# **Supplementary Figures**

## (A) 16S rRNA genes (Bacteria)











Supplementary Fig. 1. Rarefaction analysis of OTUs at different taxonomic resolutions.



**Supplementary Fig. 2. Relationships between taxon richness and several other soil and climate variables.** Line represents least squares regression fit and shaded area represents 95% confidence. The relationships of taxon richness with temperature, pH and moisture were seen in Fig. 1. The relationships of microbial biodiversity (taxon richness, Shannon diversity and phylogenetic diversity) to all soil, climate and plants were summarized in Table S5.



**Supplementary Fig. 3**. **Relationships between taxon richness of individual samples and temperature**. The sequences of individual samples in a site were used for estimating theoretical Chao1 value for a given sample at the taxonomic resolution of 97%. The natural log of the estimated Chao1 values of individual samples were calculated for examining the relationships between taxon richness and temperature, which was expressed as the inverse of annual mean temperature in degree of Kelvin. Line represents least squares regression fit and the shaded area represents their 95% confidence limits (A) Bacteria based on16S rRNA gene; (B) Fungi based on ITS; and (C) N fixers based on nifH gene. The relationships between microbial taxon richness of individual sites and temperature were seen in Fig. 3.

## **Supplementary Tables**

| Sites <sup>a</sup> | Latitude | Elevation<br>(m) | Mean<br>temperature<br>(°C) | Precipitation<br>(mm) | Soil<br>moisture <sup>b</sup><br>(%) | Soil pH   | Total<br>carbon<br>content<br>(mg/g) | Total<br>nitrogen<br>content<br>(mg/g) | Plant<br>species<br>number <sup>c</sup> |
|--------------------|----------|------------------|-----------------------------|-----------------------|--------------------------------------|-----------|--------------------------------------|--|---|
| BCI                | 9.16     | 157              | 25.71                       | 2383.0                | 31.43±6.77                           | 5.87±0.42 | 3.99±2.09                            | 0.31±0.12                              | 263                                     |
| LUQ                | 18.32    | 386              | 23.62                       | 3069.2                | 40.53±4.42                           | 5.06±0.39 | 7.54±3.05                            | 0.47±0.17                              | 93                                      |
| CWT                | 35.05    | 864              | 12.62                       | 1853.8                | 30.28±6.05                           | 4.72±0.37 | 6.68±4.07                            | 0.25±0.08                              | 49                                      |
| AND                | 44.23    | 860              | 8.94                        | 1587.4                | 36.88±13.79                          | 5.28±0.64 | 19.36±13.87                          | 0.40±0.18                              | 17                                      |
| HFR                | 42.54    | 356              | 8.27                        | 1128.7                | 34.35±10.45                          | 3.84±0.29 | 19.21±8.60                           | 0.62±0.29                              | 25                                      |
| NWT                | 40.04    | 3,186            | 2.50                        | 481.6                 | 16.00±8.74                           | 4.83±0.37 | 11.46±7.03                           | 0.33±0.18                              | 5                                       |

#### Supplementary Table 1. Summary of soil and climate characteristics for the 6 sampling sites.

<sup>a</sup>The symbols for these sites are: BCI, Barro Colorado Island, Panama; LUQ: Luquillo, El Yunque National Forest, Puerto Rico; CWT: Coweeta Hydrological Laboratory, North Carolina; AND: H.J. Andrews Experimental Forest, Oregon; HFR: Harvard Forest, Massachusetts; NWT: Niwot Ridge, Colorado. <sup>b</sup>Average individual soil variables (moisture, pH, total C and N) over 21 samples.

<sup>c</sup>Plant species is the total of five 0.1 ha Gentry plots within the 25 ha plot at each site.

# Supplementary Table 2. Pairwise Pearson correlations (r values, n=6) between major soil and site variables. For the soil variables, the site averages among 21 samples were used for calculating correlations.

