Appendix S1: detailed descriptions of methods

Section 1: the Oklahoma long-term field warming experiment

Site description. We initiated a long-term field experiment to investigate the responses of a tall grass prairie ecosystem to warming in November, 1999 (Supplementary Fig. 1a). The experimental site is located at the Kessler's Farm Field Laboratory in McClain County (34° 58' 54" N, 97° 31' 14" W), 40 km from the Norman campus of the University of Oklahoma, OK, USA. Annual mean temperature is 16.3°C and annual mean precipitation is 914 mm. Before the start of this experiment, the field had not been grazed for at least 40 years. The plant community composition is dominated by C_4 grasses (*Andropogon gerardii*, *Panicum virgatum*, and *Sorghastrum nutans*) and C_3 forbs (*Ambrosia psilostachyia*, *Solidago rigida*, *Solidago nemoralis*, and *hemiachyris dracunculoides*). The soil is part of the Nash-Lucien complex, characterized by a high water holding capacity and a deep and moderately penetrable root zone with a neutral pH (Zhou et al 2007).

Experimental design. The field experiment was a paired factorial design with warming as the main factor (n = 6) nested by the clipping factor. Each main plot was 2 m × 2 m in size (Supplementary Figs. 1a and 1b). In each pair of main treatments, one plot has been warmed continuously since November, 1999, to the present, and the other has been treated as control with ambient temperature. In each warmed plot, a single 165 cm × 15 cm infrared heater (Kalglo Electronics Inc., Bethlehem, PA, USA), suspended 1.5 m above the ground, was used to elevate temperature in each warmed plot. In each control plot, meanwhile, a 'dummy' heater with the same shape and size as the infrared heater was also suspended

1.5 m high to simulate the shading effects of the heater of warmed plots. Within each pair, the control plot was at least 5 m away from the warming one to avoid the heating effects. The distance between different paired plots ranged from 20 to 60 m. Each plot was split into four 1 m × 1 m subplots. Plants within two diagonal subplots were clipped at a height of 10 cm above the ground once a year to mimic hay harvesting or biofuel feedstock production, while the other two diagonal subplots remained unclipped (Supplementary Fig. 1b). During the course of experiment, daily mean air temperature, daily mean soil temperature, and soil moisture have been continuously monitored (Luo et al 2001, Xu et al 2015, Zhou et al 2012, Zhou et al 2007). In our system, experimental warming significantly enhanced the daily mean air temperature by 1.27 °C and daily mean soil temperature by 1.71 °C under unclipping and by 2.18 °C under clipping over the experimental duration. The heating effect of infrared heaters on soil temperature has been shown to reach soil depth as deep as 50 cm in the literature (Rui et al 2011, Xue et al 2015, Zhang et al 2015) (also see Supplementary Data Sets).

Deep soil collars. We installed a deep soil collar (a PVC tube with 10 cm in diameter × 70 cm in depth) in an unclipped subplot of each main treatment plot in October, 2001. The schematic map of the deep collar is shown in the Supplementary Figure 1c. The purpose of the installation of the deep collar was to cut off the existing roots and to exclude the influences of mycorrhizae, root exudation and the growth of new roots on soil C decomposition. In the winter of 2015, we also measured soil temperature adjacent to the deep collar at both 5 cm and 25 cm soil depths using a LI-COR 8100 gas flux system coupled with a thermo-probe sensor. Soil temperature had been monitored from 9:00 am to 1:00 pm each day. Warming increased daily soil temperature on average by 3.0 °C and 2.8 °C, respectively, at 5 cm and 25 cm soil

depths, suggesting the magnitude of heating effect on soil temperature was comparable across the soil profile. We also monitored the CO_2 efflux from the deep collar twice a month from October, 2001 to the present (Xu et al 2015, Zhou et al 2007).

