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## Supplementary information

# Reduction of microbial diversity in grassland soil is driven by long-term climate warming

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# Supplementary information for

## Climate warming reduces microbial biodiversity in a temperate grassland

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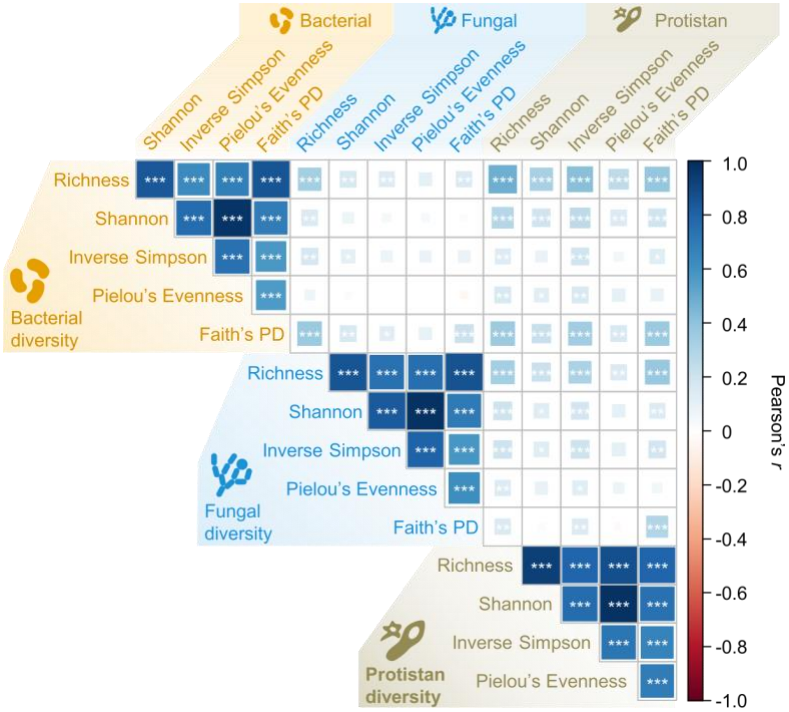


**Fig. S1. Pairwise correlations between different diversity indices.** The color gradient on the right indicates Pearson's correlation coefficients, with more positive values (dark blue) indicating stronger positive correlations. The asterisks '*' denote the significance levels of the Pearson's correlation coefficients based on two-sided t-tests (n = 360): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. The richness index was highly correlated with other diversity indices such as Shannon, inverse Simpson and Pielou's evenness and Faith's phylogenetic diversity (PD) (Pearson's r > 0.63 for bacteria; Pearson's r > 0.74 for fungi).

## Supplementary Tables

**Table S1. Treatment effects on microbial diversity based on linear mixed-effects models.** Statistical significance is based on Wald type II $\chi^2$ tests (n = 360). Significant effects ($p < 0.05$) are given in bold. All estimated effect sizes (β) are based on rescaled response variables. PD, phylogenetic diversity. W: Warming; P: Precipitation level; C: Clipping. The Shannon index considers both the total number of taxa individuals as well as the number of individuals within each taxon. It varies from 0 for communities with only a single taxon to high values for communities with many taxa, each with a few individuals. The inverse Simpson index gives more weight to abundant taxa as compared to Shannon index, with low values indicating that only a few taxa dominating in the community. Pielou's evenness index represents the degree to which individuals are split among taxa, with low values indicating that one or a few taxa dominate, and high values indicating that relatively equal numbers of individuals belong to each taxon.