|                       | Elevation | Mean<br>temperature | Precipitation | Soil<br>moisture | Soil<br>pH | Total carbon | Total<br>nitrogen | Plant<br>richness |
|-----------------------|-----------|---------------------|---------------|------------------|------------|--------------|-------------------|-------------------|
| Latitude              | 0.447     | -0.934**            | -0.774        | -0.252           | -0.656     | 0.816*       | 0.252             | -0.916**          |
| Elevation             |           | -0.706              | -0.722        | -0.876*          | -0.106     | 0.088        | -0.320            | -0.511            |
| Mean<br>temperature   |           |                     | 0.929**       | 0.571            | 0.587      | -0.673       | -0.106            | 0.852*            |
| Precipitation         |           |                     |               | 0.740            | 0.482      | -0.551       | -0.044            | 0.608             |
| Soil moisture         |           |                     |               |                  | 0.057      | 0.127        | 0.452             | 0.197             |
| Soil pH               |           |                     |               |                  |            | -0.550       | -0.612            | 0.690             |
| Total carbon          |           |                     |               |                  |            |              | 0.648             | -0.688            |
| Total nitrogen        |           |                     |               |                  |            |              |                   | -0.282            |
| * p<0.05<br>** p<0.01 |           |                     |               |                  |            |              |                   |                   |

\*\* \*\*\*

p<0.001

# Supplementary Table 3. Summary of sequence statistics

| MiSeq sequencing                  | Targets                     | Total base pairs<br>(bp) | bp per sample | Total No. of reads | Average No. of reads per sample |
|-----------------------------------|-----------------------------|--------------------------|---------------|--------------------|---------------------------------|
| 16S-rRNA gene                     | Soil bacteria +<br>archaea* | 2.05 G                   | 16.3 M        | 8.10 M             | 64±18 K                         |
| ITS (internal transcribed spacer) | Soil fungi                  | 1.39 G                   | 11.0 M        | 4.94 M             | 39±11 K                         |
| nifH (nitrogenase<br>subunit H)   | Nitrogen fixing<br>bacteria | 1.17 G                   | 9.3 M         | 3.75 M             | 30±9 K                          |

\*Very small percentages of sequences from Archaea were obtained.

## Supplementary Table 4. OTU classifications at different cutoffs.

| Gene targets | Similarity thresholds | No of OTUs without resampling <sup>a</sup> | No of OTUs with resampling <sup>b</sup> |  |
|--------------|-----------------------|--|---|--|
| 165          | 0.97                  | 257,867                                    | 188,620                                 |  |
| 105          | 0.99                  | 789,214                                    | 524,047                                 |  |
| ITC          | 0.95                  | 13,762                                     | 12,942                                  |  |
| 115          | 0.97                  | 18,442                                     | 17,239                                  |  |
| e:fII        | 0.95                  | 102,941                                    | 87,938                                  |  |
| miH          | 0.97                  | 160,082                                    | 133,192                                 |  |

<sup>a</sup>Estimated numbers of OTUs based on all sequences obtained, without resampling.

<sup>b</sup>Estimated numbers of OTUs after sampling the minimum numbers of sequences from each sample. The minimum numbers of sequences resampled are 25,901 for 16S rRNA genes, 13,688 for ITS and 16,000 for nifH.

**Supplementary Table 5. Changes of microbial biodiversity with latitudes**. The changes of taxon richness and Shannon diversity with latitudes were fitted with the linear model: y=a + bx. The significance of the relationships was evaluated based correlation (*r*) and probability (*p*) values.

| Tovonomia grouns <sup>a</sup> | Taxon 1 | richness                   | Shanno | Shannon diversity          |  |  |
|-------------------------------|---------|----------------------------|--------|----------------------------|--|--|
| Taxononine groups             | r       | р                          | r      | р                          |  |  |
| Bacteria (16S rRNA)           | -0.550  | $2.6 \times 10^{-11}$      | -0.389 | 6.9x10 <sup>-6</sup>       |  |  |
| Fungi (ITS)                   | -0.744  | $< 2.2 \mathrm{x10}^{-16}$ | -0.454 | 9.3x10 <sup>-8</sup>       |  |  |
| Nitrogen fixers (nifH)        | -0.808  | $< 2.2 \mathrm{x10}^{-16}$ | -0.746 | $< 2.2 \mathrm{x10}^{-16}$ |  |  |

<sup>a</sup>The taxonomic resolution used in this analysis is 97% for 16S rRNA gene and ITS, and 95% for nifH gene.