Soil sampling. We used a 5-cm diameter soil corer to take soil samples from the soil layer of 0-25 cm in the deep collar by the end of 11^{th} year growth season. Soil samples were split into two soil layers (0-20 and 20-25 cm) and then sealed in plastic bags, stored in a cooler with dry ice and transported to the laboratory. In the laboratory, field moist soil of the 20-25 cm layer was mixed thoroughly and passed through a 4-mm sieve. Subsamples (~ 10 g) were then taken immediately, frozen and stored at -80 °C for the DNA extraction and the rest of soils were stored at 4 °C for the laboratory incubation and other analyses. A 5-g subsample was oven-dried at 65 °C for 48 h and weighed for the determination of the water content. Soil pH was measured in water using a ratio of 5 g soil to 5 ml DI water. Warming had no impact on soil pH (Warming plots: 7.34 ± 0.25 (s.e.m.); control plots: 7.33 ± 0.25 (s.e.m.)).

Soil organic carbon and radiocarbon analyses. Subsamples (10 g) from both top- and sub-soil layers were pretreated with acid (0.1 M HCl) for 8 hours to eliminate carbonate (Jenkinson et al 2008), then were oven-dried at 60 °C and ground into powder. The soil C concentrations were determined with a CHN elemental analyzer (Carla Erba and model 2400, Perkin Elmer Co., Norwalk, CT, USA). The bulk density of soil samples was determined and used to calculate the soil organic C stocks of the 0-25 cm soil profile. Subsamples from the subsoil layer of 20-25 cm were also used to determine the $\Delta^{14}C$ age of soil organic matter (SOM).The soils for radiocarbon dating were analyzed at the University of Arizona

NSF-AMS facility (Donahue 1995). The radiocarbon data were shown as conventional years Before Present (BP, relative to 1950).

Section 2: carbon pool partitioning

Theory. The ^{14}C nucleus is not stable, decaying with a half-life of 5730 years. The remaining amount (N) of ^{14}C in a sample after a certain period of decaying can be described by:

$$N = N_0(\frac{1}{2})^{\frac{t}{5730}} \quad (1)$$

where N_0 is the initial amount of ^{14}C, and t is the time of decaying (years). If organic matter in a soil sample is homogeneous at steady state, each C atom would have the same probability of leaving (Torn et al 2009). In reality, however, SOM in the bulk soil is enormously heterogeneous. Even so, it is plausible to divide SOM of a soil sample into several C age pools with each pool assuming to be homogeneous (Knorr et al 2005, Torn et al 2009). As such, the total amount of ^{14}C in a n-pool C model can be described by:

$$N_{Total} = \sum_{i=1}^{n} N_i w_i \quad (2)$$

where N_i is the ^{14}C content of the i^{th} C age pool, w_i is the proportion of the i^{th} C age pool. We then used the Arrhenius equation (Arrhenius 1889, Davidson and Janssens 2006) to estimate the warming effects on the dynamics of each C pool. The Arrhenius equation of the i^{th} C age pool can be expressed as

$$v_i = Ae^{-\frac{E_i}{RT}} \quad (3)$$

where v_i is the reaction rate of the i^{th} pool, E_i is the activation energy of the SOM of the i^{th} pool, R is the gas constant, T is the temperature in Kelvin, and A is the pre-exponential factor which equals to the theoretical decomposition rate of a C pool when the activation energy is zero. We can then use the Arrhenius equation to compare the changes in the reaction rate of a given C pool under two different temperatures (T_1 and T_2):

$$\frac{v_i(T_1)}{v_i(T_2)} = e^{\frac{E_i}{R}(\frac{1}{T_2}-\frac{1}{T_1})} \quad (4)$$

Because decomposition is a repertoire of enzymatic reactions, the decomposition rate of a single C pool can be also described by the Michaelis–Menten equation

$$\frac{dc}{dt} = -\frac{V_{\max,i} \times c}{k_{m,i} + c} \quad (5)$$

where $V_{max,\,i}$ is the maximum reaction rate of the i^{th} C age pool at a given temperature, c is the substrate concentration, and $k_{m,\,i}$ is the half-saturated constant which equals to the substrate concentration at half of the maximum reaction rate. To introduce the effect of temperature into the Michaelis-Menten kinetics (Davidson et al 2012), we substituted equation (3) into equation (5) to obtain a temperature-dependent equation as follows:

$$\frac{dc}{dt} = -\frac{A_{v\,\max,i}e^{-\frac{E_i}{RT}} \times c}{k_{m,i} + c} \quad (6)$$

