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Research

Coupling temperature-dependent spatial turnover of microbes and plants using the metabolic theory of ecology

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Summary

- There is an urgent need to understand the coupled relationship between belowground microbes and aboveground plants in response to temperature under climate change. The metabolic theory of ecology (MTE) provides a way to predict the metabolic rate and species diversity, but the spatial scale dependence and connections between plants and microorganisms are still unclear.
- Here, we used two independent datasets to address this question. One is from comprehensive sampling of paddy fields targeting bacteria and microbial functional genes, and the other is a global metadata of spatial turnover for microorganisms (bacteria, fungi and archaea, $n = 139$) and plants ($n = 206$).
- Results showed that spatial turnover of bacterial communities and microbial functional genes increased with temperature and fitted MTE. Through meta-analysis, the temperature-dependent spatial scale pattern was further extended to the global scale, with the spatial turnover of microorganisms and plants being consistent with MTE. Belowground microorganisms and aboveground plants were closely linked with each other even when controlling for temperature, suggesting that factors other than shared relationships with temperature also contribute to their linkages.
- These results implied a broad application of MTE in biology and have important implications for predicting the ecological consequences of future climate warming.

Introduction

The metabolic theory of ecology (MTE) predicts a quantitative relationship between the metabolic rate of organisms and environmental temperature, where higher temperatures will increase individual performance, such as the development rate, mortality rate and life span (Brown *et al.*, 2004; Zhou *et al.*, 2016). This dynamic is described by an Arrhenius/Boltzmann exponential relationship ($R \propto e^{-E/kT}$), where R is the rate of a given biological process such as the mortality rate, e is the base of the natural logarithm, k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}), T is the temperature in Kelvin, and E is the 'activation energy' that characterizes the temperature dependence of a given biological process. This framework can be further extended to the population and community levels of ecological organization, as many features of population and community organization rely on the performance of individual organisms, such as species diversity (α diversity). For example, in accordance with the MTE, Allen *et al.* (2002) reported that the species richness of macroorganisms increases with environmental temperature, which is supported by data from European trees, North American tiger beetles and European amphibians. The species diversity–temperature relationships for microorganisms were also confirmed for forest soil microbes across a large latitudinal temperature gradient, from subalpine Colorado to tropical Panama, but their temperature dependence ($E = 0.13$–0.47 electron volts (eV)) was lower than that recorded for trees and animals (*c.* 0.65 eV; Zhou *et al.*, 2016). Despite the applicability of the MTE to the α diversity of both macro- and microorganisms, much less is known about how patterns of their spatial scaling (β diversity) vary across broad temperature gradients and whether these patterns also fit the MTE.

Biological metabolism influences the fates of energy and materials in ecosystems (Brown *et al.*, 2004); thus, the scope of the MTE continues to expand to ecosystem processes, such as biomass production. Yvon-Durocher & Allen (2012) reported the temperature dependency of short-term gross primary production (i.e. gross photosynthetic flux) based on aquatic mesocosm experimental data ($E = 0.27$). Temperature dependence of biomass accumulation rates following disturbances in forests was also detected, and the biomass accumulation rate was a function of inverse temperature ($1/kT$; $E = 0.32$) (Anderson *et al.*, 2006). Surprisingly, few studies have evaluated whether microbial biomass production fits the MTE, even though we can easily find many cases showing the sensitivity of microbial biomass carbon to environmental temperature (Curtin *et al.*, 2012; Xu & Yuan, 2017). For example, after 14 d of incubation of four different soils along a temperature gradient (5–45°C), an apparent increase in soil microbial biomass carbon was observed before the breakpoint temperatures, whereas a decrease was observed after the breakpoint temperatures (Šantrůčková *et al.*, 2003; Čapek *et al.*, 2019). However, in an 85-d laboratory incubation, Curtin *et al.* (2012) detected substantial reductions in soil microbial biomass carbon (18–35%) between 5°C and 25°C, which significantly enhanced the temperature response of carbon mineralization. Thus, empirical evidence is necessary to uncover the potential linkage between environmental temperature and soil microbial biomass across broad temperature gradients considering the critical role of microorganisms in climate feedback.

In this study, we first hypothesize that the distribution of species spatial turnover (β diversity) fits the MTE and that this theory is applicable to both belowground microbes (i.e. soil main microbial group – bacterial community and microbial functional genes) and aboveground macroscopic plants on a global scale (H_1; Fig. 1). This is because species spatial turnover reflects community levels of ecological organization, which should be influenced by temperature (Brown *et al.*, 2004). Second, we hypothesize that belowground microbial biomass and aboveground plant biomass, as reflections of ecosystem processes, also fit the MTE (H_2), consistent with the widely studied pelagic ocean ecosystem (Regaudie-de-Gioux & Duarte, 2012; Schramski *et al.*, 2015). Meanwhile, we expect the interdependency of the spatial scaling or functioning of the above- and belowground ecosystem (De Deyn & Van der Putten, 2005; Bardgett & Van Der Putten, 2014). Third, we hypothesize that the community-level feature (i.e. spatial turnover) relates to the ecosystem process (i.e. biomass production; H_3), as they are inextricably linked (Yvon-Durocher & Allen, 2012).

To test these hypotheses, we selected 39 paddy fields across 13 regions throughout the main rice cropping areas in China (19.75°N to 47.58°N, 110.41°E to 126.92°E) and investigated the distribution of the spatial turnover of soil bacterial community and microbial functional genes, together with the biomass of both belowground microbes (i.e. total microbial biomass) and aboveground rice plants. Paddy soil ecosystems were selected to test these hypotheses as the soil environment is less heterogeneous in different paddy fields compared with upland soils, such as the soil moisture, oxygen content and organic components (Maie *et al.*, 2002; Chen *et al.*, 2021). Such habitats are expected to have advantages for determining the relationships between ecological processes and temperature because of the partially reduced effects of soil heterogeneity, which also mediates the ecological processes (Ranjard *et al.*, 2013). In addition, we compiled a large number of spatial turnover results for both microorganisms (including bacteria, fungi and archaea) and plants from the literature and tested our hypotheses on a global scale. The results indicated that, consistent with the MTE, belowground spatial turnover of bacterial communities and microbial functional genes, as well as the soil total microbial biomass and aboveground rice plant biomass, are related to environmental temperature on the continental scale. Through meta-analysis, the temperature-dependent spatial scale pattern of belowground microbes (including bacteria, fungi and archaea) and aboveground plants was further extended to the global scale. Moreover, belowground microbes contribute to the aboveground spatial scaling and the functioning of ecosystems.