**Supplementary Table 6.** Summary of determination coefficients ( $\mathbb{R}^2$ ) from fitting a linear univariate model(y=a + bx) for predicting microbial biodiversity (taxon richness, Shannon diversity, phylogenetic diversity) as a function of various site and soil variables. Taxon richness, and Shannon diversity were calculated based on the observed sequences after resampling. Since the relative roles of pH and temperature on controlling microbial communities are controversial, their values were particularly bolded in the Tables.

| X7 11                      | 16S            | (97%)  | ITS   | 5 (97%) | nif            | nifH (95%)   R <sup>2</sup> AIC   0.65 1986.5   0.17 2096.7 <b>0.63 1994.8</b> 0.49 2034.6   0.13 2103.2 <b>0.34 2067.4</b> |  |
|----------------------------|----------------|--------|-------|---------|----------------|---|--|
| variables                  | $\mathbf{R}^2$ | AIC    | $R^2$ | AIC     | $\mathbf{R}^2$ | AIC   |  |
| Latitude                   | 0.30           | 2161.9 | 0.55  | 1656.4  | 0.65           | 1986.5  |  |
| Elevation                  | 0.22           | 2175.3 | 0.14  | 1738.8  | 0.17           | 2096.7  |  |
| Annual average Temperature | 0.41           | 2141.8 | 0.48  | 1675.5  | 0.63           | 1994.8  |  |
| Annual precipitation       | 0.39           | 2144.8 | 0.27  | 1718.5  | 0.49           | 2034.6  |  |
| Soil moisture              | 0.20           | 2179.2 | 0.01  | 1756.4  | 0.13           | 2103.2  |  |
| pH                         | 0.35           | 2152.8 | 0.40  | 1693.7  | 0.34           | 2067.4  |  |
| Total Carbon               | 0.12           | 2190.9 | 0.44  | 1685.6  | 0.28           | 2077.9  |  |
| Total Nitrogen             | 0.04           | 2202.6 | 0.18  | 1733.6  | 0.00           | 2119.7  |  |
| Plant richness             | 0.30           | 2162.0 | 0.65  | 1626.0  | 0.51           | 2030.6  |  |

#### (A) Taxon richness

#### (B) Shannon diversity

| Wasiahlar                  | 16S            | (97%) | ITS            | 5 (97%) | nifl           | H (95%) |
|----------------------------|----------------|-------|----------------|---------|----------------|---------|
| variables                  | $\mathbb{R}^2$ | AIC   | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC     |
| Latitude                   | 0.15           | 89.4  | 0.21           | 270.3   | 0.56           | 303.6   |
| Elevation                  | 0.21           | 80.7  | 0.01           | 297.7   | 0.13           | 388.3   |
| Annual average temperature | 0.28           | 68.5  | 0.16           | 278.1   | 0.53           | 312.3   |
| Annual precipitation       | 0.37           | 50.9  | 0.09           | 287.4   | 0.39           | 344.5   |
| Soil moisture              | 0.29           | 66.5  | 0.00           | 299.3   | 0.11           | 391.5   |
| рН                         | 0.19           | 84.1  | 0.20           | 271.1   | 0.35           | 350.9   |
| Total carbon               | 0.04           | 105.0 | 0.23           | 265.7   | 0.18           | 381.8   |
| Total nitrogen             | 0.00           | 109.6 | 0.16           | 277.8   | 0.00           | 406.2   |
| Plant richness             | 0.11           | 95.7  | 0.22           | 268.7   | 0.46           | 328.8   |

## (C) Inverse Simpson diversity

| We wight a                 | 16S            | (97%)  | ITS            | 5 (97%) | nif            | H (95%) |
|----------------------------|----------------|--------|----------------|---------|----------------|---------|
| Variables                  | $\mathbf{R}^2$ | AIC    | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC     |
| Latitude                   | 0.00           | 1817.0 | 0.19           | 1129.5  | 0.56           | 1400.3  |
| Elevation                  | 0.07           | 1807.7 | 0.01           | 1155.0  | 0.14           | 1485.0  |
| Annual average temperature | 0.05           | 1811.2 | 0.14           | 1137.0  | 0.52           | 1411.6  |
| Annual precipitation       | 0.18           | 1792.6 | 0.08           | 1145.5  | 0.37           | 1445.6  |
| Soil moisture              | 0.24           | 1782.6 | 0.00           | 1156.5  | 0.08           | 1492.7  |
| рН                         | 0.00           | 1817.1 | 0.15           | 1136.5  | 0.28           | 1461.8  |
| Total carbon               | 0.00           | 1817.3 | 0.20           | 1129.1  | 0.23           | 1470.2  |
| Total nitrogen             | 0.04           | 1812.0 | 0.09           | 1144.3  | 0.00           | 1503.7  |
| Plant richness             | 0.02           | 1815.0 | 0.19           | 1130.6  | 0.46           | 1426.1  |