| **Treatment effects** | | **Bacteria diversity** | | | | | **Fungi diversity** | | | | | **Protist diversity** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Richness** | **Shannon** | **Inverse Simpson** | **Pielou's evenness** | **Faith's PD** | **Richness** | **Shannon** | **Inverse Simpson** | **Pielou's evenness** | **Faith's PD** | **Richness** | **Shannon** | **Inverse Simpson** | **Pielou's evenness** | **Faith's PD** |
| Warming | β | **-0.83** | **-0.39** | **-0.18** | **-0.11** | **-0.49** | **-0.84** | **-0.42** | **-0.23** | **-0.24** | **-0.64** | **-0.99** | **-0.89** | **-0.72** | **-0.79** | **-0.80** |
| | t | **-3.51** | **-1.35** | **-0.60** | **-0.38** | **-2.04** | **-3.22** | **-1.39** | **-0.75** | **-0.77** | **-2.53** | **-3.40** | **-2.93** | **-2.39** | **-2.57** | **-2.90** |
| | *p* | **1.6E-17** | **1.1E-08** | **6.5E-04** | **2.1E-05** | **1.2E-08** | **3.1E-08** | **4.3E-04** | **6.0E-04** | **6.1E-03** | **1.1E-05** | **9.9E-07** | **2.0E-04** | **3.8E-04** | **2.2E-03** | **3.0E-03** |
| Precip. level | β | **0.27** | 0.15 | -0.04 | 0.09 | 0.05 | 0.03 | 0.04 | 0.28 | 0.04 | 0.06 | **0.08** | -0.01 | -0.05 | -0.05 | **0.14** |
| | t | **2.14** | 0.98 | -0.26 | 0.58 | 0.37 | 0.23 | 0.27 | 1.68 | 0.25 | 0.41 | **0.52** | -0.04 | -0.30 | -0.28 | **0.95** |
| | *p* | **5.1E-07** | 0.16 | 0.85 | 0.96 | 0.095 | 0.050 | 0.61 | 0.37 | 0.96 | 0.07 | **0.009** | 0.090 | 0.530 | 0.230 | **0.006** |
| Clipping | β | 0.02 | -0.08 | -0.16 | -0.09 | **-0.16** | **0.03** | -0.11 | 0.28 | -0.15 | **0.12** | -0.40 | -0.51 | -0.29 | -0.53 | **-0.01** |
| | t | 0.08 | -0.27 | -0.50 | -0.30 | **-0.67** | **0.11** | -0.36 | 0.89 | -0.49 | **0.46** | -1.37 | -1.67 | -0.94 | -1.74 | **-0.02** |
| | *p* | 0.11 | 0.90 | 0.76 | 0.53 | **0.041** | **0.035** | 0.27 | 0.31 | 0.44 | **0.04** | 0.23 | 0.36 | 0.45 | 0.46 | **1.4E-04** |
| W × P | β | 0.03 | -0.17 | -0.10 | -0.27 | -0.08 | 0.14 | -0.15 | -0.27 | -0.24 | 0.10 | 0.16 | 0.16 | 0.14 | 0.15 | 0.17 |
| | t | 0.15 | -0.79 | -0.42 | -1.15 | -0.41 | 0.71 | -0.64 | -1.14 | -1.02 | 0.52 | 0.71 | 0.70 | 0.61 | 0.62 | 0.82 |
| | *p* | 0.37 | 0.50 | 0.37 | 0.20 | 0.67 | 0.081 | 0.96 | 0.56 | 0.68 | 0.11 | 0.58 | 0.73 | 0.69 | 0.83 | 0.49 |
| W × C | β | 0.05 | -0.08 | 0.02 | -0.15 | 0.20 | **0.15** | **0.12** | -0.01 | **0.08** | 0.04 | **0.84** | **0.91** | **0.59** | **0.88** | **0.82** |
| | t | 0.16 | -0.19 | 0.04 | -0.34 | 0.58 | **0.41** | **0.28** | -0.01 | **0.18** | 0.11 | **2.02** | **2.11** | **1.37** | **2.02** | **2.10** |
| | *p* | 0.10 | 0.66 | 0.60 | 0.95 | 0.13 | **0.024** | **0.016** | 0.057 | **0.020** | 0.07 | **5.5E-04** | **9.9E-04** | **0.041** | **0.002** | **3.3E-04** |
| P × C | β | -0.02 | 0.02 | 0.21 | 0.03 | 0.18 | -0.04 | -0.01 | -0.31 | 0.00 | -0.08 | 0.16 | 0.23 | 0.14 | 0.26 | 0.03 |
| | t | -0.11 | 0.09 | 0.87 | 0.12 | 0.96 | -0.18 | -0.06 | -1.33 | -0.02 | -0.43 | 0.73 | 1.02 | 0.59 | 1.11 | 0.12 |
| | *p* | 0.59 | 0.57 | 0.36 | 0.60 | 0.13 | 0.62 | 0.39 | 0.40 | 0.32 | 0.80 | 0.56 | 0.42 | 0.71 | 0.37 | 0.77 |
| W× P× C | β | 0.17 | 0.14 | -0.11 | 0.11 | 0.04 | 0.21 | 0.31 | 0.34 | 0.35 | 0.23 | -0.14 | -0.21 | -0.15 | -0.22 | -0.14 |
| | t | 0.69 | 0.44 | -0.32 | 0.35 | 0.16 | 0.74 | 0.96 | 1.03 | 1.03 | 0.85 | -0.45 | -0.64 | -0.47 | -0.67 | -0.46 |
| | *p* | 0.49 | 0.66 | 0.75 | 0.73 | 0.88 | 0.46 | 0.34 | 0.31 | 0.30 | 0.39 | 0.65 | 0.52 | 0.64 | 0.50 | 0.65 |

**Table S2. Treatment effects on microbial biomass based on linear mixed-effects models.** Statistical significance is based on Wald type II $\chi^2$ tests (n = 360). Significant effects ($p < 0.05$) are given in bold. All estimated effect sizes (β) are based on rescaled response variables. PLFA, phospholipid fatty acids. W: Warming; P: Precipitation level; C: Clipping.

| Treatment effects | | Total PLFA | Bacteria | Gram-Negative | Gram-Positive | Fungi | AM Fungi | DNA yield |
|---|---|---|---|---|---|---|---|---|
| Warming | β | **-0.83** | **-0.84** | **-0.81** | -0.74 | -0.69 | **-0.54** | **-0.72** |
| | t | **-3.03** | **-3.10** | **-3.00** | -2.67 | -2.27 | **-2.06** | **-2.44** |
| | *p* | **0.046** | **0.035** | **0.021** | 0.15 | 0.75 | **0.013** | **1.9E-03** |
| Precipitation level | β | **0.02** | **0.03** | **0.03** | **0.04** | -0.15 | **0.16** | -0.23 |
| | t | **0.17** | **0.24** | **0.20** | **0.26** | -0.95 | **1.16** | -1.40 |
| | *p* | **8.4E-03** | **6.4E-03** | **0.015** | **0.013** | 0.51 | **1.1E-03** | 0.82 |
| Clipping | β | 0.13 | 0.20 | 0.20 | 0.21 | -0.42 | 0.15 | **-0.40** |
| | t | 0.46 | 0.76 | 0.75 | 0.77 | -1.40 | 0.58 | **-1.34** |
| | *p* | 0.52 | 0.48 | 0.70 | 0.24 | 0.91 | 0.68 | **0.019** |
| W × P | β | **0.44** | **0.45** | **0.45** | **0.39** | 0.39 | **0.20** | **0.34** |
| | t | **2.15** | **2.24** | **2.22** | **1.86** | 1.73 | **1.04** | **1.51** |
| | *p* | **5.4E-04** | **1.6E-04** | **5.0E-04** | **5.2E-04** | 0.16 | **0.030** | **0.029** |
| W × C | β | 0.12 | 0.03 | 0.04 | 0.02 | 0.78 | -0.06 | 0.01 |
| | t | 0.31 | 0.08 | 0.09 | 0.04 | 1.82 | -0.16 | 0.02 |
| | *p* | 0.16 | 0.20 | 0.40 | 0.098 | 0.058 | 0.34 | 0.92 |
| P × C | β | -0.17 | -0.22 | -0.21 | -0.22 | 0.19 | -0.17 | 0.14 |
| | t | -0.81 | -1.07 | -1.02 | -1.05 | 0.83 | -0.86 | 0.59 |
| | *p* | 0.46 | 0.36 | 0.27 | 0.51 | 0.90 | 0.60 | 0.38 |
| W × P × C | β | 0.12 | 0.17 | 0.10 | 0.25 | -0.34 | 0.19 | 0.01 |
| | t | 0.41 | 0.60 | 0.35 | 0.84 | -1.05 | 0.70 | 0.04 |
| | *p* | 0.68 | 0.55 | 0.73 | 0.40 | 0.29 | 0.49 | 0.97 |

**Table S3. The contribution of soil and plant variables on the response variable of bacterial richness.** The relative contribution of each variable was determined by bootstrap forest partitioning, which was conducted with the function of 'Predictor Screening' in JMP 15.0 (SAS Institute).