By integrating equation (6), we obtained that the relationship between the remaining SOM content and decomposition time t at a given temperature T in the i^{th} homogeneous C pool meets the following equation:

$$k_{m,i} \ln \frac{c_0}{c_{t,i}} + c_0 - c_{t,i} = A_{v\max,i} e^{-\frac{E_i}{RT}} t \quad (7)$$

where c_0 is the initial SOM content of the i^{th} C pool, $c_{t,i}$ is the remaining SOM content after decomposition time t in the i_{th} C pool. Although elevated temperature may also increase $k_{m,i}$ for most enzymes (Davidson and Janssens 2006), the relative changes in $k_{m,i}$ can be negligible for a given C pool under the current warming scenarios (Davidson et al 2012).Considering a stable $k_{m,i}$ in decomposition of a given homogeneous C pool, we rearranged equation (7) by substituting the '$c_{t,i}$'into '$c_0 - c_{consumed,T,i}$' to obtain:

$$k_m \ln\left(\frac{c_0}{c_0 - c_{consumed,T,i}}\right) + c_{consumed,T,i} = A_{v\max} e^{-\frac{E_i}{RT}} t \quad (8)$$

where $c_{consumed,T,i}$ is the consumed SOM content after decomposition time t at a given temperature T in the i_{th} C pool. Let's define a function $f(x)$ as follows:

$$f(x) = -\frac{1}{c_0} \frac{\ln(1-x)}{x} \quad (9)$$

By taking a derivative of $f(x)$, we can see $f(x)$ increases with x ($0< x <1$). Suppose $x = \frac{c_{consumed,T,i}}{c_0}$, and when T_1 aT_2, as we have $c_{consumed,T_1,i} \geq c_{consumed,T_2,i}$, we can obtain:

$$f\left(\frac{C_{consumed,T_1,i}}{C_0}\right) \geq f\left(\frac{C_{consumed,T_2,i}}{C_0}\right) \quad (10)$$

Equation (10) can also be expressed by:

$$\frac{\ln\left(\frac{C_0}{C_0 - C_{consumed,T_1,i}}\right)}{\ln\left(\frac{C_0}{C_0 - C_{consumed,T_2,i}}\right)} \geq \frac{C_{consumed,T_1,i}}{C_{consumed,T_2,i}} \quad (11)$$

We then rearranged equation (8), and inserted it into inequality (11) to obtain a new inequality as follows, which shall be used to infer the warming effects on the decomposition of different C pools in soil samples in our study.

$$\frac{C_{consumed,T_1,i}}{C_{consumed,T_2,i}} \leq e^{\frac{E_i}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)} \quad (12)$$

Parameterization. SOM constitutes numerous C pools with a continuum of ages, and each C pool may be deemed homogeneous at steady sate. Previous studies have often considered SOM one-, or two- or three-C pools (Davidson and Janssens 2006, Knorr et al 2005, Schaedel et al 2013). Knorr *et al* found that the three-pool C model was sufficient and a better choice to interpret the data from the soil warming experiment in comparison with one- and two-pool C models (Knorr et al 2005). In our current study, we had precluded new C inputs into deep collars for nearly 10 years, and thus, there were rarely SOM with the age < 9 yr remaining in deep collars at the sampling time. Thus, we did not consider a C pool with the average age of less than 10 years (that is annual age C pool). We then restricted equation (2) to a four-pool C model in which we assumed the average ages of each C pool to be 50 yr, 500 yr, 5000 yr

and ≥ 50000 yr, respectively. These four C pools were used to refer to the pools of decadal, centennial, millennial and 'immortal' age SOM in soil. The decadal age C pool often constitutes the largest C pool, followed by centennial, millennia and immortal C pools, in soil (Davidson and Janssens 2006, Rumpel et al 2002), which shall be used to set a range of the proportions of each C age pools in our model. We also assumed that current warming scenarios did not affect the decomposition of the oldest C pool.

