***New Phytologist* Supporting Information**

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

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The following Supporting Information is available for this article:

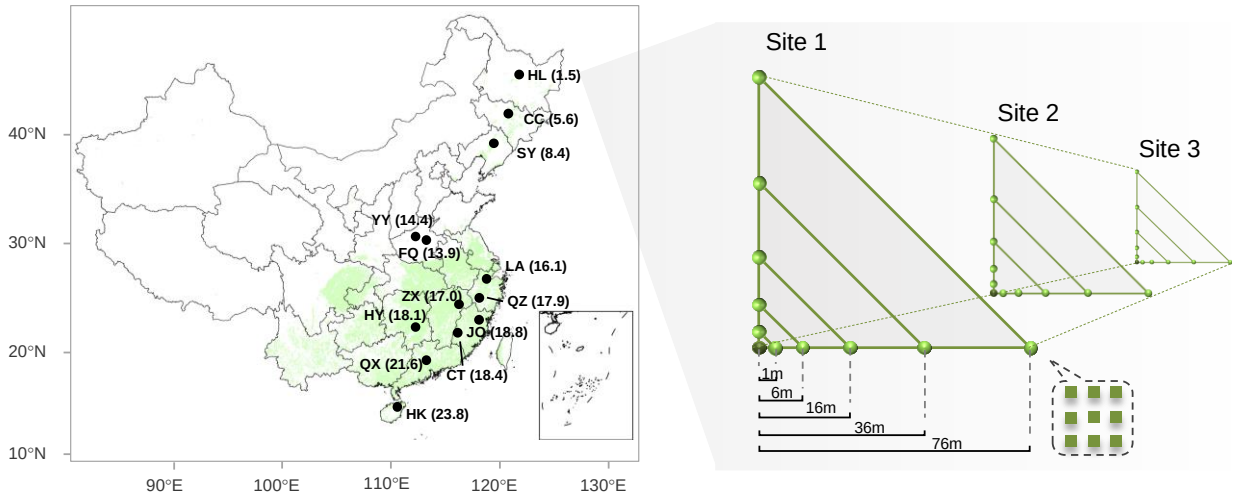

Fig. S1 Sampling strategy with a nested design. A total of 429 soil samples were taken from 39 paddy soil sites located in 13 regions of China. The number in parentheses represents the mean annual temperature (°C) in the sampling region. At each site, 11 nested samples were collected at distances of 1, 6, 16, 36, and 76 m.

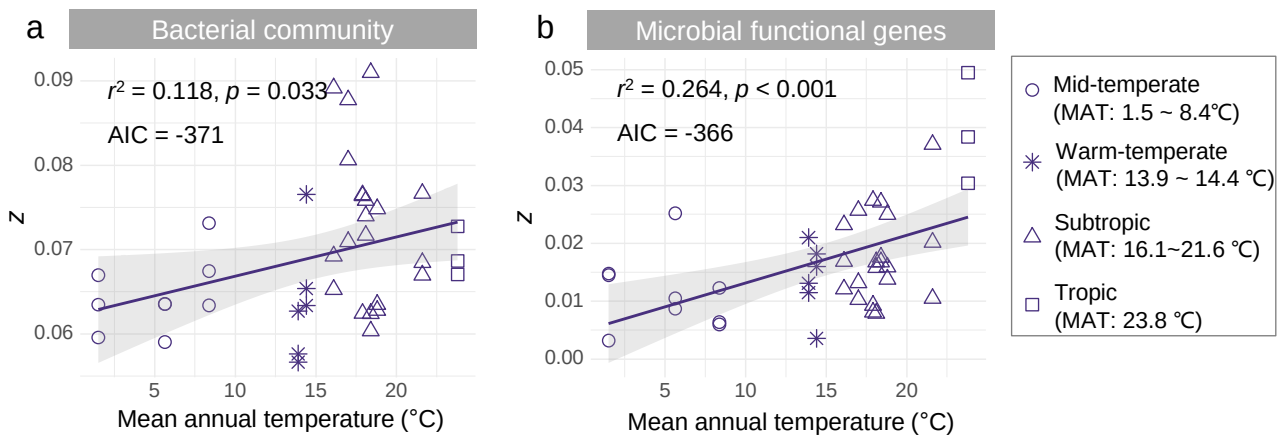

Fig. S2 Relationships between the mean annual temperature and the spatial turnover (*z*) of both the soil bacterial community (a) and microbial functional genes (b) in 39 paddy fields across China. Both the bacterial community (r^2 = 0.118, p = 0.033, AIC = -371) and microbial functional genes (r^2 = 0.264, p < 0.001, AIC = -366) showed a significantly increasing trend of *z* values with mean annual temperature. Numbers in the brackets of the legend indicate the mean annual temperature ranges of the sampling area. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits. MAT = mean annual temperature; AIC = Akaike information criterion.