| Predictor | Contribution | Portion | Rank |
|---|---|---|---|
| Soil pH | 13093568.0 | 0.2445 | 1 |
| Soil temperature | 7753382.7 | 0.1448 | 2 |
| Soil $NO_3^-$-N content | 7296757.5 | 0.1363 | 3 |
| Soil moisture (annual mean) | 6468401.2 | 0.1208 | 4 |
| $C_3$ plant biomass | 3683432.7 | 0.0688 | 5 |
| Soil moisture (sampling month) | 3078593.3 | 0.0575 | 6 |
| $C_4$ plant biomass | 2488043.2 | 0.0465 | 7 |
| Soil $NH_4^+$ -N content | 2391011.4 | 0.0447 | 8 |
| Total plant biomass | 2221703.3 | 0.0415 | 9 |
| Plant richness | 2197815.3 | 0.0410 | 10 |
| Soil total nitrogen content | 1738889.2 | 0.0325 | 11 |
| Soil total carbon content | 1131116.7 | 0.0211 | 12 |

**Table S4. The contribution of soil and plant variables on the response variable of fungal richness.** The relative contribution of each variable was determined by bootstrap forest partitioning, which was conducted with the function of 'Predictor Screening' in JMP 15.0 (SAS Institute).

| Predictor | Contribution | Portion | Rank |
|---|---|---|---|
| Soil moisture (annual mean) | 437374 | 0.2879 | 1 |
| Soil $NH_4^+$ -N content | 272000 | 0.1791 | 2 |
| Soil moisture (sampling month) | 177691 | 0.1170 | 3 |
| Soil pH | 157512 | 0.1037 | 4 |
| $C_4$ plant biomass | 143715 | 0.0946 | 5 |
| Soil $NO_3^-$-N content | 119891 | 0.0789 | 6 |
| $C_3$ plant biomass | 56201 | 0.0370 | 7 |
| Plant richness | 49231 | 0.0324 | 8 |
| Soil temperature | 36675 | 0.0241 | 9 |
| Soil total nitrogen content | 25693 | 0.0169 | 10 |
| Total plant biomass | 22134 | 0.0146 | 11 |
| Soil total carbon content | 20939 | 0.0138 | 12 |

**Table S5.** Pearson correlation coefficients between predictor variables.

| | Soil temperature | Soil moisture (sampling month) | Soil moisture (annual mean) | Soil $NO_3^-$-N | Soil $NH_4^+$ -N | Soil TN | Soil TC | Soil pH | Total plant biomass | C4 plant biomass | C3 plant biomass |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil temperature | | | | | | | | | | | |
| Soil moisture (sampling month) | -0.138 | | | | | | | | | | |
| Soil moisture (annual mean) | -0.244 | 0.530 | | | | | | | | | |
| Soil $NO_3^-$-N | 0.432 | -0.092 | -0.176 | | | | | | | | |
| Soil $NH_4^+$ -N | 0.178 | 0.097 | -0.107 | 0.558 | | | | | | | |
| Soil TN | 0.184 | 0.033 | -0.068 | 0.531 | 0.512 | | | | | | |
| Soil TC | 0.143 | 0.050 | -0.063 | 0.474 | 0.482 | 0.983 | | | | | |
| Soil pH | -0.286 | 0.725 | 0.475 | -0.428 | -0.117 | -0.141 | -0.106 | | | | |
| Total plant biomass | -0.070 | -0.153 | 0.287 | -0.157 | -0.124 | -0.008 | 0.011 | -0.019 | | | |
| $C_4$ plant biomass | -0.065 | 0.025 | 0.285 | -0.166 | -0.156 | -0.065 | -0.047 | 0.040 | 0.522 | | |
| $C_3$ plant biomass | -0.014 | -0.189 | 0.040 | -0.012 | 0.014 | 0.053 | 0.056 | -0.059 | 0.588 | -0.384 | |
| Plant richness | -0.101 | 0.189 | 0.178 | -0.162 | -0.130 | -0.040 | -0.054 | 0.195 | 0.062 | 0.109 | -0.037 |

**Table S6. Comparison of linear mixed models for the response variable of bacterial richness**. The predictor variables were obtained via strategies 1-3 as described in Methods, for the full data set. All estimated effect sizes (β) were based on rescaled predictors. Empty cells indicated the respective predictor was not included in the model. Model 1 had the lowest AIC and was therefore selected as the preferred model. All models included sampling year and block as random intercept factors.

| | **Model 1** | **Model 2** | **Model 3** |
|---|---|---|---|
| AIC | 5314.19 | 5342.35 | 5317.38 |
| Effect size | β | β | β |
| Soil pH | 263.19 | | 260.85 |
| $C_3$ plant biomass | 258.09 | 262.88 | 268.73 |
| Soil moisture (annual mean) | 162.75 | | 127.41 |
| Soil $NO_3^-$-N | -117.52 | | -125.27 |
| Total plant richness | 99.28 | 98.03 | |
| Soil temperature | -81.03 | -152.89 | -91.69 |
| Soil $NH_4^+$ -N | | -69.57 | |
| Soil moisture (sampling month) | | 331.33 | 69.81 |
| $C_4$ plant biomass | | 7.37 | |

**Table S7. Comparison of linear mixed models for the response variable of fungal richness**. The predictor variables were obtained via strategies 1-3 as described in Methods, for the full data set. All estimated effect sizes (β) were based on rescaled predictors. Empty cells indicate the respective predictor was not included in the model. Model 1 had the lowest AIC and was therefore selected as the preferred model. All models included sampling year and block as random intercept factors.

| | **Model 1** | **Model 2** | **Model 3** |
|---|---|---|---|
| AIC | 4144.03 | 4168.08 | 4144.32 |
| Effect size | β | β | β |
| Soil moisture (annual mean) | 46.87 | | 55.23 |
| Soil pH | 26.35 | | 30.48 |
| Soil $NH_4^+$ -N | -18.71 | -20.822 | -18.75 |
| Soil temperature | -13.50 | -25.81 | |
| $C_4$ plant biomass | 6.99 | 10.57 | 5.57 |
| Total plant richness | 6.30 | 4.223 | |
| Soil moisture (sampling month) | | 42.05 | -17.83 |
| $C_3$ plant biomass | | -8.35 | |
| Soil $NO_3^-$-N | | | -6.81 |

