Supplementary Information

Gene-informed decomposition model predicts lower soil carbon loss due to persistent microbial adaptation to warming

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Includes Supplementary Tables (1-11) Supplementary Figures (1-16)

| Sequencing/ GeoChip | Targets | Numbers of samples analyzed | Total base pairs (bp) | Average No. of reads/probes per sample | OTUs/ genes |
|---|-----------------------|-----------------------------------|--------------------------|--|----------------|
| 16S rRNA gene amplicon sequencing | Bacteria + archaea | 56 | 0.74G | 51,415±2,696 | 26,158 |
| ITS amplicon sequencing | Fungi | 56 | 0.43G | $3,1203\pm4,017$ | 5,336 |
| Shotgun sequencing | Functional genes | 56 | 0.96T | 127.83±2.89M | 98,682 |
| GeoChip | Functional genes | 56 | NA | 35,425±468 | 35,425 |

Supplementary Table 1. Summary of sequence and GeoChip statistics. The microbial samples from each year were analyzed with various molecular approaches.

Supplementary Table 2. The correlations between the structure of each functional gene group involved soil C decomposition and N cycling processes and each environmental attribute revealed by CCA analysis. Significance is adjusted by false discovery rate (FDR) and the p values are shown here.

⁺Abbreviation of environmental attributes: R_h , heterotrophic respiration; R_t , soil total respiration; Q_{10} , temperature sensitivity of heterotrophic respiration.

Supplementary Table 3. The correlations between the structure of each functional gene group involved soil C decomposition and N cycling processes and each environmental attribute revealed by Mantel test. Significance is adjusted by false discovery rate (FDR) and the p values are shown here.

 $+$ Abbreviation of environmental attributes: R_h , heterotrophic respiration; R_t , soil total respiration; Q_{10} , temperature sensitivity of heterotrophic respiration.

Supplementary Table 4. The enzyme/protein encoded by biogeochemical cycling genes shown in Fig. 2, Supplementary Figures 9 and 10, and Supplementary Tables 2 and 3.

| Function description | Equation Eq# | | |
|--|---|-------|--|
| Reaction rate (v) at a specific soil water potential (ψ) , soil temperature (T) , and soil pH (pH) | $v = v_0 \cdot f(\psi) \cdot f(T) \cdot f(pH)$ | (E1) | |
| Response function of soil pH | $f(pH) = \exp\left[-\left(\frac{pH - pH_{opt}}{pH_{sen}}\right)^2\right]$ | | |
| Temperature sensitivity of carbon use efficiency (Y_g) | $Y_{\rm g}(T) = Y_{\rm g}(T_{\rm ref}) - k_{Y\rm g} (T - T_{\rm ref})$ | (E3) | |
| Arrhenius equation or Q_{10} method to simulate simulate the response of other parameters to changes in temperature | $f(T) = \exp\left[-\frac{Ea}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]$ | (E4) | |
| | $f(T) = Q_{10}^{\frac{T-T_{\text{ref}}}{10}}$ | (E5) | |
| | $Q_{10} = \exp \left[\frac{Ea}{R \cdot T_{\text{ref}}} \cdot \frac{10}{T} \right]$ | (E6) | |
| Soil moisture response function for SOM decomposition by oxidative enzymes | 0. $\psi \le -10^{2.5}$ $0.625 - 0.25 \times \log_{10}(-\psi)$, $-10^{2.5} < \psi \le -10^{1.5}$ $1, -10^{1.5} < \psi \le -10^{-2.5}$ $[2.5 + 0.4 \times \log_{10}(-\psi)]/1.5$, $-10^{-2.5} < \psi \le -10^{-4}$ $f_{\text{lig}}(\psi) = \left\{$ | (E7) | |
| Soil moisture response function for SOM decomposition by hydrolytic enzymes | 0.6, $\psi > -10^{-4}$ 0, $\psi \le \psi_{\min}$ $\label{eq:fcel} f_{\rm cel}(\psi) = \left\{ \begin{array}{c} 1 - \left[\frac{\ln(\psi/\psi_{\rm FC})}{\ln(\psi_{\rm min}/\psi_{\rm FC})}\right]^\flat, \quad & \psi_{\rm min} < \psi \leq \psi_{\rm FC} \\ 1, \quad & \psi > \psi_{\rm FC} \end{array} \right.$ | (E8) | |
| Soil moisture response function for microbial mortality, dormancy & resuscitation | $f_{A2D}(\psi) = \frac{(-\psi)^{\omega}}{(-\psi)^{\omega} + (-\psi_{A2D})^{\omega}}$ $f_{D2A}(\psi) = \frac{(-\psi_{D2A})^{\omega}}{(-\psi)^{\omega} + (-\psi_{D2A})^{\omega}}$ | (E9) | |
| | | (E10) | |

Supplementary Table 5. Response functions of soil pH, temperature and moisture in MEND model.