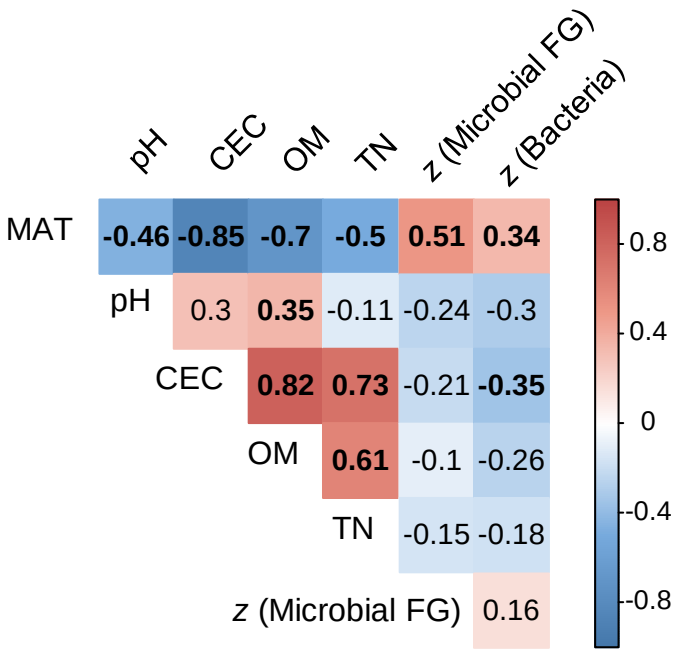

Fig. S3 The correlations between environmental factors and the spatial turnover (*z*) of both the soil bacterial community and microbial functional genes based on Pearson's correlation. Significant correlations were marked in bold ($p < 0.05$). MAT= mean annual temperature; CEC = cation exchange capacity; OM = organic matter; TN = total nitrogen; FG = functional genes.

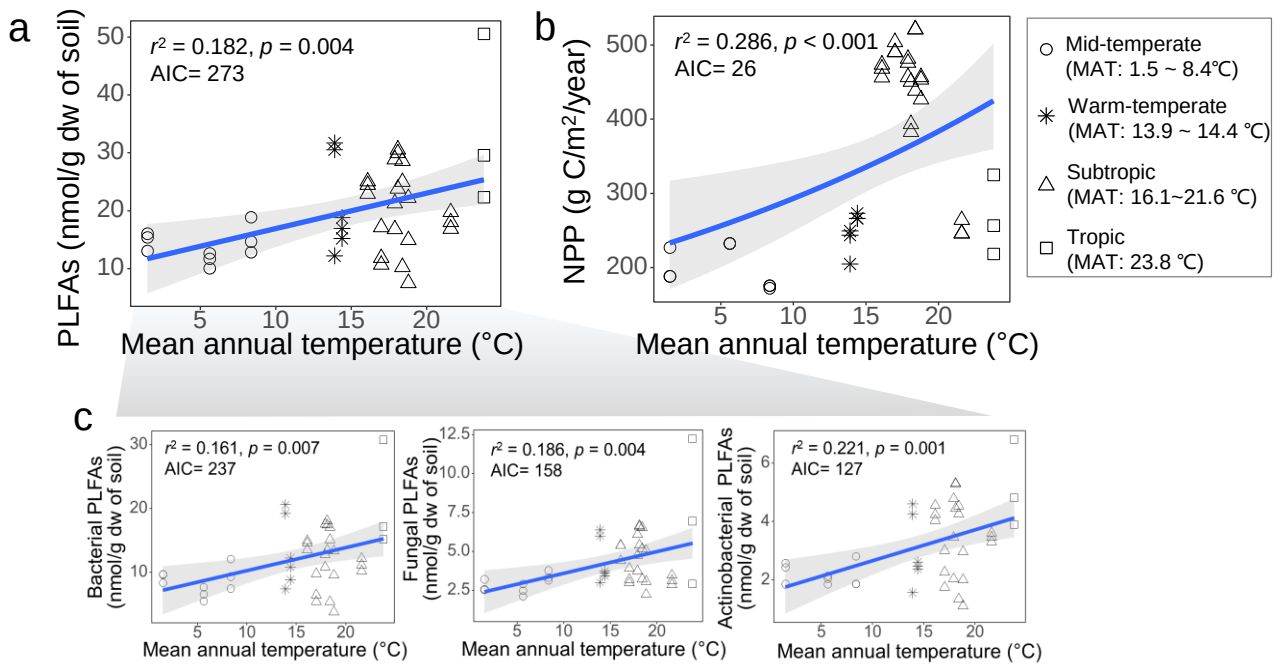

Fig. S4 Relationships between the mean annual temperature and belowground microbial biomass (a, c) and aboveground plant biomass (b) in 39 paddy fields across China. The sum of the microbial PLFAs was used as a measure of the total microbial biomass, including the biomass of bacteria, fungi, and actinobacteria. The NPP was used as a measure of paddy field aboveground biomass. There is significant increasing trend of microbial phospholipid fatty acids (PLFAs) and net primary productivity (NPP) with mean annual temperature (MAT). Numbers in the brackets of the legend indicate the mean annual temperature ranges of the sampling area. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits. AIC = Akaike information criterion.