## Supplementary Notes

### A. Treatment effects on soil and plant variables

Linear mixed-effects models were employed to test the effects of treatments and their interactions on soil variables and plant communities, in which the regression coefficients represent the magnitudes of treatment effects, namely effect sizes (β). Across years, warming significantly increased surface soil temperature by 2.2 $^{o}$C (β = 2.2, $p < 0.0001$; Fig. S2a). The impacts of other treatments on soil temperature were not significant, except clipping (β = -0.1, $p = 0.005$). Also, warming significantly decreased soil moisture by 1.5% (absolute) (β = -1.5, $p < 0.0001$; Fig. S2b). Compared with warming, both precipitation level and clipping had less influence on soil moisture (β = 0.7, $p < 0.0001$ for precipitation level; β = 0.22, $p$ =0.007 for clipping; Fig. S2b). That is, the double precipitation treatment only caused a 0.7% (absolute) increase in soil moisture as compared to the ambient precipitation condition, while half precipitation decreased soil moisture by 0.35% roughly analogous to the effect of clipping. In addition, warming decreased soil pH (β = - 0.2, $p <$ 0.0001; Fig. S2c), but increased soil $NO_3^-$-N content ($log_{10}$ value, β = 0.17, $p < 0.0001$; Fig. S2d) with no significant effects on soil $NH_4$-N content (Fig. S2e). Moreover, there was a significant, albeit very weak, negative effect of warming (β = -0.04, $p = 0.01$) on plant richness. In contrast, clipping was the main treatment influencing above-ground plants with negative effects on plant biomass (β = -17, $p = 0.0001$; Fig. S2f), but positive effects on plant richness (β = 0.88, $p < 0.0001$; Fig. S2g). There was also a positive interactive effect of precipitation level and clipping on plant biomass (β = 50, $p = 0.006$; Fig. S2f). Collectively, these results indicated that experimental warming had predominant effects on soil microclimate (i.e., temperature, moisture) and biogeochemistry (i.e., soil pH, $NO_3^-$) compared to altered precipitation and clipping.

## B. Treatment effects on microbial diversity and biomass

### 1. Overall effects of various treatments on microbial diversity

We quantified the effects of warming, altered precipitation level and clipping and their interactions on soil microbial biodiversity using effect sizes yielded from the similar linear mixed-effects models as for soil and plant variables (Fig. 1b-f; Table S1). While species richness (Fig. 1b), Shannon-Weaver information (Fig. 1c), inverse Simpson (Fig. 1d), Pielou's evenness (Fig. 1e), and Faith's phylogenetic diversity (Fig. 1f) were strongly decreased by warming for bacterial ($\beta$ = -0.11 ~ -0.83, $p < 0.0007$), fungal ($\beta$ = -0.23 ~ -0.84, $p < 0.007$), and protistan ($\beta$ = -0.72 ~ -0.99, $p < 0.003$) communities, these biodiversity metrics were not substantially changed by precipitation level or clipping or treatment interactions ($\beta$ = -0.27 ~ 0.27 for bacteria; $\beta$ = -0.31 ~ 0.35 for fungi; $\beta$ = -0.53 ~ -0.01 for protist; Table S1). For richness, warming had strong negative effects on bacteria ($\beta$ = -0.83, $p < 0.0001$), fungi ($\beta$ = -0.84, $p < 0.0001$), and protists ($\beta$ = -0.99, $p < 0.0001$), whereas precipitation level had relatively small, but positive, effects on bacteria ($\beta$ = 0.27, $p < 0.0001$), and protists ($\beta$ = 0.08, $p = 0.009$) but no significant effects on fungi ($\beta$ = 0.03, $p = 0.050$) (Fig. 1b). It was also noted that, the effect sizes of warming were much larger than that of precipitation level. A significant, but negligible, effect of clipping on fungal richness was observed ($\beta$ = 0.03, $p = 0.035$) but not on bacterial or protistan richness ($\beta$ = 0.02, $p = 0.11$; Fig. 1b). For Shannon-Weaver information, inverse Simpson and Pielou's evenness (Fig. 1c, e), similar patterns of effect sizes were observed for warming, precipitation levels, and clipping treatments. Warming had significant negative effects on bacterial ($\beta$ = - 0.39, $p < 0.0001$ for Shannon; $\beta$ = -0.18, $p < 0.001$ for inverse Simpson; $\beta$ = -0.11, $p < 0.0001$ for Pielou's evenness), fungal ($\beta$ = -0.42, $p < 0.001$ for Shannon; $\beta$ = -0.23, $p < 0.001$ for inverse Simpson; $\beta$ = -0.24, $p = 0.0061$ for Pielou's evenness) and protists ($\beta$ = -0.89, $p < 0.001$ for Shannon; $\beta$ = -0.72, $p < 0.001$ for inverse Simpson;

72 β = -0.79, $p$ = 0.0022 for Pielou's evenness) communities (Fig. 1c, e). In contrast, the effects of
73 altered precipitation level and clipping on these two metrics were not significant ($p$ > 0.09) (Fig.
74 1c, e). As for Faith's phylogenetic diversity (Fig. 1f), strong negative effects of warming were
75 observed for bacterial (β = -0.49, $p$ < 0.0001), fungal (β = -0.47, $p$ = 0.0027), and protistan (β = -
76 0.80, $p$ = 0.003) communities. Interestingly, clipping had negative impacts on Faith's phylogenetic
77 diversity of bacteria (β = -0.16, $p$ = 0.041), and protists (β = -0.01, $p$ < 0.001), but had a positive
78 effect on that of fungi (β = 0.13, $p$ = 0.040) (Fig. 1f). Altogether, our results indicated that
79 experimental warming, rather than altered precipitation level and clipping, was the predominant
80 treatment associated with the biodiversity decrease of soil bacterial and fungal communities.
81
82 The effects of warming, altered precipitation level, clipping and their interactions on microbial
83 biomass were further tested by linear mixed-effects models (Fig. 1g; Table S2). Experimental
84 warming significantly decreased the total microbial biomass measured by PLFA (β = - 0.83, $p$ =
85 0.046) and DNA yield (β = - 0.72, $p$ = 0.0019) from DNA extraction (Fig. 1g; Table S2). Warming
86 also negatively impacted the biomass of arbuscular mycorrhiza fungi (AMF) (β = - 0.54, $p$ = 0.013;
87 Fig. 1g). In contrast, minor but significant positive effects of altered precipitation level were
88 observed for total microbial biomass by PLFA (β = - 0.02, $p$ = 0.0084) and biomass of AMF (β =
89 0.16, $p$ = 0.0011) (Fig. 1g; Table S2). Clipping had strong negative effects on DNA yield (β = -
90 0.40, $p$ = 0.019) but not on total biomass by PLFA or biomass of AMF ($p$ > 0.52) (Fig. 1g; Table
91 S2). In addition, the effects of warming, altered precipitation levels, and the interactions of
92 warming and altered precipitation levels on bacteria and Gram-Negative bacteria were roughly
93 similar (β = - 0.84 and - 0.81, $p$ = 0.035 and 0.021 for warming; β = 0.03 and 0.03, $p$ = 0.0064 and

