationships for N gene community similarity based on Bray-Curtis over geographic distance (A), long-term environmental distance based on long-term environmental variables (B) or short-term environmental distance based on short-term environmental variables (C).

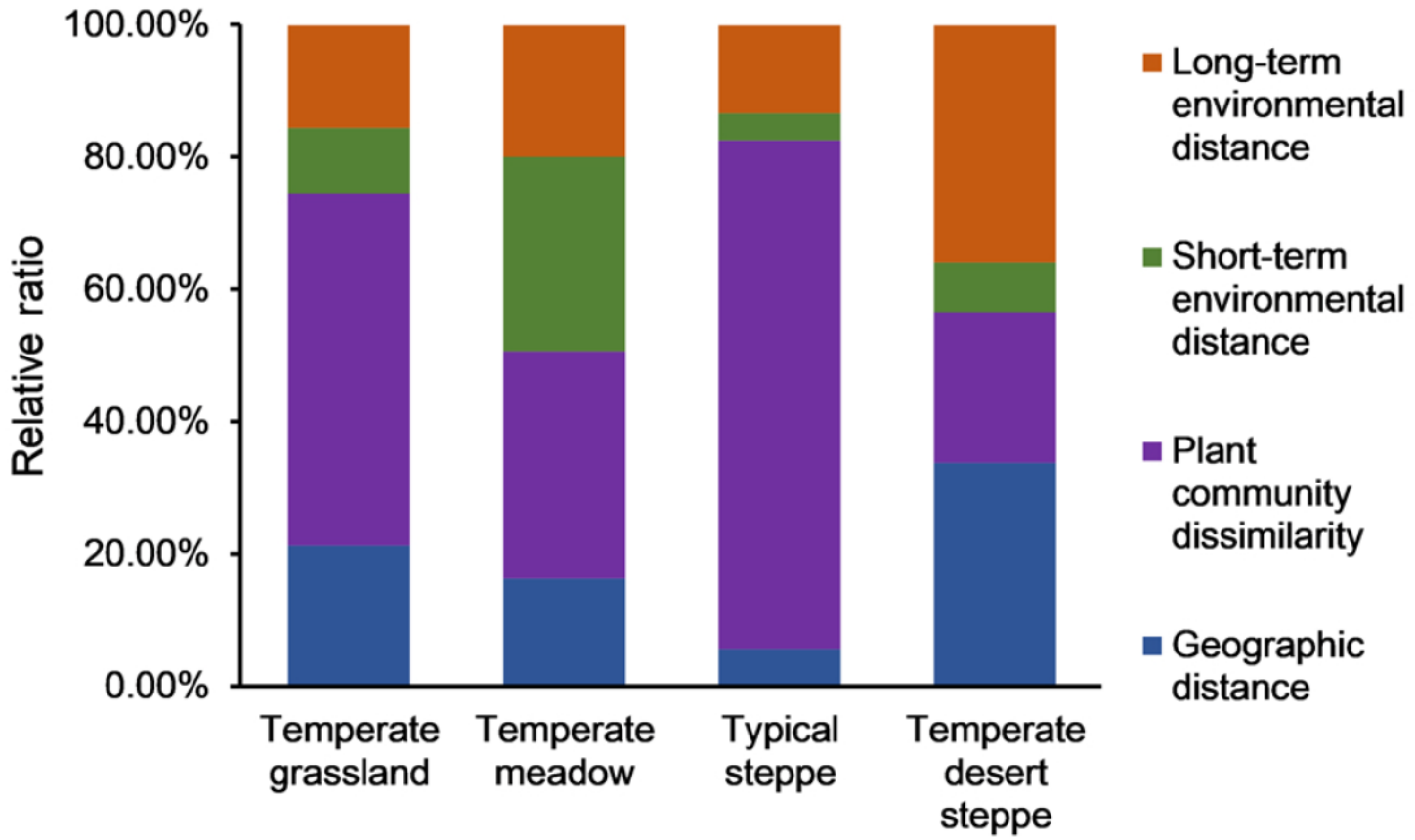

Figure 4

Attributions of geographic distance, plant community dissimilarity, short-term environmental distance based on short-term environmental variables, long-term environmental distance based on long-term environmental variables to N gene community similarity in different ecosystems.