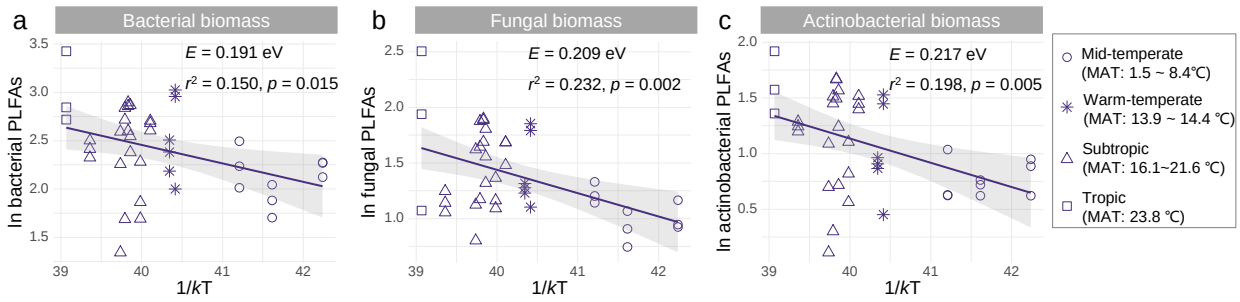

Fig. S5 Temperature-dependent belowground microbial biomass in 39 paddy fields across China. (a), Bacterial phospholipid fatty acids (PLFAs); **(b),** fungal PLFAs; **(c),** actinobacterial PLFAs. The line represents the least squares regression fit, and the shaded area represents the 95% confidence limits. Numbers in the brackets of the legend indicate the mean annual temperature ranges of the sampling area.

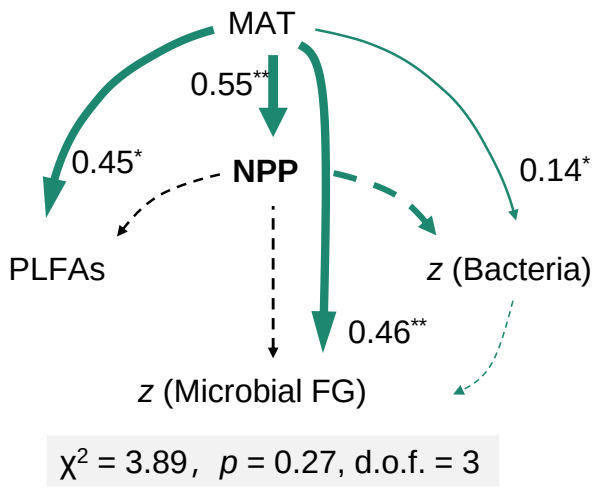

Fig. S6 Direct and indirect effects of mean annual temperature and aboveground biomass on the belowground spatial turnover and biomass estimated by structural equation modelling. The aboveground biomass (i.e., NPP in paddy fields) had weak direct impacts on both belowground total microbial biomass and spatial turnover of bacterial communities and microbial functional genes ($p > 0.05$). 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 significance levels of each predictor are as follows: *$p < 0.05$ and **$p < 0.01$. *z* (Bacteria) = spatial turnover of bacterial communities; *z* (Microbial FG) = spatial turnover of microbial functional genes; MAT = mean annual temperature; PLFAs = phospholipid fatty acids; NPP = net primary productivity; and FG = functional genes.

Table S1 Information on the 39 paddy fields across China.

Mean annual temperature	Mean annual precipitation	Soil type	Rice cultivation and yield			Plot	Coordinates	NPP	pH	CEC	OM	TN
(°C)	(mm)		N rate (kg N/ha)	P rate (kg P/ha)	Yield (Mg/ha)			g C/m^2/year	/	(cmol/kg)	(g/kg)	(g/kg)
1.5	556	Neutral black soil derived from loamy loess	362.45	95.19	7.08	HLa	47.579°N, 126.884°E	188.01	5.87	36.12	58.32	3.04
						HLb	47.566°N, 126.892°E	188.01	5.84	36.93	66.10	3.20
						HLc	47.554°N, 126.929°E	226.87	6.57	35.85	64.05	2.77
5.6	520	Neutral black soil derived from loamy loess	473.18	46.89	7.59	CCa	44.207°N, 125.572°E	232.30	7.59	34.25	45.60	2.49
						CCb	44.205°N, 125.576°E	232.30	7.97	36.38	40.10	2.32
						CCc	44.207°N, 125.569°E	232.30	8.02	31.62	39.15	2.65
8.4	399	Neutral black soil derived from loamy loess	497.73	86.86	8.50	SYa	41.516°N, 123.353°E	171.95	7.62	16.05	25.11	1.35
						SYb	41.525°N, 123.322°E	175.55	7.17	17.35	22.69	1.31