Materials and Methods

Study sites and sampling strategy

A total of 429 soil samples were collected between June and October 2013 after rice was harvested from 39 paddy fields across 13 regions throughout the main rice cropping areas in China (19.75°N to 47.58°N, 110.41°E to 126.92°E; Supporting Information Fig. S1). The sampling area covered five temperature zones from North to South China, namely the cold temperate zone, middle temperate zone, warm temperate zone, subtropical zone and tropical zone, with the mean annual temperature (MAT) ranging widely from 1.5°C to 23.8°C. Three sites in the same region were located in relatively close proximity (within 20 km) to ensure that the climate, soil type and farming practices, such as cropping system, fertilization and irrigation scheme, were relatively similar. Eleven soil samples were taken from within a 100 m × 100 m plot in each field using a spatially explicit 'L-shaped' sampling design. Details of the 'L-shaped' sampling design and soil collection and storage were described in our previous study (Xiao *et al.*, 2021). Hourly temperature and annual precipitation data were collected by the nearest weather stations on site, and the MAT and average annual precipitation were calculated thereafter. The net primary productivity (NPP) of the paddy fields was simulated by remote sensing in 2014 based on a light-use efficiency model (Yuan *et al.*, 2007). Soil properties, including pH, cation exchange capacity (CEC), organic matter (OM) and total nitrogen (TN), were measured as in Methods S1. Detailed field information is provided in Table S1.

Illumina sequencing of 16S rRNA gene amplicons

The soil bacterial community was analysed by amplicon sequencing of the 16S rRNA gene using the Illumina MiSeq 2 × 150 bp sequencing platform (Illumina, San Diego, CA, USA). The primer set including 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) was selected, targeting the bacterial V4 region of the 16S rRNA gene (Caporaso *et al.*, 2012). Details of the sample preparation and sequencing are provided in Methods S2.

GeoChip analysis of microbial functional genes

GeoChip 5.0 (Tu *et al.*, 2014) was used to target the soil microbial functional genes. An aliquot of DNA (800 ng) from each

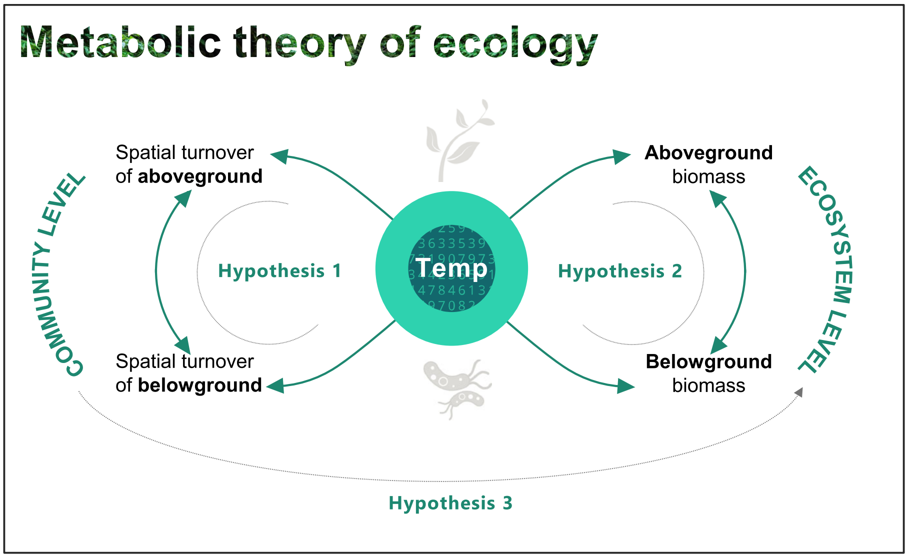

Fig. 1 Schematic of the working hypotheses. Hypothesis 1: The distribution of species spatial turnover fits the metabolic theory of ecology (MTE), and this theory is applicable to both belowground microbes and aboveground macroscopic plants. Hypothesis 2: Belowground microbial biomass and aboveground plant biomass, as reflections of ecosystem processes, also fit the MTE. We expect the interdependency of the spatial scaling or functioning of the above and belowground ecosystem. Hypothesis 3: The community-level feature (i.e. spatial turnover) relates to the ecosystem process (i.e. biomass production), as they are inextricably linked.

sample was directly labelled with the fluorescent dye Cy-3 (GE Healthcare, Los Angeles, CA, USA), purified, resuspended in 27.5 μl of DNase/RNase-free distilled water and then mixed completely with 42 μl of a hybridization solution containing 1× CGH blocking agent, 1× HI-RPM hybridization buffer, 10 pM universal standard DNA (Liang *et al.*, 2010), 0.05 μg μl^{-1} Cot-1 DNA, and 10% formamide (final concentrations). Then, the solution was denatured at 95°C for 3 min, incubated at 37°C for 30 min and then hybridized with GeoChip 5.0 (180k; Agilent, Agilent Technologies Inc., Santa Clara, CA, USA). GeoChip hybridization was conducted at 67°C in an Agilent hybridization oven for 24 h. After hybridization, the slides were washed using Agilent washing buffers at room temperature. Then, the arrays were scanned at 633 nm with a laser power of 100% and a photomultiplier tube gain of 75% with a NimbleGen MS200 Microarray Scanner (Roche NimbleGen Inc., Madison, WI, USA). The image data were extracted using the Agilent Feature Extraction programme. The microarray data were preprocessed with the Microarray Data Manager system at the Institute for Environmental Genomics (IEG) website (http://ieg.ou.edu/microarray), which was described previously (Liang *et al.*, 2015). Spots with signal-to-noise ratios lower than 2.0 were removed before statistical analysis. Finally, 123 804 functional genes involved in C/N/P/S cycling were detected by GeoChip, stemming from bacterial, fungi and archaea.