## (D) Faith's phylogenetic diversity

|                            | 16S   | (97%)  | ITS            | 5 (97%) | nifH           | I (95%)* |
|----------------------------|-------|--------|----------------|---------|----------------|----------|
| Variables                  | $R^2$ | AIC    | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC      |
| Latitude                   | 0.36  | 1383.7 | 0.69           | 1280.7  | 0.04           | 1444.3   |
| Elevation                  | 0.23  | 1408.2 | 0.23           | 1394.7  | 0.00           | 1449.7   |
| Annual average temperature | 0.47  | 1361.9 | 0.65           | 1296.2  | 0.04           | 1444.3   |
| Annual precipitation       | 0.44  | 1367.9 | 0.42           | 1360.1  | 0.04           | 1444.6   |
| Soil moisture              | 0.20  | 1413.0 | 0.06           | 1420.4  | 0.02           | 1447.9   |
| pH                         | 0.43  | 1370.8 | 0.43           | 1356.4  | 0.14           | 1431.4   |
| Total carbon               | 0.16  | 1418.9 | 0.48           | 1345.7  | 0.00           | 1449.6   |
| Total nitrogen             | 0.05  | 1433.9 | 0.13           | 1410.4  | 0.00           | 1449.7   |
| Plant richness             | 0.36  | 1384.9 | 0.74           | 1255.6  | 0.04           | 1445.4   |

\*The phylogenetic diversity of nifH gene fits poorly with all variables. Most likely, this is due to the difficulty in obtaining reliable sequence alignments for constructing phylogenetic tree to calculate phylogenetic diversity.

# (E) NRI phylogenetic diversity

| We week to a               | 16             | δS    | ΓΙ             | TS    | n     | ifH   |
|----------------------------|----------------|-------|----------------|-------|-------|-------|
| variables                  | $\mathbf{R}^2$ | AIC   | $\mathbf{R}^2$ | AIC   | $R^2$ | AIC   |
| Latitude                   | 0.48           | 569.7 | 0.26           | 457.2 | 0.64  | 473.1 |
| Elevation                  | 0.19           | 625.8 | 0.14           | 477.2 | 0.18  | 574.7 |
| Annual average temperature | 0.53           | 556.7 | 0.28           | 453.7 | 0.60  | 485.9 |
| Annual precipitation       | 0.47           | 572.4 | 0.24           | 460.6 | 0.44  | 526.9 |
| Soil moisture              | 0.18           | 627.0 | 0.09           | 484.2 | 0.08  | 589.9 |
| pH                         | 0.45           | 577.1 | 0.01           | 494.7 | 0.16  | 578.1 |
| Total carbon               | 0.17           | 627.6 | 0.14           | 477.2 | 0.40  | 535.5 |
| Total nitrogen             | 0.02           | 649.7 | 0.02           | 493.7 | 0.01  | 599.4 |
| Plant richness             | 0.41           | 585.9 | 0.16           | 474.6 | 0.48  | 518.2 |

**Supplementary Table 7.** Summary of determination ( $\mathbb{R}^2$ ) coefficients from fitting a non-linear univariate model( $y=a+bx+cx^2$ ) for predicting microbial biodiversity (taxon richness, diversity) as a function of pH and temperature

## (A) Taxon richness

| Variables                  | 16S (          | (97%)  | ITS            | (97%)  | nifH (9        | 95%)   |
|----------------------------|----------------|--------|----------------|--------|----------------|--------|
| v artables                 | $\mathbf{R}^2$ | AIC    | $\mathbf{R}^2$ | AIC    | $\mathbf{R}^2$ | AIC    |
| Annual average temperature | 0.41           | 2142.7 | 0.51           | 1671.4 | 0.71           | 1966.2 |
| Soil pH                    | 0.36           | 2151.5 | 0.51           | 1669.3 | 0.40           | 2058.1 |

#### (B) Shannon index

| Variables                  | 16S ( | (97%) | ITS   | (97%) | nifH (9        | 95%)  |
|----------------------------|-------|-------|-------|-------|----------------|-------|
| variables                  | $R^2$ | AIC   | $R^2$ | AIC   | $\mathbf{R}^2$ | AIC   |
| Annual average temperature | 0.32  | 62.8  | 0.18  | 277.1 | 0.65           | 277.3 |
| Soil pH                    | 0.19  | 86.0  | 0.21  | 272.3 | 0.43           | 337.5 |