We used equation (1) to estimate the relative content of ^{14}C of the centennial, millennia and even older carbon pool in comparison with N_0. Atmospheric nuclear weapon testing almost doubled the amount of ^{14}C in the atmosphere in Northern Hemisphere in the 1960s (Currie 2004, Levin et al 1994), leading to the substantial amount of bomb radiocarbon incorporated into the decadal C pool. Because the decayed bomb radiocarbon during the 50 years was negligible compared to the total ^{14}C, we assumed that the relative amount of ^{14}C in the decadal C pool was 1.5 times of N_0. The activation energy for each C pool was estimated based on Knorr's model(Knorr et al 2005), with the activation energy for decadal, centennial and millennia C pools being 61.6, 67.1 and 72.7 kJ mol^{-1}, respectively. Notice that Knorr's model (Knorr et al 2005) used the turnover time instead of the average age to describe each C pool. For a homogeneous SOM pool at steady state as assumed in our model, the turnover time and average age of a SOM pool could be assumed equal (Torn et al 2009), so it is possible to estimate the activation energy of each age pool according to Knorr's model (Knorr et al 2005). We set the range of the initial total C content according to measurements of the current work and our previous published work (Niu et al 2010, Schaedel et al 2013). We then combined equation (2) and inequality (12) to estimate the proportions of each C pool as well as the consumed C in each pool under warming and control, and then calculated the

possible range of the ratio of consumed carbon in different temperature conditions (see results in the Supplementary Table 2). The four-C age pool model can be also reduced into two- or three-C age pool models. In addition, the centennial C age pool can also be set as the largest pool. Altering these did not change the results that warming increased decomposition of decadal to millennial SOM within deep collars in our Oklahoma soil plots. All calculations were performed using the MATLAB software (The MathWorks, Inc. USA), and the matlab codes are available upon request.

Section 3: the meta-analysis study

Data extraction. We conducted a meta-analysis study to examine the effects of warming on SOM decomposition across the soil profile in the field. We used Web of Science for a thorough search of relevant peer-reviewed articles published before November 2015. The search terms included either "warming" or "elevated temperature", and "soil", and "carbon" or "C" or "soil organic matter". We then selected published studies (He et al 2012, Heng et al 2011, Li et al 2010, Li et al 2011, Pan et al 2008, Rinnan et al 2008, Rui et al 2011, Sistla and Schimel 2013, Xu et al 2015, Xue et al 2015, Yu et al 2015, Zhang et al 2015, Zhou et al 2013) that included changes of soil organic C in different soil layers (≥ 2) under warming in the field. The definition of "topsoil" has been ascribed to be either 0-10 cm or 0-20 cm or 0-30 cm soil layers in the literature(Hiederer and Köchy 2011, Jobbagy and Jackson 2000). In this meta-analysis work, we placed the surface one into the "topsoil" category, while the following one the "subsoil" category, if a published study had only two soil layers. For those studies that had more than two soil layers, we considered the top soil layers (< 20 cm) the category of "topsoil", while the rest of

them the category of "subsoil". We have also slightly varied our choice of soil layers being the "topsoil" category, and found that it did not change the conclusion that warming had a similar effect on soil C changes in topsoil and subsoil. To determine the effects of temperature elevation on soil C changes, we grouped independent observations into two categories: temperature elevations ≥ 2 °C and temperature elevations < 2 °C. We also determined the influences of experimental duration on soil C changes by grouped observations into two categories: experiments that had been last ≥ 5 years and those < 5 years. We required that the observations included in the meta-analysis model meet the statistical assumption of independence (Hedges et al 1999). In the current study, measurements of soil C from different soil layers, precipitation levels, managements, plant species, and ecosystems within a single study were considered independent observations. For measurements on soil C through time at the same temperature level, the overall means across different measurement time points were computed and treated as a single independent observation. Data presented in the figures of published papers were extracted using Engauge Digitizer (https://digitizer.sourceforge.net).