						SYc	41.502°N, 123.324°E	175.55	6.49	20.95	30.00	1.56
14.4	550	Alkaline fluvo-aquic soil derived from alluvial sediments of the Yellow River	471.57	233.77	7.56	YYa	35.003°N, 114.129°E	273.16	8.41	11.48	13.81	0.98
						YYb	35.001°N, 114.052°E	266.24	8.45	10.45	16.70	1.13
						YYc	35.003°N, 114.089°E	266.24	8.43	10.45	23.84	1.44
13.9	615	Alkaline fluvo-aquic soil derived from alluvial sediments of the Yellow River	490.06	243.81	6.60	FQa	34.958°N, 114.574°E	249.21	8.31	17.02	23.29	1.42
						FQb	34.804°N, 114.457°E	243.46	8.44	14.17	28.38	1.54
						FQc	34.933°N, 114.573°E	204.61	8.40	8.85	13.11	0.89
16.1	783	Hydromorphic paddy soils derived from sediments of lakes	382.80	86.60	7.29	LAa	30.230°N, 119.796°E	468.20	7.10	12.82	2.81	0.36
						LAb	30.097°N, 119.766°E	456.48	4.93	9.19	2.95	1.02
						LAc	30.084°N, 119.732°E	473.57	5.01	11.19	3.49	1.23

17.9	1971	Hydromorphic paddy soils derived from sediments of lakes	358.13	81.26	6.78	QZa	28.834°N, 118.869°E	476.00	4.53	14.77	4.34	2.46
						QZb	27.083°N, 118.432°E	456.81	4.71	13.15	4.88	2.58
						QZc	28.855°N, 119.014°E	481.52	5.53	11.88	4.14	2.06
17	1934	Acidic red soil derived from Quaternary red clay	213.98	110.87	1.73	ZXa	27.661°N, 117.012°E	490.26	5.11	10.93	16.86	1.15
						ZXb	27.755°N, 116.995°E	490.45	4.99	9.58	27.47	1.61
						ZXc	27.742°N, 116.976°E	503.92	4.83	9.79	31.93	1.56
18.8	1664	Acidic red soil derived from Quaternary red clay	417.74	149.38	6.07	JOa	27.067°N, 118.367°E	453.31	5.82	10.89	2.48	0.86
						JOb	27.050°N, 118.303°E	426.79	4.07	9.18	3.28	1.04
						JOc	27.083°N, 118.432°E	456.81	4.86	13.44	4.43	1.75
18.4	1700	Acidic red soil derived from Quaternary red clay	487.20	174.30	6.08	CTa	25.861°N, 116.176°E	521.20	4.88	9.53	3.66	1.78
						CTb	25.882°N, 116.142°E	438.27	6.58	9.57	3.06	1.67

						CTc	25.866°N, 116.183°E	521.20	5.13	10.34	3.58	1.84
18.1	1500	Acidic red soil derived from Quaternary red clay	355.80	90.56	6.34	HYa	26.856°N, 113.019°E	393.48	4.93	9.94	3.40	1.60
						HYb	26.898°N, 113.054°E	450.57	4.77	10.03	3.36	1.69
						HYc	26.813°N, 113.016°E	382.74	4.63	10.42	3.37	1.93
21.6	2216	Submergienc paddy soil derived from neritic sediment	567.70	123.66	5.72	QXa	23.634°N, 112.842°E	245.70	6.61	10.76	24.54	1.58
						QXb	23.618°N, 112.813°E	245.70	5.61	9.25	20.93	1.49
						QXc	23.685°N, 112.930°E	264.17	6.30	12.21	34.39	1.94
23.8	1686	Submergienc paddy soil derived from marine sediment	550.19	131.42	4.73	HKa	19.897°N, 110.425°E	256.52	5.43	11.08	19.61	1.24
						HKb	19.841°N, 110.417°E	218.22	5.00	11.96	21.89	1.45
						HKc	19.758°N, 110.512°E	324.89	5.59	15.67	29.78	2.01

NPP = net primary productivity.