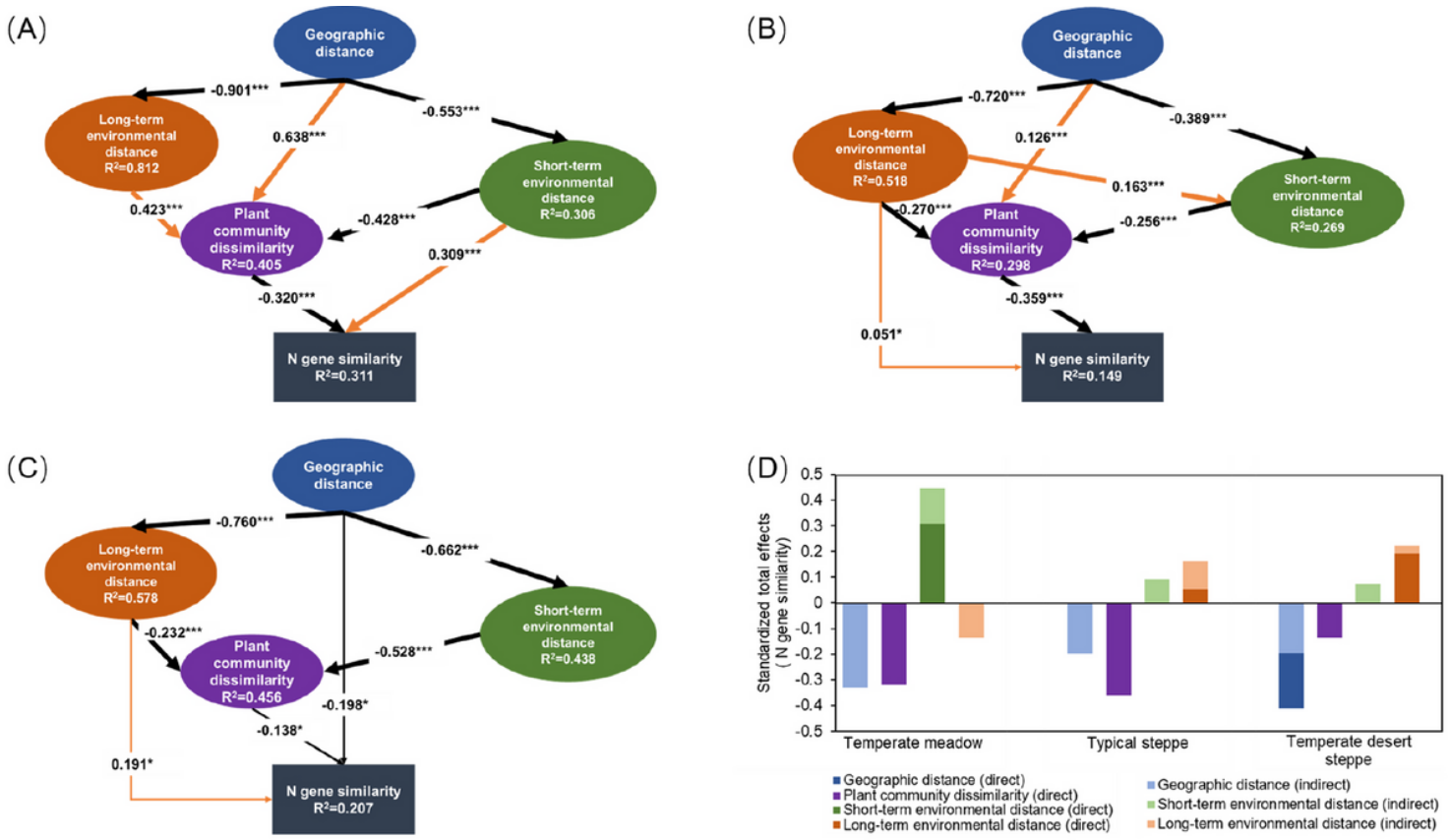

Figure 5

Structural equation models (SEM) for effects of geographic distance, plant community dissimilarity, short-term environmental distance based on short-term environmental variables, and long-term environmental distance based on long-term environmental variables on N gene similarity in temperate meadow (A), typical steppe (B), and temperate desert steppe (C). Significance levels are as follows: *P < 0.05, **P < 0.01 and ***P < 0.001. Standardized total effects (direct plus indirect effects) derived from the structural equation model were presented (D).