Microbial phospholipid fatty acids

A modified Bligh-Dyer procedure was used to extract the microbial phospholipid fatty acids (PLFAs) from moist soil equivalent to 2 g of dry soil (Frostegård *et al.*, 1993). Briefly, raw lipids were extracted with a mixed solution of methanol, chloroform and citric acid (2 : 1 : 0.8). The glycolipid and neutral lipid fractions were then removed via passage through silicic acid-bonded solid-phase extraction columns (Waters, Milford, MA, USA). The resulting phospholipids were saponified and methylated to fatty acid methyl esters (FAMEs), which were subsequently analysed using the MIDI Sherlock microbial identification system (MIDI, Newark, DE, USA) with FAME 19:0 as the internal standard. The sum of the PLFAs was used as a measure of total microbial biomass, and the biomasses of bacteria, fungi and actinobacteria were quantified as described previously (Frostegård *et al.*, 1993; Bååth & Anderson, 2003).

Microbial spatial turnover computation and the metabolic theory of ecology

To quantify belowground microbial spatial turnover, taxon–area relationships (TARs), describing changes in taxonomic (or species) richness with area, were evaluated. Since a spatially explicit 'L-shaped' sampling scheme was used to collect the soil samples, five nested triangular areas were obtained for the microbial spatial pattern analysis in each paddy field. The power-law form of the TARs ($S = cA^z$) was converted by logarithmic transformation. The exponent z was estimated by linear regression:

$$\log_e S = \log_e c + z\log_e A \qquad \text{Eqn 1}$$

where S is the observed gene or taxon richness, A is the area in the nested design (0.5, 18, 128, 648 and 2888 m^2), $\log_e c$ is the intercept in log–log space, and the taxa-area exponent, z, is a measure of the rate of species change in space, typically used to describe the spatial turnover of organisms. The log-based linear equation was applied to both the bacterial community and functional genes (Table S2), as well as to the taxonomic groups or subgroups of functional genes.

The MTE predicts that the biological metabolic rate varies with temperature (Brown *et al.*, 2004). The relationship between community/ecosystem processes and temperature can be quantitatively calculated as follows:

$$\log_e I = -E\,(1/kT) + c_T \qquad \text{Eqn 2}$$

where I is the community-level process (species spatial turnover) or ecological-level process (biomass accumulation), k is Boltzmann's constant ($k = 8.62 \times 10^{-5}$ eV K^{-1}), T is the absolute temperature, c_T is a normalization constant, and E_T is the activation energy for all of these processes. Eqn 2 predicts that the natural logarithm of the organismal metabolic rate or diversity should be a linear function of the inverse absolute temperature ($1/kT$).

Meta-analysis of temperature-dependent spatial turnover in microbes and macroscopic plants

To obtain general insights into the spatial pattern of community composition among different organisms, we conducted a meta-analysis of spatial turnover (*z*-value) for both microbes and macroscopic plants. Here, we collected all available published *z*-values based on TARs as effect sizes in the meta-analysis based on the search strings 'taxa-area relationship*', 'taxa-area curve*', 'species-area relationship*' and 'species-area curve*', with and without hyphens. We first obtained a dataset comprising 1043 *z*-values from all available TARs published in the literature and from this study. Then, the dataset was filtered by the *z*-values derived only from microbes and plants. We further filtered the dataset by the availability of MAT data at these sites; the data were either obtained from the literature or inferred from the latitude and longitude coordinates. In addition, for the most diverse microbial communities, we included only high-throughput sequencing and microarray techniques with relatively high resolution to limit the influence of the method. In total, MAT data for 206 plants and 139 microbial *z*-values were available from 41 countries, representing all continents except Antarctica, including the bacteria, fungi and archaea. We obtained sampling variances to conduct a weighted meta-analysis (Gurevitch & Hedges, 1993). For *z*-values, the sampling variance is the squared standard error. The weighted dataset was further used to obtain general insights into temperature-driven spatial turnover for both macroscopic plants and microbes.

Statistical analyses

For the linear and nonlinear regressions, the goodness of fit was assessed using the Akaike information criterion (AIC) and r^2 values. Pairwise comparisons of the slopes of the linear regressions were achieved by bootstrapping (999 times) followed by a pairwise *t*-test (Liang *et al.*, 2015). To investigate the contributions of belowground components to aboveground ecosystem function (i.e. NPP) independent of temperature, MAT and belowground *z*-values of bacterial communities and microbial functional genes and the total microbial biomass were used to predict the aboveground biomass in paddy fields by random forest modelling using the RandomForest package (Liaw & Wiener, 2002) in R (v.3.5.1; http://www.r-project.org/). RandomForest modelling evaluated the importance of each predictor by examining how much the mean square error (MSE) increased when the data for that predictor were permuted randomly while the other data remained unchanged.

Structural equation models (SEMs) were further fitted to illustrate the direct and indirect effects of MAT and belowground features on aboveground ecosystem function in paddy fields. First, an *a priori* model was established based on the known relationships between MAT, belowground *z*-values of bacterial communities and microbial functional genes and the total microbial biomass, and aboveground biomass. Second, nonnormally distributed data were log$_e$-transformed. Then, we parameterized the *a priori* model with our dataset to test the overall goodness of model fit. Improved model fit was assessed with the chi-squared test, goodness-of-fit index and root mean square error of approximation as described previously (Xiao *et al.*, 2018). With a reasonable model fit, we then interpreted the path coefficients of the model and the associated *P*-values. We also linked the spatial turnover of microbes (including bacteria, fungi and archaea) to that of plants using 19 sets of metadata with adjacent geographical locations by SEM. Since this model is a saturated model, with all parameters to be estimated being exactly equal to the elements in the covariance matrix, the fit index was no longer estimated, and only the path coefficients were interpreted in our study. All SEM analyses were carried out with IBM® SPSS® AMOS 20.0 (AMOS IBM, Armonk, NY, USA).