Supplementary Table 6. Soil carbon pools (state variables) in the MEND model.

| Governing Equation | Eq# |
|---|-------|
| $\frac{dP_1}{dt} = I_{P1} + (1 - g_D) \cdot F_{12} - F_1$ | (S1) |
| $\frac{dP_2}{dt} = I_{P2} - F_2$ | (S2) |
| $\frac{dM}{dt} = (1 - f_D) \cdot (F_1 + F_2) - F_3$ | (S3) |
| $\frac{dQ}{dt} = F_4 - F_5$ | (S4) |
| $\frac{dD}{dt} = I_D + f_D(F_1 + F_2) + g_D \cdot F_{12} + F_3 + (F_{14,EP1} + F_{14,EP2} + F_{14,EM}) - F_6 - (F_4 - F_5)$ | (S5) |
| dBA $\frac{1}{\text{dt}} = F_6 - (F_7 - F_8) - (F_9 + F_{10}) - F_{12} - (F_{13,\text{EP1}} + F_{13,\text{EP2}} + F_{13,\text{EM}})$ | (S6) |
| $\frac{dBD}{dt} = (F_7 - F_8) - F_{11}$ | (S7) |
| $\frac{dE P_1}{dt} = F_{13,\text{EP1}} - F_{14,\text{EP1}}$ | (S8) |
| $\frac{dE P_2}{dt} = F_{13,\text{EP2}} - F_{14,\text{EP2}}$ | (S9) |
| $\frac{dEM}{dt} = F_{13,EM} - F_{14,EM}$ | (S10) |
| $\frac{dC_2}{dt} = (F_9 + F_{10}) + F_{11}$ | (S11) |
| $\frac{d}{dt}(P_1 + P_2 + M + Q + D + BA + BD + EP_1 + EP_2 + EM) = I_{P1} + I_{P2} + I_D - (F_9 + F_{10} + F_{11})$ | (S12) |

Supplementary Table 7. Governing equations of each soil carbon pool in the MEND model

The state variables (C pools) are described in Table S6; Eq. S11 indicates the total heterotrophic respiration flux and Eq. S12 expresses the overall mass balance of the system. The transformation fluxes are elucidated by Eqs. S13–S26 in Table S8.

Supplementary Table 8. Component fluxes in the MEND model (parameters are described in

Table S9)

Notes: Italic symbols like F_i represent component fluxes in equations. Italic symbols P_1 , P_2 , M , Q , *D*, BA, BD, EP₁, EP₂, and EM are state variables (soil carbon pools, see Supplementary Table 6) in equations.

Notes: The column "Eq#" lists the major equation # (see Supplementary Table 7 and 8) in which each parameter is used.

Supplementary Table 10. Objective functions used for different response variables in the MEND model parameterization.

Notes: RR is the response ratio of gene abundance under warming to that under control. R^2 denotes the coefficient of determination, MARE is the mean absolute relative error, see Methods Eqs. 3–4.