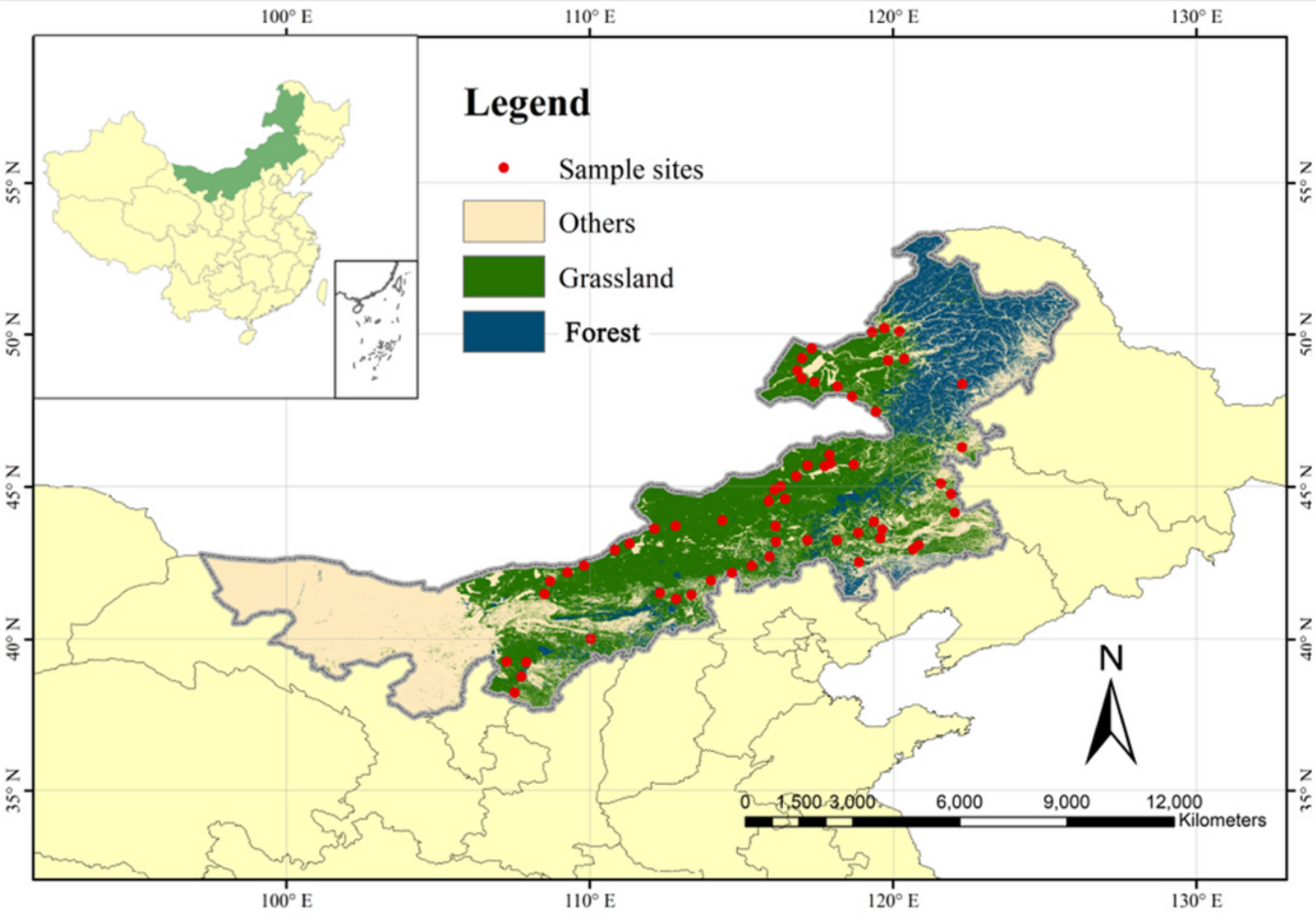

Figure 6

Sampling sites in the temperate grassland of Inner Mongolia, China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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