Results

The distribution of belowground microbial spatial turnover in paddy fields fits the MTE

Species spatial turnover is an important concept used to describe turnover in species composition across a wide range of spatial scales, and it underpins much of conservation theory and practice (Gering *et al.*, 2003). Here, the spatial turnover (*z*-values) of both the soil bacterial community and microbial functional genes was estimated. The belowground microbial spatial turnover of 39 paddy fields located in 13 regions across China varied greatly, with *z* (bacteria) values ranging between 0.003 and 0.050 and between 0.057 and 0.091 for *z* (microbial functional genes; Fig. S2). Interestingly, both the bacterial community ($r^2 = 0.118$, $P = 0.033$, AIC = −371) and microbial functional genes ($r^2 = 0.264$, $P < 0.001$, AIC = −366) and their subgroups showed a significantly increasing trend of *z*-values with MAT (Fig. S2; Table S3). Though paddy soil properties (i.e. pH, OM, CEC and TN) covary with MAT, they rarely related to the microbial spatial turnover according to the Pearson's correlation analyses (Fig. S3).

To test hypothesis 1, the temperature dependence of *z* (bacteria) and *z* (microbial functional genes) was estimated based on the MTE. The activation energies (*E*) for belowground spatial turnover were estimated as slopes of linear regressions between the log$_e$-transformed *z*-values and the reciprocal of absolute temperature ($1/kT$). Notably, we found strong linear relationships between the log-transformed *z*-values and $1/kT$ for the bacterial community ($E = 0.047$ eV, $r^2 = 0.128$, $P = 0.025$) and microbial functional genes ($E = 0.341$ eV, $r^2 = 0.255$, $P = 0.001$; Fig. 2). The *E*-values of microbial functional genes were significantly higher than those of bacterial communities according to bootstrapping (999 times) followed by a pairwise *t*-test

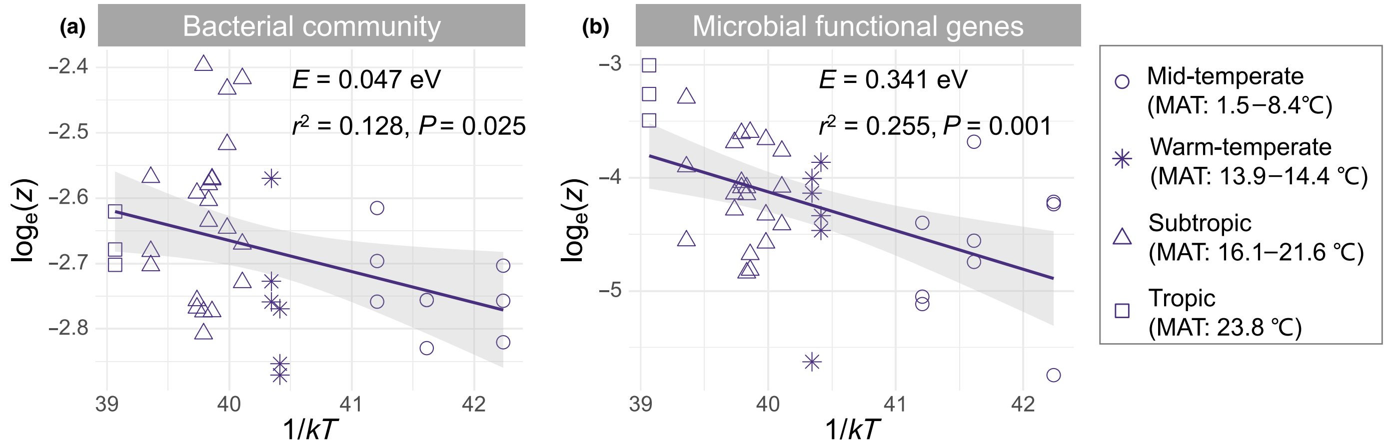

Fig. 2 Temperature-dependent spatial turnover (z) of both the soil bacterial community (a) and microbial functional genes (b) in 39 paddy fields across China. In accordance with the metabolic theory of ecology, strong linear relationships between the log-transformed *z*-values and the reciprocal of absolute temperature (1/*kT*) for the bacterial community and microbial functional genes were observed. *E* is the 'activation energy' estimated as slopes of linear regressions between the $\log_e$-transformed *z*-values and 1/*kT*. Numbers in the brackets of the legend indicate the mean annual temperature (MAT) ranges of the sampling area. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits.

($P < 0.01$). The *z*-values of bacterial subgroups also showed strong linear relationships with $1/kT$, with the highest activation energy observed for Bacteroidetes (E = 0.168 eV, r^2 = 0.213, P = 0.003), followed by Actinobacteria (E = 0.120 eV, r^2 = 0.159, P = 0.012) and Chloroflexi (E = 0.114 eV, $r^2 = 0.180$, $P = 0.007$; Table 1). The *z*-values of all soil microbial functional subgroups also fit the MTE, with the highest activation energy observed for the functional genes involved in methane generation ($E = 0.428$ eV).

Both belowground microbial biomass and aboveground biomass fit the MTE

An understanding of the relationship between biomass and temperature is useful for predicting the impacts of the future course of climate change on carbon stores (Stegen *et al.*, 2011). The belowground total microbial biomass of paddy fields was evaluated with the PLFA technique, including bacteria, fungi and actinobacteria. The total microbial PLFAs of 39 paddy fields varied greatly from 7.49 to 50.56 nmol g^{-1} DW of soil. Increases in belowground microbial total biomass and bacterial, fungal and actinobacterial biomass indices with MAT were also observed on the basis of PLFA measurements (r^2 = 0.161–0.221, $P < 0.05$; Fig. S4a,c). We further estimated aboveground biomass in the paddy fields based on NPP with remote sensing, which varied from 171.95 to 521.20 g C m^{-2} yr^{-1}. Similarly, NPP increased significantly with MAT from the best-fit prediction (exponential model: r^2 = 0.246, $P < 0.001$; Fig. S4b), though there is a decrease at the tropical sites.

Due to the significantly increasing trend of PLFAs and NPP with MAT (Fig. S4), we further evaluated whether the influence of temperature on biomass followed the prediction of the MTE (hypothesis 2). As expected, significant temperature dependence of both PLFAs and NPP was found (Figs 3, S5). The activation energies were 0.191–0.217 eV (r^2 = 0.150–0.232, $P < 0.05$) for belowground microbial total PLFAs and bacterial, fungal and actinobacterial PLFAs and 0.240 eV ($r^2 = 0.313$, $P < 0.001$) for NPP.