Meta-analysis. We assessed data sets using meta-analysis methods described by Hedges et al. (1999) (Hedges et al 1999). The effect sizes of warming for each individual observation were estimated by transforming the response ratio (R) with the natural log: $\ln R = (\overline{X_W}/\overline{X_C})$, where $\overline{X_W}$ is the mean for warming treatment, $\overline{X_W}$ is the mean for ambient treatment. A mixed model of the meta-analytical software was employed to calculate the mean effect sizes and 95% confidence intervals (CIs) (Rosenberg et al 2000).The warming effect on a response variable was deemed statistically significant if

the 95% CI did not overlap with 0. Data obtained on the mean effect sizes ($\overline{lnR}$) and 95% CIs were unlogged and reported as percentage changes under warming (that is, 100 × (|R-1|)).

Section 4: the stable isotope probing microcosm experiment

Laboratory incubation. We designed a laboratory microcosm experiment to ascertain the effects of warming on microbial communities and decomposition. Each field plot was treated as the level of replication for the laboratory incubation study. Soil from the 20-25 cm soil layer collected from the deep collar of each plot was split into two 20-g dry mass equivalent aliquots, adjusted to moisture levels of 60% water holding capacity, and then placed in 165-ml jars. The uniformly (U) ^{13}C-labeled whole shoot biomass of *Triticum aestivum* (ground, ^{13}C abundance > 97%, IsoLife BV, Wageningen, The Netherlands) were added into one jar with a mass ratio of 150:1 (soil: U-^{13}C plant material) and then mixed thoroughly with soil. The other aliquot of soil without the addition of U-^{13}C plant material was also subjected to the same mixing process to control for soil disturbance. All soils were incubated at 25 °C in the dark for 9 weeks.

Stable isotope probing microcosms. DNA-based stable isotope probing (e.g. DNA-SIP) is a powerful tool to identify active microorganisms that incorporate specific substrates without the prerequisite of cultivation (Dumont and Murrell 2005, Friedrich 2006, Radajewski et al 2000). In this study, we used U-^{13}C plant material to label the members of microbial communities active in organic matter decomposition in soils collected from warming and control treatments. The plant material consisted of a wide range of organic compounds with a range of resistance to microbial attack (that is, from the labile

starch to recalcitrant lignin). Soils were incubated for 9 weeks, and this length of incubation time was reasonable because it allowed for detection of microbial utilization of both labile and recalcitrant organic compounds while minimizing as much as possible cross-feeding of isotope labelled intermediates of metabolism produced during the course of the experiment (Dumont and Murrell 2005, Radajewski et al 2000).

Gas sampling, measurements and calculations. During the SIP incubations, headspace gas samples were taken at days 0, 3, 7, 14, 21, 35, 49 and 63. At each sampling point, a 12-ml aliquot of headspace gas was extracted by a gas-tight syringe from each jar, and then injected into a 12-ml Exetainer evacuated vial (Labco International Inc., Houston, TX, USA). After each gas sampling, the jars without labeled plant residue addition were first vented for a while, and then followed by those with residue addition. Gas samples were analyzed at the stable isotope facility of University of California, Davis. The stable isotope ratio of ^{13}C in CO_2 was determined with a SerCon Cryoprep TGII trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

A single isotope, two-source mixing model was used to estimate the proportion of CO_2 respired from soil organic matter and ^{13}C-labelled plant material (Phillips and Gregg 2001). The linear mixing model was described by the following mass balance equations:

$$\overline{AP}_M = f_R \ \overline{AP}_R \ + f_S \ \overline{AP}_S \quad (13)$$

$$f_R + f_S = 100\% \quad (14)$$

where f_R and f_S represent the mean proportion of CO_2 respired from residue and soil, respectively, and $\overline{AP}_M$, $\overline{AP}_R$ and $\overline{AP}_S$ denote the mean isotopic signatures (*AP* or atom % ^{13}C, which is equal to the fractional abundance of ^{13}C times 100) for the mixture *M* (headspace CO_2), source *R* (residue) and source *S* (soil). To calculate the mean proportion of source *S* in the mixture, equation (2) was rearranged and inserted into equation (1) that was further rearranged to solve for f_S: the mean proportion of source soil in the mixture can be calculated as

$$f_S = \frac{\overline{AP}_M - \overline{AP}_R}{\overline{AP}_S - \overline{AP}_R} \quad (15)$$