| ID | Category | Enzyme | Ea | Q_{10} | Reference |
|--------------|------------|----------------------|------|----------|---|
| $\mathbf{1}$ | Cellulases | β -glucosidase | 30.8 | 1.52 | Eivazi and Tabatabai, 1988 ¹ |
| 2 | Cellulases | β -glucosidase | 25.3 | 1.41 | Deng and Tabatabai, 1994 ² |
| 3 | Cellulases | β -glucosidase | 53.2 | 2.05 | Chauve et al., 2010 ³ |
| 4 | Cellulases | β -glucosidase | 39.0 | 1.70 | Vila-Real et al., 2010 ⁴ |
| 5 | Cellulases | β -glucosidase | 39.7 | 1.71 | Han and Srinivasan, 1969 ⁵ |
| 6 | Cellulases | β -glucosidase | 54.3 | 2.09 | Plant et al., 1988 ⁶ |
| 7 | Cellulases | β -glucosidase | 59.6 | 2.24 | Patchett et al., 19877 |
| 8 | Cellulases | β -glucosidase | 31.0 | 1.52 | Patchett et al., 1987 ⁷ |
| 9 | Cellulases | β -glucosidase | 41.0 | 1.74 | Patchett et al., 19877 |
| 10 | Cellulases | β -glucosidase | 29.4 | 1.49 | Patchett et al., 1987 ⁷ |
| 11 | Cellulases | β -glucosidase | 79.5 | 2.93 | Patchett et al., 19877 |
| 12 | Cellulases | β -glucosidase | 44.3 | 1.82 | Ait et al., 1979 ⁸ |
| 13 | Cellulases | β -glucosidase | 24.7 | 1.40 | McClaugherty and Linkins, 1990 ⁹ |
| 14 | Cellulases | β -glucosidase | 61.1 | 2.29 | McClaugherty and Linkins, 1990 ⁹ |
| 15 | Cellulases | β -glucosidase | 43.1 | 1.79 | McClaugherty and Linkins, 1990 ⁹ |
| 16 | Cellulases | β -glucosidase | 33.2 | 1.57 | McClaugherty and Linkins, 1990 ⁹ |
| 17 | Cellulases | β -glucosidase | 41.3 | 1.75 | McClaugherty and Linkins, 1990 ⁹ |
| 18 | Cellulases | β -glucosidase | 39.3 | 1.70 | McClaugherty and Linkins, 1990 ⁹ |
| 19 | Cellulases | β -glucosidase | 57.0 | 2.16 | Rajoka et al., 2004 ¹⁰ |
| 20 | Cellulases | β -glucosidase | 15.0 | 1.23 | Yague and Estevez, 1988 ¹¹ |
| 21 | Cellulases | β -glucosidase | 52.0 | 2.02 | Rajoka et al., 2006 ¹² |
| 22 | Cellulases | β -glucosidase | 46.0 | 1.86 | Calsavara et al., 2001 ¹³ |
| 23 | Cellulases | β -glucosidase | 30.1 | 1.50 | Li et al., 1965 ¹⁴ |
| 24 | Cellulases | Cellobiohydrolase | 22.2 | 1.35 | Maguire, 1977 ¹⁵ |
| 25 | Cellulases | Cellobiohydrolase | 79.4 | 2.93 | Saharay et al., 2010 ¹⁶ |
| 26 | Cellulases | Cellobiohydrolase | 13.8 | 1.21 | Rouau and Odier, 1986 ¹⁷ |
| 27 | Cellulases | Cellobiohydrolase | 25.9 | 1.42 | Nikolova et al., 1997 ¹⁸ |
| 28 | Cellulases | Cellobiohydrolase | 17.5 | 1.27 | Banka et al., 1998 ¹⁹ |
| 29 | Cellulases | Cellobiohydrolase | 52.0 | 2.02 | Eriksen and Goksoyr, 1977 ²⁰ |
| 30 | Cellulases | Cellobiohydrolase | 14.7 | 1.22 | Eriksen and Goksoyr, 1977 ²⁰ |
| 31 | Cellulases | Endo-glucanase | 26.1 | 1.42 | Eriksen and Goksoyr, 1977 ²⁰ |
| 32 | Cellulases | Endo-glucanase | 47.2 | 1.89 | Eriksen and Goksoyr, 1977 ²⁰ |
| 33 | Cellulases | Endo-glucanase | 22.8 | 1.36 | Onyike et al., 2008 ²¹ |
| 34 | Cellulases | Endo-glucanase | 45.0 | 1.84 | Petre et al., 1986 ²² |
| 35 | Cellulases | Endo-glucanase | 26.9 | 1.44 | Warner et al., 2010 ²³ |
| 36 | Cellulases | Endo-glucanase | 3.3 | 1.05 | Javed et al., 2008 ²⁴ |
| 37 | Cellulases | Endo-glucanase | 51.0 | 1.99 | Saqib et al., 2010 ²⁵ |
| 38 | Cellulases | Endo-glucanase | 32.7 | 1.56 | Saqib et al., 2010 ²⁵ |
| 39 | Cellulases | Endo-glucanase | 36.2 | 1.63 | Jabbar et al., 2008 ²⁶ |
| 40 | Cellulases | Endo-glucanase | 35.5 | 1.62 | Perez-Avalos et al., 2008 ²⁷ |

Supplementary Table 11. Activation energy (Ea: kJ mol⁻¹) and Q_{10} values^{*} for cellulases and ligninases

* *Q*¹⁰ values are calculated from Ea with a temperature increase from 20 °C to 30 °C.