#### (C) Inverse Simpson index

| X7 ' 11                    | 16S (97%)      |         | ITS            | (97%)   | nifH (95%)     |         |
|----------------------------|----------------|---------|----------------|---------|----------------|---------|
| variables                  | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC     |
| Annual average temperature | 0.15           | 1798.41 | 0.17           | 1134.89 | 0.61           | 1388.30 |
| Soil pH                    | 0.08           | 1808.52 | 0.15           | 1137.48 | 0.36           | 1448.88 |

#### (D) ln(Faith's phylogenetic diversity)

| <b>X</b> 7 ' 11               | 16S (97%)      |        | ITS            | (97%)  | nifH (95%)*    |        |
|-------------------------------|----------------|--------|----------------|--------|----------------|--------|
| v ariables                    | $\mathbb{R}^2$ | AIC    | $\mathbf{R}^2$ | AIC    | $\mathbb{R}^2$ | AIC    |
| Annual average<br>Temperature | 0.47           | 1363.4 | 0.67           | 1291.9 | 0.08           | 1441.8 |
| Soil pH                       | 0.44           | 1369.2 | 0.56           | 1326.0 | 0.14           | 1433.4 |

\*The phylogenetic diversity of nifH gene fits poorly with all variables. Most likely, this is due to the difficulty in obtaining reliable sequence alignments for constructing phylogenetic tree to calculate phylogenetic diversity.

#### (E) ln(NRI phylogenetic diversity)

| Variables                     | 16S (97%)      |       | ITS            | (97%) | nifH (95%)*    |       |
|-------------------------------|----------------|-------|----------------|-------|----------------|-------|
|                               | $\mathbb{R}^2$ | AIC   | $\mathbf{R}^2$ | AIC   | $\mathbb{R}^2$ | AIC   |
| Annual average<br>Temperature | 0.55           | 552.7 | 0.29           | 455.4 | 0.63           | 477.0 |
| Soil pH                       | 0.47           | 572.8 | 0.05           | 491.7 | 0.23           | 569.0 |

**Supplementary Table 8.** Summary of multiple linear regressions by including additional environmental or plant variables beyond temperature based on observed OTU richness.

| Madal  | 16S     |                | ITS     |                | nifH    |                |
|--|---------|----------------|---------|----------------|---------|----------------|
| Widdei   | AIC     | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ | AIC     | $\mathbf{R}^2$ |
| richness ~ temperature   | 2141.84 | 0.41           | 1675.49 | 0.48           | 1994.82 | 0.63           |
| richness ~ temperature+ precipitation  | 2142.06 | 0.41           | 1646.41 | 0.59           | 1993.64 | 0.64           |
| richness ~ temperature+ pH   | 2127.36 | 0.48           | 1657.02 | 0.56           | 1989.34 | 0.65           |
| richness ~ temperature+ pH + precipitation   | 2123.65 | 0.50           | 1633.54 | 0.64           | 1989.72 | 0.66           |
| richness ~ temperature+ pH + precipitation + plant<br>richness                                 | 2121.70 | 0.52           | 1621.91 | 0.68           | 1961.63 | 0.73           |
| richness ~ temperature+ pH + precipitation + plant<br>richness + total carbon + total nitrogen | 2118.08 | 0.54           | 1614.83 | 0.70           | 1960.97 | 0.73           |

**Supplementary Table 9.** Summary of the fitting results to different statistical models to evaluate the relationships between temperature and taxa richness estimated with Chao1.

| Model                  | 16S     |                | ITS     |                | nifH    |                |
|------------------------|---------|----------------|---------|----------------|---------|----------------|
|                        | AIC     | $\mathbf{R}^2$ | AIC     | $\mathbb{R}^2$ | AIC     | $\mathbf{R}^2$ |
| Linear                 | -191.36 | 0.42           | -212.99 | 0.42           | -88.47  | 0.61           |
| Quadratic polynomial   | -190.17 | 0.42           | -211.44 | 0.42           | -100.20 | 0.65           |
| Piecewise relationship | *       | -              | -       | -              | -102.44 | 0.66           |

\* There is no turnover point observed