The variance for f_S can be estimated by a first-order Taylor series approximation using partial derivatives as:

$$\sigma^2_{f_S} = \frac{1}{(\overline{AP}_S - \overline{AP}_R)^2} [\, \sigma^2_{\overline{AP}_M} + f_S^2\, \sigma^2_{\overline{AP}_S} + (1- f_S)^2 \sigma^2_{\overline{AP}_R} \,] \quad (16)$$

where $\sigma^2_{\overline{AP}_M}$, $\sigma^2_{\overline{AP}_S}$ and $\sigma^2_{\overline{AP}_R}$ represent variances of the mean isotopic signatures for the mixture *M*, and source *S* and *R*, respectively. Using the equation (4), we considered the variability of isotopic signatures of both sources and mixture in estimating the variance of f_S.

Soil DNA extraction. Upon the completion of laboratory incubations, soil samples were immediately stored in a freezer at -80 °C. Genomic DNA was extracted from 5-g aliquots of soil samples using the PowerMax Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). DNA quality was quantified by the ratios of 260/280 nm and 260/230 nm using the NanoDrop (ND-100

Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE, USA), and the DNA concentrations were determined using the Quant-iT PicoGreen dsDNA Assay Kit (Molecular Probes, Inc., Eugene, OR, USA) with FLUOstar Optima microplate reader (BMG Labtech Inc., Cary, NC, USA).

^{13}C-DNA separation. We separated ^{13}C-labelled DNA from unlabelled community DNA using the gradient fractionation method described by Neufeld and colleagues (Neufeld et al 2007). Briefly, 3 µg genomic DNA was mixed with gradient buffer (0.1 M tris, 0.1 M KCl AND 1 Mm EDTA) in 1-ml aliquots. The mixture of DNA/gradient buffer was then combined with CsCl solution (1.870 g ml^{-1}) and loaded in a polyallomer bell-top Quick-Seal centrifuge tube (Beckman Coulter Inc., Fullerton, CA). Centrifuge tubes were sealed and spun in a Vti-65 rotor in an Optima LE-80k ultracentrifuge (Beckman Coutler) at 44,100 rpm and 20 °C for 40 h. Gradients were fractionated using the Beckman Fraction Recovery System (Beckman Coutler) in connection with a syringe pump (model R-100E, Razel, Georgia, VT). The buoyant density of gradient fractions was determined using a refractometer (AR200, Reichert, Depew, NY). DNA from gradient fractions was precipitated with isopropanol for 2 h at 25 °C and then resuspended in sterilized nuclease-free H_2O.

16S rRNA gene sequencing

Amplification and pyrosequencing of targeted 16S gene. We amplified 16*S* rRNA genes of each sample by targeting the V4-V8 hypervariable regions of *Escherichia coli* 16*S* rRNA genes (positions 515-1391). The forward primer (NNNNNNNNTCGTGCCAGCMGCCGCGG) contained a unique 8-nt error-correcting barcode to tag each PCR product as designated by “NNNNNNNN”, a “TC” linker