Table 1 Activation energy (*E*)-values of soil microbial spatial turnover in paddy fields across China.

	E (eV)	r^2	*P*	AIC
Taxonomic groups				
Bacterial communities	0.047	0.128	0.025	57.069
Acidobacteria	/	/	ns	/
Actinobacteria	0.120	0.159	0.012	5.679
Bacteroidetes	0.168	0.213	0.003	17.831
Chloroflexi	0.114	0.180	0.007	−4.338
Firmicutes	/	/	ns	/
Verrucomicrobia	/	/	ns	/
Proteobacteria	/	/	ns	/
Functional groups				
Microbial functional genes	0.341	0.255	0.001	63.975
C cycling	0.348	0.255	0.001	65.203
C fixation	0.333	0.225	0.002	68.484
C degradation	0.350	0.261	< 0.001	64.585
Methane generation	0.428	0.337	< 0.001	66.165
Methane oxidation	0.368	0.247	0.001	71.432
N cycling	0.332	0.229	0.002	67.256
N fixation	0.314	0.188	0.006	72.555
Ammonification	0.333	0.243	0.001	64.369
Nitrification	0.366	0.187	0.006	84.874
Denitrification	0.311	0.183	0.007	72.999
P cycling	0.339	0.240	0.002	66.542
S cycling	0.351	0.255	0.001	66.014

'/' denotes no significant relationship between $\log_e$-transformed species spatial turnover and the reciprocal of absolute temperature (1/*k*T). AIC, Akaike information content; ns, not significant; *P*, Monte Carlo derived probabilities.

Linking the below- and aboveground components of paddy fields

Given that belowground microbes sustain life aboveground, we explored how belowground microbes, that is the spatial turnover

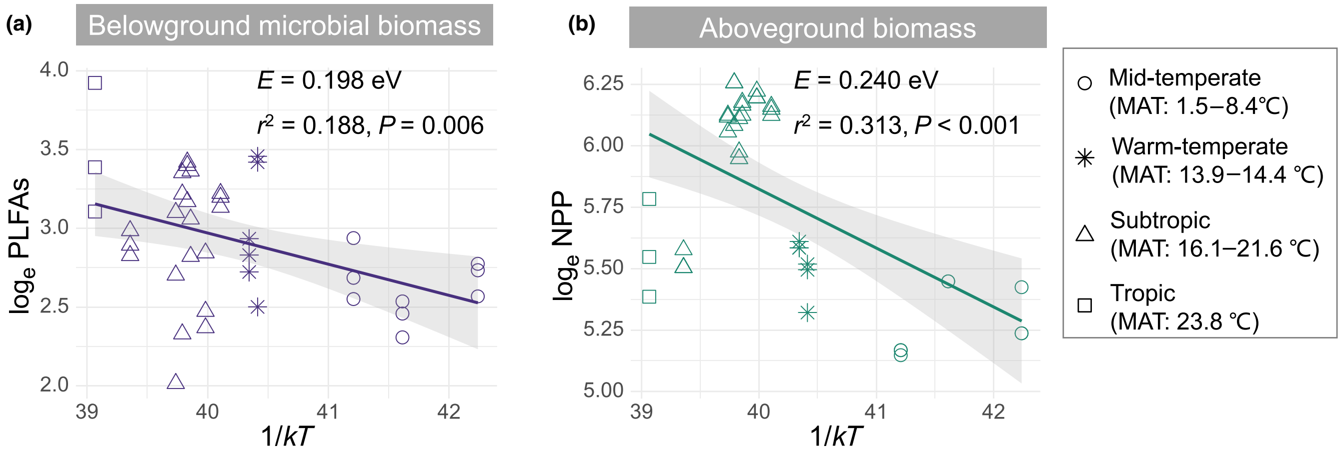

Fig. 3 Temperature-dependent belowground microbial biomass (a) and aboveground plant biomass (b) in 39 paddy fields across China. The sum of the microbial phospholipid fatty acids (PLFAs) was used as a measure of total microbial biomass, including bacteria, fungi and actinobacteria; net primary productivity (NPP) was used as a measure of paddy field aboveground biomass. The influence of temperature on belowground microbial biomass and aboveground plant biomass followed the prediction of the metabolic theory of ecology, as there were strong linear relationships between the log-transformed biomass values and the reciprocal of absolute temperature ($1/kT$). E is the 'activation energy' estimated as slopes of linear regressions between the $\log_e$-transformed biomass and $1/kT$. Numbers in the brackets of the legend indicate the mean annual temperature (MAT) ranges of the sampling area. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits.

of bacterial communities and microbial functional genes and the microbial biomass, contribute to aboveground ecosystem functions independent of temperature across a broad temperature gradient. Mean annual temperature and belowground species spatial turnover (z) and biomass (PLFAs) were used to predict the NPP in paddy fields by RandomForest modelling. In total, these predictors explained 69.81% of the variation in NPP. Mean annual temperature played the most important role in shaping the variation in NPP in paddy fields across the broad temperature gradient (Fig. 4a). The z (bacteria) also affected NPP, followed by PLFAs and z (microbial functional genes).

The direct and indirect effects of MAT and belowground species spatial turnover and biomass on aboveground biomass were further investigated with SEMs, with the final model explaining 38% of the variation in aboveground biomass (Fig. 4b). The results revealed the strongest direct effect of MAT on aboveground biomass ($r = 0.53$, $P < 0.01$). Furthermore, MAT also affected aboveground biomass indirectly by influencing the spatial turnover of the bacterial community ($r = 0.27$, $P < 0.05$). Although belowground biomass and the spatial turnover of microbial functional genes were also mediated by temperature, they had weak direct impacts on aboveground biomass. These results highlighted the importance of belowground bacterial spatial turnover in determining aboveground biomass in addition to temperature, which supported hypothesis 3.