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

Sites	Bacterial community			Microbial functional genes		
	z	r^2	*p*	*z*	r^2	*p*
HLa	0.063	0.999	<0.001	0.015	0.947	0.003
HLb	0.06	0.999	<0.001	0.003	0.969	0.001
HLc	0.067	0.999	<0.001	0.015	0.936	0.005
CCa	0.064	0.999	<0.001	0.011	0.955	0.003
CCb	0.064	0.999	<0.001	0.025	0.968	0.002
CCc	0.059	0.998	<0.001	0.009	0.926	0.006
SYa	0.067	0.998	<0.001	0.006	0.928	0.005
SYb	0.063	0.997	<0.001	0.006	0.865	0.014
SYc	0.073	0.996	0.001	0.012	0.941	0.004
YYa	0.063	0.995	<0.001	0.016	0.938	0.004
YYb	0.065	0.997	<0.001	0.018	0.84	0.018
YYc	0.077	0.985	0.004	0.004	0.973	0.001
FQa	0.069	0.996	0.001	0.013	0.977	<0.001
FQb	0.064	0.998	<0.001	0.021	0.85	0.017
FQc	0.063	0.992	<0.001	0.012	0.976	0.001
LAa	0.089	0.98	0.003	0.023	0.885	0.011
LAb	0.065	0.997	0.001	0.017	0.679	0.05
LAc	0.069	0.998	<0.001	0.012	0.76	0.034
QZa	0.077	0.997	<0.001	0.027	0.758	0.035
QZb	0.076	0.968	0.003	0.009	0.913	0.007
QZc	0.062	0.999	<0.001	0.008	0.962	0.002
ZXa	0.071	0.977	0.007	0.01	0.975	0.001
ZXb	0.081	0.994	0.002	0.026	0.971	0.001
ZXc	0.088	0.964	0.004	0.013	0.923	0.006
JOa	0.063	0.997	<0.001	0.016	0.9	0.009
JOb	0.064	0.997	0.001	0.025	0.944	0.004
JOc	0.075	0.993	0.001	0.014	0.908	0.008
CTa	0.062	0.997	<0.001	0.017	0.874	0.013
CTb	0.091	0.994	0.002	0.018	0.975	0.001
CTc	0.06	0.999	<0.001	0.027	0.977	<0.001
HYa	0.076	0.98	0.002	0.016	0.973	0.001
HYb	0.074	0.993	0.002	0.008	0.893	0.01
HYc	0.072	0.999	0.001	0.017	0.926	0.006
QXa	0.067	0.999	<0.001	0.011	0.963	0.002
QXb	0.068	0.997	<0.001	0.02	0.967	0.002
QXc	0.077	0.996	0.002	0.037	0.795	0.027
HKa	0.073	0.997	0.001	0.05	0.901	0.009
HKb	0.067	0.974	<0.001	0.038	0.895	0.01
HKc	0.069	0.995	<0.001	0.03	0.989	<0.001

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

		r	*p*
Taxonomic groups	**Bacterial communities**	**0.343**	**0.033**
	Acidobacteria	0.174	0.290
	Actinobacteria	**0.358**	**0.025**
	Bacteroidetes	**0.457**	**0.003**
	Chloroflexi	**0.379**	**0.017**
	Firmicutes	0.179	0.275
	Verrucomicrobia	0.105	0.526
	Proteobacteria	0.273	0.092
Functional groups	**Microbial functional genes**	**0.514**	**< 0.001**
	C cycling	**0.516**	**< 0.001**
	C fixation	**0.486**	**0.002**
	C degradation	**0.523**	**< 0.001**
	Methane generation	**0.565**	**< 0.001**
	Methane oxidation	**0.529**	**0.001**
	N cycling	**0.491**	**0.001**
	N fixation	**0.497**	**0.001**
	Ammonification	**0.497**	**0.001**
	Nitrification	**0.449**	**0.004**
	Denitrification	**0.452**	**0.004**
	P cycling	**0.508**	**< 0.001**
	S cycling	**0.510**	**< 0.001**

$p < 0.05$ indicates significant correlations and is listed in bold font.

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

Taxonomic groups	Ecosystem	Latitude (°N)	Longitude (°E)	Continent / Ocean	Country	References
Plants	Island	27.50	-175.50	Pacific Ocean	United States	Amerson and Binion et al. 1975 [1]
Plants	Grassland	27.67	-98.20	North America	United States	Archer and Scifres et al. 1988[2]
Plants	Grasslands	53.00	-1.47	Europe	United Kingdom	Archibald. 1949[3]
		53.50	-1.57	Europe	United Kingdom	
		54.30	-1.58	Europe	United Kingdom	
		52.00	1.00	Europe	United Kingdom	
		51.80	1.00	Europe	United Kingdom	
Plants	Island	45.50	-92.10	North America	United States	Barnes and William et al. 1991[4]
Plants	Western Australia	-15.00	128.00	Australia	Australia	Beard and Chapman et al. 2000[5]
Plants	Island	66.50	21.50	Europe	Sweden	Berglund and Jonsson et al. 2001[6]
Plants	Forest	36.00	79.00	Asia	China	Billings 1938[7]
Plants	Island	-33.90	22.75	Africa	South Africa	Bond and Midgley et al. 1988[8]
Plants	Island	43.50	-113.30	North America	United States	Carter-Lovejoy et al. 1982[9]
Plants	Human	-24.00	152.00	Pacific Ocean	Australia	Chaloupka and Domm et al. 1986[10]
Plants	Island	23.50	-166.20	Pacific Ocean	United States	Amerson. 1971[11]
Plants	Island	27.50	-166.20	Pacific Ocean	United States	Amerson. 19752[2]
Plants	Island	42.80	10.10	Europe	Italia	Baroni. 1971[12]
Plants	Island	20.60	-155.30	Pacific Ocean		Carlquist. 1974[13]
Plants	Island	27.00	-111.00	Pacific Ocean	Mexico	Cas. 1975[14]
Plants	Island	-0.09	-91.32	Pacific Ocean	Ecuador	Johnson and Raven. 1973[15]
Plants	Island	56.08	-6.20	Europe	United Kingdom	Johnson and Simberloff et al. 1974[16]
Plants	Forest	34.02	-119.74	North America	United States	Hughes and Inouye et al. 2008[17]
Plants	Island	31.00	-118.00	Pacific Ocean	United States	Power. 1972[18]
Plants	Forest	51.50	-0.63	Europe	United Kingdom	Crawley and Harral et al. 2001[19]
Plants	Park	7.00	81.00	Asia	Sri Lanka	Santiapillai and de Silva et al. 2000[20]
Plants	Forest	1.75	31.50	Africa	Uganda	Eggeling. 2006[21]
Plants	Forest	42.46	-83.95	North America	United States	Evans and Clark. 1995[22]
Plants	Forest	-33.47	26.82	Africa	South Africa	Geldenhuys. 1997[23]