0.015 for precipitation levels; β = 0.45 and 0.45, $p < 0.001$ for warming × precipitation levels) (Table S2), likely due to the dominance of Gram-Negative bacteria in the bacterial community.

## 2. Temporal variations of treatments on microbial diversity

Linear mixed-effects models were further used to test the effects of warming, altered precipitation level, clipping and their interactions on soil bacterial and fungal richness in each year. The detrimental effects of warming on bacterial richness changed over time (Fig. S5a). In the first year (i.e., 2010), warming had no significant (β = -0.39, $p = 0.52$) impacts on bacterial richness, just like precipitation alternation and clipping. In the subsequent years (i.e., from 2011 to 2016), warming exhibited significantly and persistently negative effects on bacterial richness (β = -1.72 ~ -0.68, $p < 0.030$). Among these years, the strongest detrimental effects of warming were observed in 2013 rather than the last year, which may be due to microbial adaptation under long-term warming or background environmental changes (i.e., climate conditions)[1]. As a result, the decreases of bacterial richness by warming in 2013 were even larger than the other years under most single and combined treatments (Fig. S5a). Notably, the altered precipitation level also exhibited significant effects on bacterial richness. However, the effects of precipitation level were negative in 2011 and 2013 (β = -0.77 ~ -0.01, $p < 0.006$), and positive in 2012 and 2014 (β = 0.37 ~ 0.45, $p < 0.002$), suggesting the impacts of altered precipitation level were relatively weak and depended on yearly background conditions. In addition, there were no more significant effects of single or combined treatments on bacterial richness in all years except significant positive effects of clipping in 2014 (β = 0.38, $p < 0.001$) and positive effects of the combined treatment of warming and clipping in 2016 (β = 1.03, $p < 0.040$).

Obvious temporal variations were also observed with the warming effects on fungal richness (Fig. S5b). No significant effects on fungal richness were observed in all single and combined treatments in 2010 ($\beta$ = -1.99 ~ 1.49, $p$ > 0.052). Warming effects on fungal richness were significant ($\beta$ = 0.37, $p$ < 0.001) in 2011 but then became insignificant ($\beta$ = -1.37, $p$ = 0.530) in 2012, suggesting the impacts of warming were not strong enough to persistently change fungal richness in the first three years (Fig. S5b). From 2013 to 2016, warming exhibited significantly and persistently negative effects on fungal richness ($\beta$ = -2.15 ~ -0.44, $p$ < 0.040), of which the largest decrease of fungal richness by warming was observed in 2015 (Fig. S5b). These results suggested that fungal diversity was persistently impacted by climate warming like bacteria, although the magnitudes of impacts varied in different years. Different from warming, clipping only showed weak positive effects on fungal richness in 2015 ($\beta$ = 0.05, $p$ = 0.020), and no significant effects of the precipitation level were observed in all years ($\beta$ = -0.54 ~ 0.70, $p$ > 0.070). However, more significant combined treatment effects of warming and precipitation levels were observed on fungal richness in 2011 and 2015, but their directions are opposite (Fig. S5b). Furthermore, a significant effect of the combined treatment of warming, precipitation levels and clipping on fungal richness was observed in 2013. These results indicated that multiple climate change factors could have more significant effects on fungal richness.

The detrimental effects of warming on protistan richness changed over time (Fig. S5c). No significant effects on protistan richness were observed in all single and combined treatments in 2010 ($\beta$ = -0.90 ~ 1.10, $p$ > 0.268). Warming effects on protistan richness were significant ($\beta$ = -.0.91, $p$ < 0.050) in 2011, but then became insignificant ($\beta$ = 0.27, $p$ = 0.441) in 2012, suggesting the impacts of warming were not strong enough to persistently change protistan richness in the

first three years like fungi (Fig. S5c). From 2013 to 2014, warming exhibited significantly negative effects on protistan richness ($\beta$ = -1.44 ~ -0.60, $p < 0.001$), of which the largest decrease of protistan richness by warming was observed in 2013 (Fig. S5c). While from 2015 to 2016, warming effects on protistan richness became insignificant ($\beta$ = -1.60 ~ -1.45, $p > 0.259$). These results suggested that protistan diversity was impacted by climate warming, although the magnitudes and persistence of impacts were relatively lower than bacteria and fungi. Different from warming, significant effects of the precipitation level were only observed in 2012 and 2014 ($\beta$ = 0.60 ~ 0.63, $p < 0.026$), and clipping only showed positive effects on protistan richness in 2014 ($\beta$ = 0.51, $p$ = 0.007). However, more significant combined treatment effects of warming and clipping were observed on protistan richness in 2016 ($\beta$ = 2.34, $p$ = 0.007), suggesting the potential synergetic effects of warming and clipping on protistan richness.