Aboveground biomass is expected to increase soil carbon input via enhanced root exudation and may therefore influence belowground communities and processes (Bartelt-Ryser *et al.*, 2005). Here, we explored the potential influence of aboveground biomass on soil microbial biomass and spatial turnover with a SEM model. Contrary to our expectation, the aboveground biomass in our study, that is the NPP in paddy fields, had weak direct impacts on both belowground biomass and microbial spatial turnover ($P > 0.05$; Fig. S6).

Meta-analysis of the temperature dependence of species spatial turnover from microbes to plants at the global scale

Given the strong temperature dependence of species spatial turnover and intimate interactions between microbes and plants (Figs 2–4), we aimed to obtain general insights into the global pattern of spatial turnover for both microbes and plants. All available species spatial turnover estimates (z-values) of the TARs from the literature (including macroscopic plants and microbes) and this study were compiled as effect sizes (Fig. 5; Table S4). In total, 465 z-values for plants and 145 z-values for microbes were obtained, of which the microbes covered the bacteria, fungi and archaea. Among all of these data, some studies (plants: $n = 206$; microorganisms: $n = 139$) could be matched with MAT data obtained directly from the original paper or inferred from latitude and longitude coordinates, including seven countries in North America, two countries in South America, 15 countries in Europe, six countries in Africa, eight countries in Asia, three countries in Australia and other ocean sites (Fig. 5a). The distribution of the raw data and weighted z-values of macroscopic plants and microbes are presented in Fig. 5(b). The weighted z-values and $1/kT$ were fitted using linear regression. Significant linear relationships between z and $1/kT$ were observed for plants ($E = 0.272$ eV, $P < 0.001$) and microbes ($E = 0.170$ eV, $P < 0.05$) at a global scale (Fig. 5c). We also noted that the r^2 value for microbes is low (0.037). We further related the spatial turnover of microbes with that of plants using 19 sets of data with adjacent geographical locations using saturated SEMs (Fig. 5d). The z-values of microbes significantly predicted the variation in the z-values of macroscopic plants ($r = 0.45$, $P < 0.05$); and we observed feedbacks of macroscopic plants on the microbes ($r = 0.50$, $P < 0.05$), even when controlling for MAT.

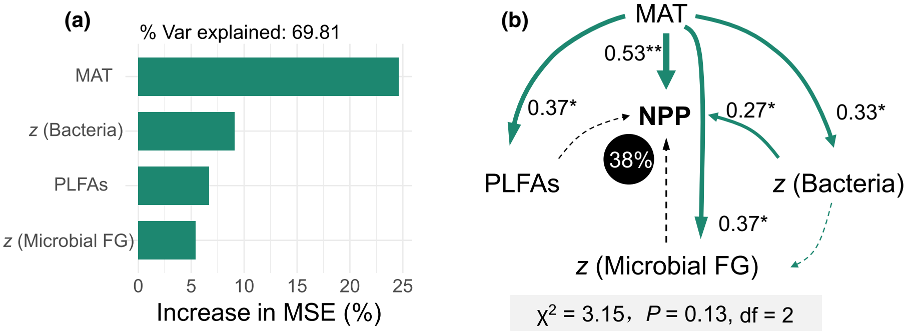

Fig. 4 Linking the below- and aboveground parts of paddy fields. (a) Mean predictor importance (% increase in the mean square error, MSE) of the mean annual temperature and belowground species spatial turnover and biomass for the aboveground biomass (i.e. net primary productivity) based on RANDOMFOREST analyses. MAT played the most important role in shaping the variation in aboveground biomass in paddy fields across. The *z* (bacteria) also affected NPP, followed by PLFAs and *z* (microbial functional genes). (b) Direct and indirect effects of mean annual temperature and belowground spatial turnover of bacterial communities and microbial functional genes and total microbial biomass on the aboveground biomass estimated by structural equation modelling. Green arrows indicate significant paths ($P < 0.05$) relationships; dotted black arrows represent nonsignificant paths ($P > 0.05$). Numbers adjacent to arrows are standardized path coefficients. The number in the black circle denotes the percentage of variation in aboveground biomass explained by the model. The significance levels of each predictor are derived from multiple testing using the Benjamini–Hochberg procedure: *, $P < 0.05$; **, $P < 0.01$. FG, functional genes; MAT, mean annual temperature; NPP, net primary productivity; PLFAs, phospholipid fatty acids.

Discussion

Spatial turnover of biodiversity is an important concept used to describe turnover in community composition across a wide range of spatial scales, and it underpins much of conservation theory and practice (Barton *et al*., 2013). Although microorganisms comprise much of the biodiversity on Earth, little is known about the temperature dependence of their spatial turnover in the present context of global warming (Ranjard *et al*., 2013; Prober *et al*., 2015; Chalmandrier *et al*., 2019). Based on the predictions of the MTE, we examined the temperature dependence of the soil bacterial community and microbial functional genes using a dataset that included 39 paddy fields across China. Our results supported our first hypothesis and showed that the temperature-dependent spatial turnover of paddy soil microbes followed the predictions of the MTE (Fig. 2). This is also applicable to microbes at a global scale, including a wide range of taxa (i.e. bacteria, fungi and archaea) and habitats, based on our meta-analysis (Fig. 5). These findings, together with those of previous studies, demonstrate that the biodiversity of forest soil microbial groups fit the MTE (Tu *et al*., 2016; Zhou *et al*., 2016), suggesting that both local species richness (α diversity) and spatial turnover (β diversity) increase with environmental temperature.