Supplementary Figure 1. Warming effects on plant and soil variables. (a) Effects of warming on aboveground plant biomass from C_3 , C_4 and total species; (**b**) Soil pH; (**c**) Soil nitrate (NO₃⁻), ammonia (NH₄⁺), total N (TN) and total organic carbon (TOC) across 7 years. Error bars represent standard error of the mean ($n = 4$ field plots examined 7 repeated measures from 2010 to 2016). The differences between warming and the control were tested by the two-sided repeated-measures ANOVA, indicated by *** when $p < 0.01$, ** when $p < 0.05$. Source data are provided as a Source Data file.

(Rh) and autotrophic respiration (Ra) from 2010 to 2016. The respiration values were displayed as mean \pm standard error ($n = 4$ biological field plots).

Supplementary Figure 3. Apparent temperature sensitivity of soil heterotrophic respiration (*Q***10).** The curve fitting method was used for the control and warming treatments in each year (2010- 2016) by exponential growth regression model. Significance was test by analysis of variance (ANOVA).

Supplementary Figure 4. Flowcharts of ecosystem models. (a) Microbial-ENzyme Decomposition (MEND) model. Soil organic matter (SOM) pools include: particulate organic matter (POM) (e.g., POM decomposed by oxidative and hydrolytic enzymes, denoted by *P*¹ and *P*² in the governing equations, respectively), mineral-associated organic matter (MOM, denoted by *M*), dissolved organic matter (DOM, *D*), adsorbed phase of DOM (QOM, *Q*), active and dormant microbes (MBA and MBD, denoted by BA & BD), POM-degraded enzymes (e.g., EP_1 and EP_2 that break down *P*¹ and *P*2, respectively), and MOM-degraded enzymes (EM). **(b)** Terrestrial ECOsystem (TECO) model.

Supplementary Figure 5. A scatterplot of BIOLOG metabolic profiles under warming and control in 2016. Values close to the reference line (red) are in good agreement with the control values. Bi-directional error bars represent standard errors of the mean under control and warming treatments. Values above the reference line have an enhanced ability to utilize that carbon source in the warmed plots, value below have an inhibited ability in the warmed plots.

Supplementary Figure 6. Pairwise comparisons of environmental factors with functional community structure based on shotgun sequencing data. The shotgun sequencing data were annotated using EcoFUN-MAP database. A color gradient denotes Pearson's correlation coefficients with functional community structure by partial Mantel tests. Edge width corresponds to the Mantel's *r* statistic for the corresponding distance correlations, and edge color denotes the statistical significance.

Supplementary Figure 7. Canonical correspondence analyses (CCA) of microbial communities. (a) Bacterial community based on 16S rRNA gene; **(b)** Fungal community based on ITS; **(c)** Functional community based on GeoChip; and **(d)** Functional community based on shotgun metagenomic sequences with EcoFUN-MAP. Phylogenetic and functional structures of microbial communities were significantly shaped by soil related factors: soil temperature (Tm), moisture, soil pH, soil total organic carbon (TOC), total nitrogen (TN), soil nitrate (NO₃⁻) and ammonia (NH₄⁺) contents; by plant related factors: C₃ and total aboveground plant biomass, and plant richness (PR); and by time.

Supplementary Figure 8. CCA-based variation partitioning analysis (VPA) of microbial communities. (a) Bacterial community based on 16S rRNA gene; **(b)** Fungal community based on ITS; **(c)** Functional community based on GeoChip; and **(d)** Functional community based on shotgun metagenomic sequences based on EcoFUN-MAP. The relative proportions of bacterial community variations that can be explained by different types of environmental factors including soil related factors: soil temperature (Tm), moisture, soil pH, soil total organic carbon (TOC), total nitrogen (TN), soil nitrate (NO_3^-) and ammonia (NH_4^+) contents; plant related factors: C_3 and total aboveground plant biomass, and plant richness (PR); and time. The unexplained variations are either due to unmeasured environmental variables and/or stochastic factors.