Plants	Forest	39.15	-112.80	North America	United States	Harner and Harper. 1976[24]
Plants	Forest	36.78	-108.13	North America	United States	Hattori and Ishida. 2000[25]
		32.02	131.35	Asia	Japan	
Plants	Grassland	32.50	-106.80	North America	United States	Hochstrasser and Kroel-Dulay et al. 2002[26]
Plants	Lake	45.23	-81.52	North America	Canada	Hogg and Morton et al. 1989[27]
Plants	Rock	33.75	-84.39	North America	United States	Houle. 1990[28]
Plants	Island	43.07	-89.38	North America	United States	Howe. 1984[29]
		-31.00	155.00	Pacific Ocean	Australia	
Plants	Coastal aqus	48.40	-122.67	North America	United States	Hruby. 1975[30]
		48.50	-122.97	North America	United States	
Plants	Desert	30.53	32.00	Africa	Egypt	Kassas and Imam. 1959[31]
Plants	Island	-45.57	167.61	Australia	New Zealand	Kelly and Wilson et al. 2006[32]
Plants	Forest	39.12	-90.33	North America	United States	Kilburn. 1966[33]
		46.92	-95.07	North America	United States	
		45.18	-92.31	North America	United States	
Plants	Forest	9.30	-79.85	North America	Panama	Knight. 1975[34]
Plants	Island	59.78	18.37	Europe	Finland	Köchy and Rydin H. 1997[35]
Plants	Tropical island	1.49	103.49	Asia	Malaysia	Koh and Sodhi et al. 2002[36]
Plants	Forest	52.72	17.97	Europe	Poland	Kwiatkowska. 1994[37]
Plants	Forest	6.20	0.00	Africa	Ghana	Lawson and Armstrong-Mensah et al. 1970[38]
Plants	Grassland	43.80	117.00	Asia	China	Li. 1993[39]
Plants	Agroecosystems	64.00	26.00	Europe	Finland	Ma and Tarmi et al. 2002[40]
Plants	Island	32.69	-117.18	North America	United States	Moody. 2000[41]
Plants	Island	25.03	-77.40	North America	Bahamas	Morrison. 1997[42]
Plants	Farmland	23.78	-77.61	Atlantic Ocean	Bahamas	Mulugeta and Stoltenberg et al. 2001[43]
		43.51	-88.74	North America	United States	
		43.51	-88.74	North America	United States	
Plants	Lake	42.50	-8.72	Europe	Spain	Niel. 1977[44]
Plants	Island	60.11	21.60	Europe	Finland	Nieminen and Hanski. 1998[45]
Plants	Island	59.58	15.47	Europe	Sweden	Nilsson and Bengtsson et al. 1988[46]
Plants	Coast	43.26	-7.60	Europe	Spain	Obeso and Aedo. 1992[47]
Plants	Tropical forest	-6.37	146.10	Australia	Papua-New Guinea	Paijmans. 1970[48]