The activation energies were 0.047 and 0.341 eV for the spatial turnover of the bacterial community and microbial functional genes, respectively. The more rapid increase in the spatial turnover of microbial functional genes with increasing temperature than in that of the bacterial community is most likely due to the finer resolution of functional genes than of species, as previous research indicates that *E*-values increase with increased taxonomic/genetic resolution (Deng *et al*., 2018). Our results, together with those of previous studies (Hawkins *et al*., 2007; Wang *et al*., 2009; Zhou *et al*., 2016), showed that the values of E for both the α and β diversities of the most diverse microbes may be lower than the predicted range of 0.60–0.70 eV (Brown *et al*., 2004; Allen *et al*., 2005). For reference, the *E*-values were expressed in terms of Q_{10}, which is defined as the rate of change with a 10°C increase in temperature and were calculated according to Liu *et al*. (2016). The *E*-values for bacteria (0.047 eV) and the functional genes (0.341 eV) are equivalent to Q_{10} values of 1.07 and 1.64; therefore, the spatial turnover of the soil microbial community increases *c*. 1.1–1.6 times for every 10°C increase in mean annual environmental temperature. Furthermore, the meta-analysis indicated that the spatial turnover of plants also fitted the MTE on a global scale; the temperature dependence of spatial turnover for microbes (E = 0.170 eV) was lower than that for plants (E = 0.272 eV). The shallower pattern of the temperature dependence of microbial spatial turnover compared with that of plants was consistent with that found for α diversity (Zhou *et al*., 2016). The lower temperature dependence of microbes may be attributed in part to their greater dispersal abilities and shorter generation time.

Based on the dataset of 39 paddy fields, we found clear support for the second hypothesis stating that belowground microbial biomass and aboveground plant biomass, as reflections of ecosystem processes, can be predicted by the MTE (Fig. 3). This temperature dependence of aboveground plant biomass agrees qualitatively with previous observations for terrestrial NPP (Lieth, 1973; Anderson *et al*., 2006). The temperature dependence of NPP is largely due to the direct effect of temperature rather than the indirect effects of growing season length and incident solar radiation (Allen *et al*., 2005). It is worth noting that a drop-off in NPP occurred at the tropical sites, suggesting the temperatures are above the optimal for growth (22–28°C) (Krishnan *et al*., 2011). Here, we observed a lower temperature dependence for aboveground plant biomass (E = 0.240 eV) than that reported previously for terrestrial ecosystems (E = 0.320 eV) (Allen *et al*., 2005; Anderson *et al*., 2006). This may be related to the intense human activities in rice plantations, such as tillage and fertilization, which might flatten the Boltzmann–Arrhenius curves. In addition, this is the first demonstration that temperature-dependent belowground microbial biomass followed the prediction of the MTE, with an *E*-value of 0.198 eV.

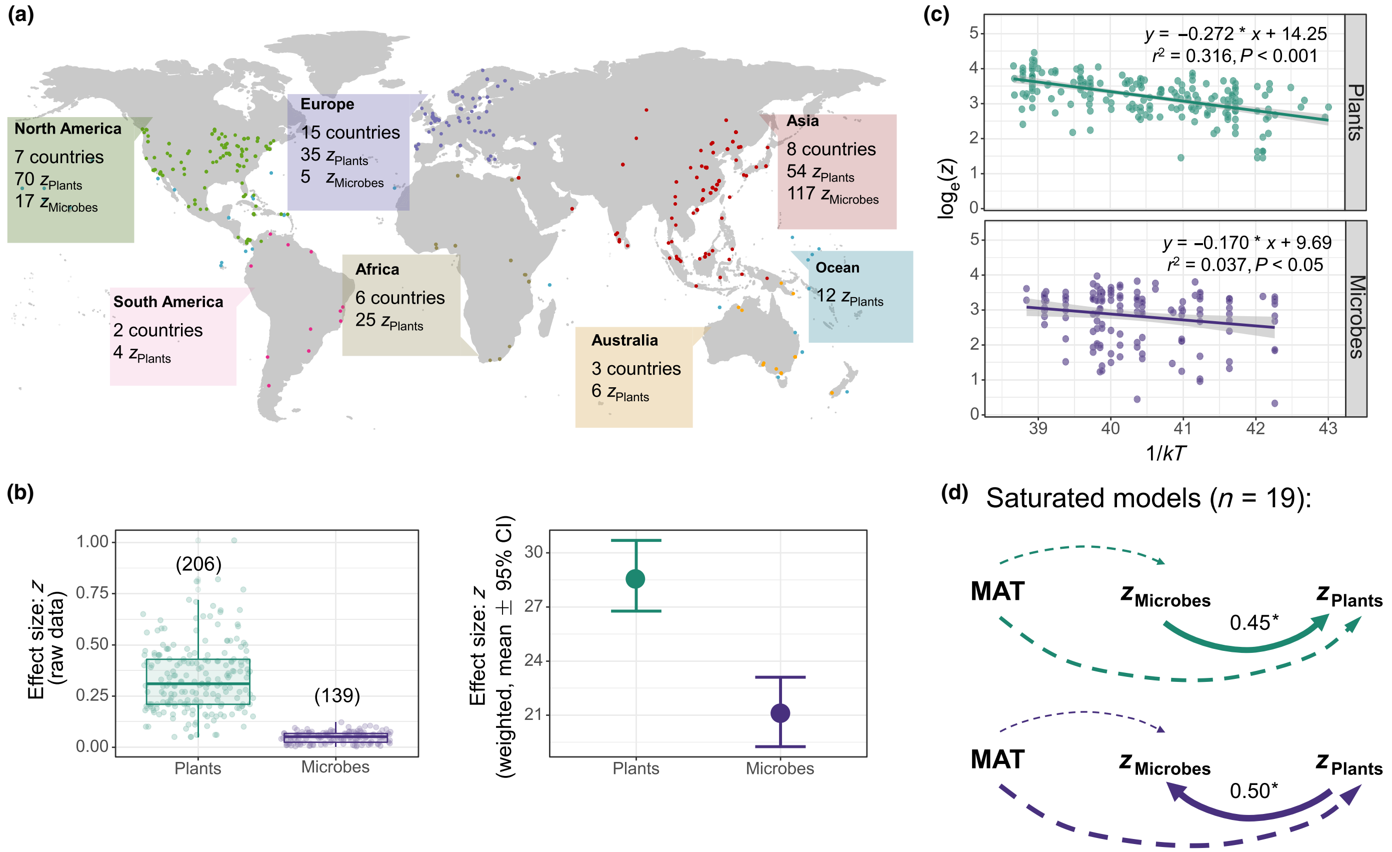

Fig. 5 Spatial turnover (*z*) of plants and microbes at the global scale and their relationships with temperature. (a) Map of locations from which *z*-values for macroscopic plants and microbes were collected. (b) Global *z*-values for plants and microbes. In total, 206 *z*-values for plants and 139 for microorganisms (including bacteria, fungi and archaea) were matched with MAT data at these sites. The average weighted *z* ± 95% confidence interval (CI) is given. Box plots show medians (horizontal lines), 25% and 75% quantiles (boxes), most extreme values sans outliers (whiskers) and outliers (samples beyond 1.5 times the interquartile range away from the median). (c) Relationships between the global *z*-values of plants and microbes and temperature. Significant linear relationships between spatial turnover (*z*) and the reciprocal of absolute temperature (1/*kT*) were observed for plants and microbes at a global scale. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits. (d) Relating the *z*-values of microbes with those of plants using 19 sets of data with adjacent geographical locations using a saturated model. The significance level of the predictor was derived from multiple testing using the Benjamini–Hochberg procedure: *, $P < 0.05$. MAT, mean annual temperature.