Time (year)

Supplementary Figure 9. Signficantly changed genes involoved in C degradation (a), N cycling (b), P utilization (c) and S metabolism (d) by warming according to GeoChip data. Significance is based on response ratio of each gene with 95% confidence intervals of abundance differences between warmed and control treatments. Dash line represents that the abundance of warming-stimulated (red) genes are in good agreement with the abundance of warming-inhibited (blue) genes. The genes involved in C degradation, N cycling, P utilization and S metabolism in this plot are listed in Supplementary Table 4.

Gene

Supplementary Figure 10. Response ratios showing significant changes in abundance of C degradation genes in each year detected by GeoChip. Warming-stimulated C degrading genes were more than warming- inhibited genes in most years. Error bars represented 95% confidence intervals of abundance differences between warmed and control treatments. The targeted substrates were arranaged in order from labile to recalcitrant C. The full names of the genes in this figure are listed in Supplementary Table 4.

Supplementary Figure 11. MEND modeling performance with gene abundance data. MENDsimulated enzyme concentrations vs. GeoChip gene abundances for (**a**) oxidative enzymes and (**b**) hydrolytic enzymes in the control plot. MEND-simulated enzyme concentrations vs. GeoChipinformed enzyme concentrations for (**c**) oxidative enzymes and (**d**) hydrolytic enzymes in the warmed plot. The model performance for the control plot is quantified by the correlation coefficient (*r*), as we cannot directly compare the absolute values between GeoChip gene abundances and MEND enzyme concentrations. The model performance for the simulations under warming is evaluated by the Mean Absolute Relative Error (MARE) (see Table S9). Lower MARE value means better performance. All data are normalized by their respective mean values.

Supplementary Figure 12 The impact of changing temperature vs. changing moisture on soil R^h estimated by the gMEND model. The negative effect on R^h due to slightly drier soil under warming treatment was considerable, but it was completely shifted by the significant positive effect by increasing soil temperature.

Supplementary Figure 13. The MEND model parameter uncertainty was quantified by the Coefficient of Variation (CV) in the (a) Control and (b) Warmed plot. The tMEND refers to the traditional MEND model parameterization without gene abundances data. The gMEND denotes the improved MEND parameterization with gene abundances. The 11 model parameters are r_E : enzyme turnover rate; p_{EP} and fp_{EM} : two coefficients controlling enzyme production rates; f_D : fraction of decomposed particulate organic matter (POM) entering dissolved organic matter (DOM) pool; *g*_D: fraction of dead microbe entering DOM pool; *V*_g: maximum specific growth rate for microbe; α : a coefficient relating specific microbial maintenance rate ($V_{\rm m}$) to growth rate ($\alpha = V_{\rm m}$) /($V_g + V_m$)); K_D : half-saturation constant for microbial uptake of DOM; Y_g : carbon use efficiency at reference temperature; *k*Yg: temperature sensitivity of *Y*g; *Q*10: temperature sensitivity of enzymecatalyzed soil organic matter decomposition. See Table S9 for detailed description of all model parameters.

Supplementary Figure 14. **Improvement of model performance with gMEND compared to the non-microbial model TECO**. (**a**) Control plots. (**b**) Warmed plots.

Supplementary Figure 15 Activation energy (Ea) and corresponding *Q***¹⁰ values from literature and our model estimates.** Literature-Ea values are pooled data from major ligninases and cellulases catalyzing the decomposition of soil organic carbon. Literature- Q_{10} values ($n = 63$) and 33 for cellulases and ligninases, respectively) are calculated from Ea with a temperature increase from 20 °C to 30 °C. Model-derived Q_{10} values are those under control ($n = 7,560$) and warming (+3°C, *n* = 2,095) treatments. Model-Ea values are calcualted from *Q*¹⁰ with a temperature increase from 20 °C to 30 °C. Boxplots depict median, first and third quartiles, and full ranges (bounded at $1.5 \times$ interquartile range).

Supplementary Figure 16 Correlation between Q_{10} and k_{Yg} (temperature sensitivity of Y_{g}). Y_g is the true growth yield, i.e., a proxy for carbon use efficiency (CUE) in the MEND model. The temperature dependence of Y_g on soil temperature (T) is described by $Y_g(T) = Y_g(T_{ref}) - k_{Yg}$. $(T - T_{\text{ref}})$, where $Y_g(T)$ and $Y_g(T_{\text{ref}})$ are the Y_g at soil temperture T and T_{ref} (reference temperature), respectively; and k_{Yg} denote the temperature sensitivity of Y_g .

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