## C. Changes of bacterial taxa in detail

### 1. Effects of warming on microbial communities at broad coarse phylogenetic scales (Phyla/Classes/guilds)

To further understand how experimental warming shifted different microbial groups, we examined the effect size of warming on the richness, phylogenetic diversity and relative abundance of major microbial groups. Warming significantly decreased the richness of most bacterial phyla (Fig. 2a), as well as their phylogenetic diversity (Fig. S6a). Warming had the largest negative effects on the richness of Acidobacteria[2], Verrucomicrobia, and Planctomycetes ($\beta$ = -1.21 ~ -1.19, $p < 0.001$; Fig. 2a) as well as their phylogenetic diversity ($\beta$ = -1.33 ~ -1.08, $p < 0.001$; Fig. S6a). Warming also decreased the richness of Bacteroidetes, Chlamydiae, Chloroflexi, and α-, β-, γ-, and δ-Proteobacteria ($\beta$ = -1.02 ~ -0.11, $p < 0.05$; Fig. 2a) as well as their phylogenetic diversity ($\beta$ = -1.07 ~ -0.10, $p < 0.01$; Fig. S6a). In contrast, warming significantly increased the richness of

Firmicutes (β = 1.52, $p < 0.01$; Fig. 2a) but not for the phylogenetic diversity of Firmicutes (β = 0.34, $p = 0.14$; Fig. S6a). Furthermore, warming significantly decreased the relative abundance of Acidobacteria, Verrucomicrobia, Planctomycetes, Chlamydiae, Nitrospirae, β-, and δ-Proteobacteria (β = -0.88 ~ -0.13 $p < 0.05$), but increased that of Actinobacteria, Firmicutes and Gemmatimonadetes (β = 0.52 ~ 1.05, $p < 0.05$; Fig. S6b), which could be due to their preference of drier soils[3-5].

Warming effects also varied among different fungal phyla and guilds classified by FUNGuild[6]. For fungal phyla, warming decreased the richness of the three major phyla, i.e., Ascomycota, Basidiomycota and Mortierellomycota (β = -0.83 ~ -0.59, $p < 0.001$; Fig. 2a). Warming also decreased the phylogenetic diversity of Basidiomycota (β = - 0.65, $p < 0.001$; Fig. S6a) and the relative abundance of Mortierellomycota (β = -0.75, $p < 0.001$; Fig. S6b). Among the fungal guilds, warming reduced the richness, phylogenetic diversity and abundance of arbuscular mycorrhiza fungi (AMF) (β = -1.05 ~ -0.42, $p < 0.01$; Fig. 2a; Fig. S6a, b). Warming decreased the richness of putative plant pathogenetic fungi and saprotrophic fungi (β = -0.73 ~ - 0.49, $p < 0.001$; Fig. 2a), but did not significantly change their phylogenetic diversity or relative abundance ($p > 0.05$; Fig. S6a, b). It was also noted that warming marginally increased the relative abundance of putative plant pathogenetic fungi (β = 0.43, $p = 0.055$; Fig. S6b).

We investigated the warming effects on the five major protist phyla, i.e., Cercozoa, Ciliophora, Lobosa, Ochrophyta and Conosa, which accounted for 80% of the total protist abundance. Warming significantly decreased the richness and phylogenetic diversity of Cercozoa and Ochrophyta (β = -1.07 ~ -0.20, $p < 0.002$) but increased that of Conosa (β = 0.05 ~ 0.12, $p < 0.02$)

(Fig. 2a; Fig. S6a). For the relative abundance, warming reduced that of Cercozoa, Lobosa and Ochrophyta ($\beta$ = -0.77 ~ -0.50, $p < 0.02$) but increased the relative abundance of Ciliophora ($\beta$ = 0.33, $p < 0.0001$) (Fig. S6b). We also assigned the major protistan lineages to their dominant mode of energy acquisition (i.e., trophic functional groups)—either phototrophic, parasitic, or as consumers. Warming significantly reduced the richness and phylogenetic diversity of consumers, phototrophs and parasites ($\beta$ = -0.98 ~ -0.39, $p < 0.04$). Warming also decreased the relative abundance of phototrophic protists but not the other two functional groups ($\beta$ = -0.17, $p = 0.01$) (Fig. 2a; Fig. S6a, b).

**2. Effects of warming on microbial communities at finer phylogenetic scale (ASVs)**

To examine the variation of warming effects on individual species of different lineages, we constructed phylogenetic trees of bacterial ASVs whose relative abundances were significantly different between warming and control (adjusted $p < 0.05$, linear mixed-effects models). A total of 14 bacterial phyla (or classes for Proteobacteria) were included in the tree (Fig. 2b), corresponding with the bacterial groups shown in Fig. 2a. Overall, warming significantly decreased the cumulated relative abundance of ASVs across all groups and for the majority of individual groups. The highest cumulated relative abundances of ASVs decreased by warming were observed with Verrucomicrobia (92.2%), Nitrospirae (87.1%), Acidobacteria (75.4%), γ-Proteobacteria (74.4%), and Chlamydia (74.0%). In comparison, the highest cumulated relative abundances of ASVs increased by warming were observed with Firmicutes (98.7%), Actinobacteria (78.4%), and Gemmatimonadetes (73.0%). Based on the number of ASVs within each group (indicated by the blue and yellow colors in the third ring), Verrucomicrobia (85.6%), γ-Proteobacteria (76.8%), Chlamydia (76.6%), δ-Proteobacteria (75.8%), Acidobacteria (72.9%),

and Planctomycetes (71.5%) had the highest proportions of ASVs decreased by warming. Among these groups, Gemmatimonadetes have been shown to prefer drier soils[7].

## D. Environmental drivers of soil microbial diversity

### 1. Correlation analyses

To identify environmental drivers for soil bacterial and fungal diversity, we correlated bacterial and fungal richness with various environmental variables (Fig. 3a). Overall, soil variables exhibited stronger correlations with bacterial, fungal, and protistan richness than plant variables. Specifically, soil moisture had the strongest positive correlation with bacterial (LMM's r = 0.21, $p < 0.001$), fungal (LMM's r = 0.24, $p < 0.001$), and protistan (LMM's r = 0.15, $p < 0.001$) richness, and soil temperature exhibited the strongest negative correlation with bacterial (LMM's r = -0.25, $p < 0.001$), fungal (LMM's r = -0.25, $p < 0.001$), and protistan (LMM's r = -0.16, $p = 0.007$) richness. Soil nitrate content also exhibited significant negative correlations with bacterial and fungal richness, though the correlation for protist was relatively weaker (LMM's r = -0.12, $p = 0.007$) than that for bacteria (LMM's r = -0.20, $p < 0.001$), and fungi (LMM's r = -0.16, $p < 0.001$). Furthermore, soil pH showed significant positive correlations with bacterial (LMM's r = 0.20, $p < 0.001$) and fungal (LMM's r = 0.15, $p < 0.001$) richness, while for protist the impacts showed insignificant (LMM's r = 0.07, $p = 0.052$). Ammonia content showed significant negative correlations with bacterial (LMM's r = -0.13, $p = 0.028$), fungal (LMM's r = -0.19, $p = 0.002$) and protistan (LMM's r = -0.13, $p = 0.033$) richness. While soil total carbon and total nitrogen marginally significantly correlated with bacterial richness (LMM's r = 0.10 ~ 0.13, $p < 0.069$), they were not correlated with fungal (LMM's r = -0.08 ~ -0.06, $p > 0.196$) and protistan (LMM's r = 0.01 ~ 0.02, $p > 0.814$) richness. For plant variables, some significant correlations were