However, contrary to our expectation, the temperature dependence of belowground microbial biomass was less than that of aboveground plant biomass. Previous studies indicated that microbial biomass, mainly controlled by respiration, should have a stronger temperature dependence than NPP, which is mainly controlled by the photosynthetic rate (Allen *et al*., 2005; Anderson *et al*., 2006). This discrepancy might partly relate to the biased determination of microbial biomass by the PLFA method used in our study, as the soil type may have significant influence on the quantity and profile of PLFA extracted from soil (Joergensen, 2022). A more standard technique for determining total microbial biomass in soils can be supplementary to our study, such as the chloroform fumigation-extraction method.

By linking the below- to aboveground biota, as well as a community-level feature (i.e. spatial turnover) to an ecosystem process (i.e. biomass production), the results based on a dataset of 39 paddy fields and a compiled meta-dataset supported our third hypothesis and revealed mutual feedbacks between aboveground plant and belowground microbes (Figs 4, 5). It has become widely accepted that vegetation dynamics are strongly influenced by the soil biota and the latter showed feedbacks on plant growth, especially at local spatial scales (Wardle *et al*., 2004; Bartelt-Ryser *et al*., 2005). Here, we extended their linkages to the global scale, and the significant relationship between below- and aboveground spatial turnover was maintained even when controlling for environmental temperature. This suggested that factors other than shared relationships with MAT also contribute to their linkages, potentially including shared evolutionary histories, plant species-specific symbioses and rhizodeposition of C compounds (Prober *et al*., 2015). The caveat here is that since the datasets used in the meta-analysis covered a wide range of habitats and were obtained using different experimental methods, the result may only roughly reflect the global distribution pattern of community spatial turnover, as can be seen from the significant but low correlation between temperature and microbial spatial turnover. Interestingly, the dataset of 39 paddy fields suggested a causal relationship between belowground spatial turnover and aboveground biomass. This suggested the potential influence of temperature-driven spatial heterogeneity in microbial diversity on plant growth, possibly directly through a variety of biotic interactions or indirectly through changes in soil nutrient availability and predation on plantfeeding organisms (Bezemer & van Dam, 2005; Bardgett & Van Der Putten, 2014). The lack of coupling between above- and belowground biomass suggests that patterns of above- and belowground biomass accumulation are governed by different mechanisms.

In conclusion, by combining the dataset of 39 paddy fields across China and the compiled global meta-dataset, we tested the MTE from below- to aboveground and from community to ecosystem processes. For both belowground microbes and aboveground macroscopic plants, the distribution of spatial turnover, reflecting community-level ecological organization, as well as biomass accumulation, reflecting an ecosystem-level process, fit the MTE. The belowground community-level process exerted a profound influence on aboveground community dynamics and ecosystem functioning, and the aboveground community dynamics showed feedbacks on the belowground community process. Our findings have important implications for the MTE and above- and belowground ecology under further climate change scenarios. We find the MTE to be useful for investigating a broad range of phenomena in biology, although our results together with those of several previous reports support the emerging generalization that there is no canonical E_T value of *c.* 0.65 eV (Hawkins *et al*., 2007; Wang *et al*., 2009; Zhou *et al*., 2016). Due to the strong temperature dependence of microbial spatial turnover and intimate above- and belowground linkages, further climate warming will intensify the vulnerability of both above- and belowground biodiversity when the habitat area is reduced, which will consequently accelerate ecosystem biomass accumulation.

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Competing interests

None declared.

Author contributions

All authors contributed intellectual input and assistance to this study and manuscript. JBZ, BS, JZZ and YTL designed the research. JZZ and YTL contributed to the GeoChip analysis. XX, ZYM and YTL contributed to the experiment and data analysis. XX and YTL wrote the manuscript.

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Data availability

Raw sequence data for 16S rRNA gene amplicons were deposited in the National Center for Biotechnology Information (NCBI) BioProject accession no. PRJNA562601. The GeoChip data are available in the repository Figshare, doi: 10.6084/m9.figshare.9746303.

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Supporting Information

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

Fig. S1 Sampling strategy with a nested design.

Fig. S2 Relationships between the mean annual temperature and the spatial turnover (*z*) of both the soil bacterial community and microbial functional genes in 39 paddy fields across China.

Fig. S3 Correlations between environmental factors and the spatial turnover (*z*) of both the soil bacterial community and microbial functional genes based on Pearson's correlation.

Fig. S4 Relationships between the mean annual temperature and belowground microbial biomass and aboveground plant biomass in 39 paddy fields across China.

Fig. S5 Temperature-dependent belowground microbial biomass in 39 paddy fields across China.

Fig. S6 Direct and indirect effects of mean annual temperature and aboveground biomass on the belowground spatial turnover and biomass estimated by structural equation modelling.

Methods S1 Measurement of soil properties.

Methods S2 Gene amplicon sequencing.

Table S1 Information on the 39 paddy fields across China.

Table S2 Summary of the taxon–area relationships of the power-law model for the bacterial community and microbial functional genes in 39 paddy fields across China.

Table S3 Pearson's correlations between the mean annual temperature and soil microbial spatial turnover in paddy fields across China.

Table S4 List of taxonomic groups and references that reported spatial turnover (*z*) for macroscopic plants and microbes.