Plants	Island	37.58	22.33	Europe	Greece	Panitsa and Tzanoudakis. 2001[49]
Plants	Forest	9.22	77.48	Asia	India	Parthasarathy and Karthikeyan. 1997[50]
Plants	Forest	8.50	77.48	Asia	India	Parthasarathy. 1999[51]
Plants	Forest	48.50	-94.00	North America	United States	Pastor and Downing et al. 2006[52]
		2.15	102.70	Asia	Malaysia	
		4.20	114.04	Asia	Malaysia	
Plants	Forest	11.57	76.64	Asia	India	Plotkin and Potts. 2000[53]
		16.86	99.72	Asia	Thailand	
		8.62	-80.37	North America	Panama	
Plants		2.49	101.89	Asia	Malaysia	Poore. 1964[54]
Plants	Forest	3.04	113.78	Asia	Malaysia	Proctor and Anderson et al. 1983[55]
Plants	Forest	5.25	117.00	Asia	Malaysia	Proctor and Lee et al. 1988[56]
Plants	Grasslands	56.68	16.42	Europe	Sweden	Rejmanek and Rosen.1992[57]
Plants	Grassland	33.47	-97.52	North America	United States	Rice. 1952[58]
Plants	Forest	7.00	8.00	Africa	Nigeria	Richards.1939[59]
Plants	Island	60.20	11.01	Europe	Norge	Rydin and Borgegard. 1988[60]
Plants	Pond	-32.77	-71.55	South America	Chile	Santelices and Hoffmann et al. 1995[61]
Plants	Alpine	43.50	41.68	Europe	Russia	Semenova and Onipchenko. 1996[62]
Plants	Pond	42.11	140.32	Asia	Japan	Shimizu. 1986[63]
Plants	Island	51.53	9.94	Europe	Germany	Steffan-Dewenter and Tscharntke. 2000[64]
Plants	Forest	18.18	-77.32	North America	Jamaica	Tanner. 1977[65]
Plants	Forest	20.20	57.50	Asia	Oman	Vaughan and Wiehe. 1941[166]
Plants	Marsh	45.42	-75.69	North America	Canada	Weiher. 1999[67]
Plants	Mountain	35.35	-81.63	North America	United States	White and Miller et al. 1984[68]
Plants	Island	54.90	21.25	Europe	Russia	Dengler. 2009[69]
Plants	Rainforests	11.00	76.00	Asia	India	Krishnamani and Kumar et al. 2004[70]
		-3.34	37.34	Africa	Tanzania	
Plants	Costa	10.00	-84.00	North America	Costa Rica	Karger and Kluge et al. 2011[71]
		-0.25	-78.58	South America	Ecuador	
Plants	Forest	52.42	-1.44	Europe	United Kingdom	Hulme. 2008[72]
Plants	Grassland	46.53	24.17	Europe	Romania	Turtureanu and Palpurina et al. 2014[73]
Plants	Subalpine	46.20	-122.18	North America	United States	Carey and Harte et al. 2006[74]
Plants	Forest	27.60	-17.75	Atlantic Ocean		Aranda and Gabriel et al. 2013[75]

Plants	Grassland	58.42	22.50	Europe	Eesti	Dengler and Boch. 2008[76]
Plants	Grassland	37.02	-93.21	North America	United States	Ryberg and Chase. 2007[77]
Plants	Forest	64.00	26.00	Europe	Finland	Tikkanen and Punttila et al. 2009[78]
Plants	Forest	-29.00	31.00	Africa	South Africa	Olivier and van Aarde et al. 2013[79]
Plants	Rainforest	3.14	101.69	Asia	Malaysia	He and Hubbell. 2013[80]
Plants	Forest	18.67	108.82	Asia	China	Qiao and Tang et al. 2012[81]
		21.95	101.20	Asia	China	
		23.17	112.50	Asia	China	
		24.50	101.20	Asia	China	
		29.80	101.67	Asia	China	
		31.40	110.32	Asia	China	
		34.90	107.70	Asia	China	
		34.27	107.70	Asia	China	
		35.45	112.40	Asia	China	
		40.30	117.25	Asia	China	
		42.38	128.90	Asia	China	
		42.18	128.15	Asia	China	
		42.20	128.07	Asia	China	
		42.32	128.95	Asia	China	
		51.83	122.83	Asia	China	
		51.87	122.72	Asia	China	
Plants	Grassland	38.22	-122.95	North America	United States	Sandel and Corbin. 2012[82]
Plants	Grassland	61.00	8.00	Europe	Norge	Vandvik and Klanderud et al. 2016[83]
Plants	Woodland	-37.00	144.00	Australia	Australia	Morgan and Wong et al. 2011[84]
Plants	Grassland	55.50	89.00	Asia	Russia	Polyakova and Dembicz et al. 2016[85]
Plants	Grassland	43.38	116.42	Asia	China	Li and Zhan et al. 2015[86]
Plants	Wetland	-35.53	138.42	Australia	Australia	Deane and Fordham et al. 2016[87]
Bacteria	Salt marsh sediment	41.70	-71.50	North America	United States	Horner-Devine and Lage et al. 2004[88]
Fungi	Tree leaves	40.73	-82.17	North America	United States	Feinstein and Blackwood. 2012[89]
Bacteria	Forest soil	35.92	-84.25	North America	United States	Zhou and Kang et al. 2008[90]
Bacteria	Grassland soil	47.00	2.00	Europe	France	Ranjard and Dequiedt et al. 2013[91]
Functional genes	Grassland soil	51.81	-0.36	Europe	United Kingdom	Liang and Wu et al. 2015[92]
Bacteria, archaea	Forest soil	39.98	-105.37	North America	United States	Deng and Ning et al. 2018[93]