observed with bacterial or fungal richness. Bacterial richness had significant positive correlations with total plant biomass, $C_3$ plant biomass and plant richness (LMM's r = 0.11 ~ 0.19, $p < 0.034$), while fungal richness only showed a significant positive correlation with $C_4$ plant biomass (LMM's r = 0.12, $p = 0.041$). However, there were no plant variables significantly correlated with protistan richness (LMM's r = 0.015 ~ 0.045, $p > 0.519$).

However, obvious collinearity was observed among soil and plant variables (Fig. 3a and Table S6). Due to warming effects on soil, the decrease of soil moisture was accompanied with the increase of soil temperature (Pearson's r = -0.24, $p < 0.001$). Soil moisture also positively associated with soil pH, total plant biomass, $C_4$ plant biomass and plant richness (Pearson's r = 0.18 ~ 0.47, $p < 0.001$), but negatively associated with soil nitrate content (Pearson's r = -0.36, $p < 0.001$). Consistently with most soil studies[8-10], there were strong and significant (Pearson's r = 0.42 ~ 0.98, $p < 0.001$) associations among soil total carbon, soil total nitrogen and available nitrogen (i.e., nitrate and ammonia contents). A significant and negative association was observed between nitrate content and plant richness (Pearson's r = -0.18, $p = 0.001$). In addition, strong and significant associations occurred among total plant biomass, $C_4$ biomass and $C_3$ biomass ($p < 0.001$).

## 2. Structural equation modeling

To discern the direct and indirect effects of the environmental drivers on microbial biodiversity, a more in-depth analysis using structural equation modelling (SEM) was performed with the presumed relationships (Fig. S8) among the selected subsets of plant and soil variables. Soil moisture, which was negatively affected by warming and half precipitation, but positively by

double precipitation, played the strongest role in shaping bacterial richness directly (standardized path coefficient, $b$ = 0.40, $p$ < 0.01; Fig. 3b). It was noted that the effect size of warming on soil moisture (standardized path coefficient, $b$ = - 0.69, $p$ < 0.001) was larger than that of half precipitation ($b$ = -0.16, $p$ = 0.01) and double precipitation ($b$ = 0.45, $p$ < 0.001), consistent with LMM analyses (Fig. S2b). Soil temperature was also mainly driven by warming treatment ($b$ = 0.88, $p$ < 0.001), but it did not affect bacterial richness directly ($b$ = 0.09, $p$ = 0.47). Soil nitrate, which was negatively correlated with soil moisture ($b$ = -0.83, $p$ < 0.001) and affected by clipping ($b$ = -0.28, $p$ = 0.001), showed a marginally direct influence on bacterial richness ($b$ = -0.20, $p$ = 0.092). However, soil nitrate could indirectly affect bacterial richness through its influence on soil pH ($b$ = -0.32, $p$ = 0.031 for nitrate effect on pH; $b$ = 0.31, $p$ = 0.004 for pH effect on bacterial richness). Plant richness and $C_3$ plant biomass, which were mainly affected by clipping ($b$ = 0.38, $p$ = 0.001 for plant richness; $b$ = -0.24, $p$ = 0.12 for $C_3$ plant biomass), were also significantly and positively ($b$ = 0.23-0.26, $p$ < 0.01) correlated with bacterial richness. Furthermore, bacterial richness positively affected protistan richness (b = 0.69, $p$ < 0.001).

For the SEM of fungal richness (Fig. S9), paths between the four treatments and soil/plant variables were largely the same as that in the bacterial SEM, except for that soil ammonium was significantly affected by warming treatment ($b$ = 0.28, $p$ = 0.033) and that the biomass of $C_4$ plants was positively correlated with clipping treatment ($b$ = 0.48, $p$ < 0.001) and soil moisture ($b$ = 0.34, $p$ = 0.007), but negatively correlated with plant richness ($b$ = -0.30, $p$ = 0.025). Among the variables which directly contributed to fungal richness, only paths of soil moisture ($b$ = 0.44, $p$ = 0.001) and plant richness ($b$ = 0.26, $p$ = 0.015) were significant (Fig. S9), suggesting that the environmental

drivers were quite different between bacteria and fungi. Overall, those variables could explain 61%, 51% and 50% of the variations in bacterial, fungal and protistan richness, respectively.

**E. Linkages between soil microbial diversity and ecosystem functional processes**

Although the terms ecosystem functioning or functions are widely used[11,12], their exact meanings and extensions are not well defined. They are generally referred to as the ecological processes controlling the fluxes of energy, matter, and information through an environment[13,14]. Ecosystem functions and services can be classified into four major types, including: (i) provisioning services such as food and fiber, (ii) regulating services such as carbon sequestration and climate regulation, (iii) cultural services such as recreational experiences, and (iv) supporting functions and services that contribute to the supply of the other services such as soil formation and nutrient cycling[15]. Consistent with the above suggestions, we used the measured C fluxes to assess ecosystem functions, including ecosystem respiration (ER), net ecosystem exchange (NEE), gross primary productivity (GPP), soil total respiration ($R_s$) and heterotrophic respiration ($R_h$), which could be considered as regulating services of climate regulation. Microbial biomass, including the total microbial biomass, bacterial or fungal biomass and the fungal to bacterial biomass ratio, were also considered as ecosystem functions, as they were indirectly related to nutrient cycling and plant growth.