		42.53	-72.18	North America	United States	
		44.20	-122.25	North America	United States	
		35.50	-83.42	North America	United States	
		18.30	-65.80	North America	United States	
		9.15	-79.85	North America	United States	
		48.52	-1.30	Europe	France	
		9.15	-79.85	North America	Panama	
Diazotrophs	Forest soil	44.20	-122.25	North America	United States	Tu, Deng. 2016[94]
		47.45	21.00	Europe	Hungary	
		41.58	-8.22	Europe	Portugal	

Methods S1 Measurement of soil properties

Soil properties, including soil pH, cation exchange capacity (CEC), organic matter (OM), and total nitrogen (TN) were measured in all soil samples. Soil pH was detected with a glass electrode in the soil with a water-to-soil ratio of 2.5:1 (Thermo Orion-868, MA, USA). CEC was measured using $BaCl_2$ extraction method (ISO11260 1994). Organic matter (OM) was measured by the dichromate oxidation method [95]. Total nitrogen (TN) were measured by the Kjeldahl method [96].

Methods S2 Gene amplicon sequencing

Microbial genomic DNA was extracted from 2 g of well-mixed soil from each sample by combining freeze-grinding and sodium dodecyl sulphate for cell lysis and purification by agarose gel electrophoresis, followed by phenol–chloroform–butanol extraction as previously described [97]. The purified DNA was qualified and quantified with agarose gel electrophoresis, an ND-1000 spectrophotometer (Nanodrop Inc., Wilmington, DE, USA) and Quant-iT™ PicoGreen ® dsDNA Reagent and Kits (Invitrogen, Carlsbad, CA, USA). The primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTA-AT-3') were selected, targeting the bacterial V4 region of the 16S rRNA gene [98]. Both the forward and reverse primers were tagged with adapter, an 8-nt index sequence, a 10-nt pad sequence, and a 2-nt linker sequences. In addition, barcode sequences (12-mer) were added to the reverse primers to allow the pooling of multiple samples in one run of MiSeq sequencing. All primers were synthesized by Invitrogen (Carlsbad, CA, USA). PCR amplification was performed in triplicate using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) in a total volume of 25 µl containing 2.5 µl of 10× PCR buffer II and 0.5 units of AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), 0.4 µM of each primer, and 10 ng template DNA. To target bacterial and archaeal 16S rRNA genes,

the first step of PCR was carried out using primers without the barcode and cycling conditions as follows: initial denaturation at 94 °C for 1 min; 10 cycles at 94 °C for 20 sec, 53 °C for 25 sec, and 68 °C for 45 sec; and a final extension at 68 °C for 10 min. The PCR products from three replicates were combined and purified using an Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA) following the manufacturer's instructions (1.8ul AMPure XP per 1.0ul of PCR product) and eluted in 50 µl of water. We used the purified PCR product (15 µl) as a template for the second step of PCR amplification with barcoded primers in three technical replicates under the same cycling conditions as the first step. The PCR products from the second step were examined by electrophoresis on a 1% agarose gel. The amplification products of three technical replicates were then combined and quantified by PicoGreen using a FLUOstar Optima (BMG Labtech, Jena, Germany). Equal molarity of the PCR products were pooled from different samples, purified with a Qiagen Gel Extraction Kit (Qiagen Sciences, Germantown, MD, USA) following the manufacturer's instructions and re-quantified by PicoGreen. According to the MiSeqTM Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA), the purified mixture was diluted and denatured to obtain an 8 pM sample DNA library and mixed with 25% of 8 pM PhiX (Illumina, San Diego, CA, USA). Finally, 600 µl of the mixed library was loaded with read 1, read 2 and index sequencing primers [98] from a 300-cycle (2×150 paired-end) kit and run on a MiSeq at the Institute for Environmental Genomics of the University of Oklahoma.

After each sequence was assigned to its sample according to its barcode, the sequences were trimmed based on quality scores using Btrim [99], and the paired-end reads were merged into longer reads by FLASH2 [100]. To test the trimming strategies, different sizes of trimming windows and cutoffs were used. Unqualified sequences were removed using the following criteria: (i)

Phred quality score Q < 20, (ii) reads that shorter than 200 bp, and (iii) reads containing ambiguous residues. Chimeric sequences were discarded based on prediction by UPARSE[101]. OTUs were clustered at the 97% similarity level. Final OTUs were generated based on the clustering results, and taxonomic annotations were assigned to each OTU's representative sequence by RDP's 16S Classifier[102]. All OTUs identified as belonging to chloroplast and mitochondria were removed from the data set. Eventually, in total, 15,129,972 sequences were obtained from the 429 samples via high-throughput sequencing analysis. After quality filtering, 14,331,470 sequences remained with 23,187 to 45,963 sequences being obtained per sample. Subsequent analyses were performed using normalized data (20,000 reads per sample). Finally,55,519 different OTUs were obtained.

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