We addressed the question whether the warming-induced decrease of soil microbial diversity affects associated ecosystem processes from different angles. First, as expected, the overall bacterial richness had significant positive correlations with the total microbial biomass, bacterial biomass, GPP, and ER ($r = 0.14 \sim 0.22$, $p < 0.002$) (Fig. 3d), and had positive, albeit

weak, correlations with $R_s$, $R_h$, and NEE (Fig. 3d). Similar positive correlation patterns were observed for most bacterial groups such as Acidobacteria, δ-Proteobacteria, γ-Proteobacteria, Plantctomycetes and Verrucomicrobia, whose richness was positively correlated with the total microbial biomass, bacterial biomass, GPP and ER ($r = 0.13 \sim 0.25$, $p < 0.04$). The richness of Actinobacteria, α-Proteobacteria, Bacteroidetes and β-Proteobacteria had significant positive correlations with the total microbial biomass and bacterial biomass ($r = 0.18 \sim 0.32$, $p < 0.003$), but not with GPP or ER. In contrast, the richness of Chlamydiae and Chloroflexi positively correlated with GPP and ER ($r = 0.13 \sim 0.18$, $p < 0.003$), but not with the total microbial biomass or bacterial biomass. It was also noted that the richness of Chlamydiae, δ-Proteobacteria, γ-Proteobacteria and Verrucomicrobia positively correlated with $R_s$ ($r = 0.11 \sim 0.13$, $p < 0.05$). However, the richness of Firmicutes showed strong negative correlations with most ecosystem functional processes, including the total microbial biomass ($r = -0.26$, $p < 0.001$), bacterial biomass ($r = -0.26$, $p < 0.001$), fungal biomass ($r = -0.13$, $p = 0.038$), fungal to bacterial biomass ratio ($r = -0.13$, $p = 0.011$) and ER ($r = -0.10$, $p = 0.011$). For the major fungal phyla (Ascomycota, Basidiomycota and Mortierellomycota) and fungal guilds (AMF, Saprotrophic and putative pathogenetic fungi), their richness showed strong positive correlations with GPP and ER ($r = 0.09 \sim 0.20$, $p < 0.03$), but not with microbial biomass, $R_h$, $R_s$ or NEE, consistent with that of the total fungal richness (Fig. 3d). In addition, the richness of Mortierellomycota was positively correlated with $R_s$ ($r = 0.12$, $p = 0.034$). It was also noted that, AMF had the strongest positive correlations with GPP ($r = 0.14$, $p < 0.001$) and ER ($r = 0.20$, $p < 0.001$) among the three fungal guilds. The overall protistan richness also had significant positive correlations with total microbial biomass, bacterial biomass, GPP and ER ($r = 0.08 \sim 0.13$, $p < 0.04$) (Fig. 3d). Among the major protistan lineages, Ciliophora and Lobosa positively correlated with total microbial biomass and bacterial

biomass (r = 0.15 ~ 0.18, $p < 0.01$), while Cercozoa and Ochrophyta positively correlated with GPP and ER (r = 0.11 ~ 0.13, $p < 0.008$). For the three protistan functional groups, consumers, and parasites were positively correlated with total microbial biomass and bacterial biomass (r = 0.12 ~ 0.22, $p < 0.03$). The richness of parasites was also positively correlated with GPP and ER (r = 0.12 ~ 0.13, $p < 0.02$) (Fig. 3d).

## F. Study site description

We conducted the warming experiment at the Kessler Atmospheric and Ecological Field Station (KAEFS) in the US Great Plains in McClain County, Oklahoma (34° 59′ N, 97° 31′ W)[16]. KAEFS is an old-field tall-grass prairie abandoned from field cropping 40 years ago and has stopped light grazing since 2008. The dominant plants are C3 forbs (*Ambrosia trifida*, *Solanum carolinense* and *Euphorbia dentate*) and C4 grasses (*Sorghum halepense* and *Tridens flavus*)[16]. Based on Oklahoma Climatological Survey data from 1948 to 1999, the air temperature ranges from 3.3 °C in January to 28.1 °C in July with mean annual temperature 16.3 °C, and the precipitation ranges from 82 mm in January and February to 240 mm in May and June with mean annual precipitation 914 mm[17]. The soil type of this site is Port–Pulaski–Keokuk complex, and soil texture class is loam with 51% of sand, 35% of silt and 13% of clay[18]. The soil has a high available water holding capacity (37%), neutral pH and a deep (about 70 cm), moderately penetrable root zone[16].

The field site experiment was established in July of 2009 with a blocked split-plot design (Fig. S1). In brief, the site has four experimental blocks, each including six plots. Each plot has the size of $2.5 \times 3.5$ m$^2$, which is further divided into one $2.5 \times 1.75$ m$^2$ clipped subplot and one $2.5 \times 1.75$ m$^2$ unclipped subplot, resulting in a total of 48 subplots (Fig. S1). Within each block,

the six plots are under one of the six randomly distributed treatments: (i) ambient temperature and normal precipitation; (ii) ambient temperature and double precipitation; (iii) ambient temperature and half precipitation; (iv) warming and ambient precipitation; (v) warming and double precipitation; and (vi) warming and half precipitation. The heating is carried out using infrared radiator (Kalglo Electronics). In warming plots, a real infrared radiator is suspended 1.5 m above the ground, while in the ambient-temperature plots dummy infrared radiator is suspended to simulate the shading effect of the device. After soil sampling in 2009, the heating is on 24 hours per day and 365 days per year in the field. For double precipitation plots, an additional fold of rainfall is received from the rainfall-collection-redistribution devices, which are angled catchments with the same size and shape as the plot[19]; For half precipitation plots, the rainout shelters are used to halve precipitation as previously described[20]. Clipping treatment is performed in the southern subplots once every year at approximately the date of peak plant biomass in the autumn (September or October), in which plants are clipped at a height of 10 cm above the ground to mimic the land use practice of mowing. The northern subplots are not clipped.

From 2009 to 2016, surface (0–15 cm) soil samples were collected annually from subplots one day before annual clipping. Each sample was mixed from three soil cores (2.5 cm diameter × 15 cm depth) from a soil sampler tube. In the first year (2009), because clipping was performed after soil sampling, we only collected 24 pre-warmed soil samples from the southern subplots, which could represent soils from the northern unclipped subplots in the first year. As for the following years, a total of 48 annual soil samples were collected from all subplots in each year. A total of 360 annual soil samples from 2009 to 2016 were collected in this study and stored in a freezer at −80 °C. To

rule out systematic bias in sampling handling, DNA extraction, PCR amplification and sequencing, all samples were reordered randomly and were further analyzed in this random order.

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