sequence and the primer 515F. The reverse primer (NNNNNNNNCAGACGGGCGGTGTGTRCA) contained a unique 8-nt barcode 'NNNNNNNN", a "CA" linker sequence and the primer 1391R. Each biological sample (field replicate) was amplified with 3 tagged primers as 3 technical replicates. Each 100-µl PCR reaction consisted of 3 Units of AccuPrime Tag DNA polymerase (Invitrogen, Carlsband, CA), 10 µl supplied 10× PCR buffer, 10 µl supplied 50 mM $MgSO_4$, 8 µl 2.5 mM dNTP (Invitrogen), 0.5 µl BSA (New England BioLabs, Ipswich, MA), 0.2 µM forward and 0.2 µM reverse primers, and 5 ng template genomic DNA. The thermocycling conditions were as follows: initial denaturation at 94 °C for 2 min, 25 cycles of 94 °C for 30 s, 56 °C for 30 s, 68 °C for 45 s and a final extension at 68 °C for 10 min. To ameliorate PCR amplification biases and get enough PCR products for sequencing, three 100-µl reactions were performed for each technical sample. The PCR products of each technical sample were pooled and purified by agarose (1.0%) gel electrophoresis. The amplified PCR products were then recovered using the QIAquick Gel Extraction Kit (QIAGEN) and quantified using Quant-iT PicoGreen dsDNA Assay Kit with the FLUOstar OPTIMA (BMG LABTECH, Aylesbury, UK). All samples were mixed in an equimolar concentration prior to emulsion PCR for 454 sequencing. Pyrosequencing was carried out on a 454 Life Sciences Genome Sequencer FLX titanium instrument (454 Life Sciences, Brandford, CT).

Pyrosequencing data preprocessing. The pyrosequencing flowgrams were converted to sequence reads using Roche GS FLX software (version 2.5.3). Both forward and reverse sequencing reads were recovered with an average length of ~450 bp. Reads trimming and downstream analyses were performed using QIIME (Caporaso et al 2010b). Briefly, we trimmed reads at an accuracy threshold of 0.5% per

base error probability (Huse et al 2007) and then removed bar codes and primers and assigned sequences to samples by examining the 8-nt barcode. Reads were removed if they did not match exactly to a bar code and primer, were less than 200 nt and contained any unresolved nucleotides (N's). All sequences were aligned using the Python Nearest Alignment Space Termination (PyNAST) algorithm (Caporaso et al 2010a). Chimeric sequences were removed from the alignment using QIIME's ChimeraSlayer wrapper. Sequences were then assigned to a taxonomy using the RDP Classifier program with a confidence cutoff of 0.8 (Wang et al 2007). The operational taxonomic units (OTUs) were defined at 97% identity (Stackebrandt and Goebel 1994). The resulting OTU tables were used for further phylogenetic and statistical analyses.

GeoChip analysis

Labelling, hybridization and imaging. We labelled purified DNA with Cy-3 using random primers and Klenow fragment of DNA polymerase I. Labelled DNA was purified and dried at 45 °C for 45 min. We then rehydrated the dried DNA with 2.68 μl sample tracking control (NimbleGen) and incubated at 50°C for 5 min. In each labelled DNA sample, we added 7.32 μl hybridization buffer containing a mixture of 4 μl formamide, 2.5 μl 20X SSC, 0.1 μl SDS(10%), 0.28 μl Cy3-labelled alignment oligo, 0.24 μl Cy5-labelled CORS target and 0.2 μl universal standard. Samples were then incubated at 95 °C for 5 min and maintained at 45 °C. Finally, samples was loaded onto the microarray placed with an HX12 mixer (Roche NimbleGen, Madison, WI, USA) and hybridized at 42 °C with 40% formamide for 16 h on a hybridization station (MAUI, BioMicro Systems, Salt Lake City, UT, USA). After hybridization,

the microarray was scanned at full laser power and 100% photomultiplier tubes gain using a NimbleGen MS 200 Microarray Scanner (Roche NimbleGen).

Data preprocessing. We obtained the signal intensity of each probe by gridding scanned images using the gridding file containing GeoChip 4 probes and NimbleGen control probes (NimbleScan Software, Roche NimbleGen). Probe spots with coefficient of variance larger than 0.8 were not included in the downstream analysis. We removed spots that with a signal to noise ratio (SNR) less than 2.0, and then performed normalization of signal intensities for each probe using methods previously described (Tu et al 2014, Zhou et al 2012). All data were further analyzed using the GeoChip 4 pipeline at the website http://ieg.ou.edu/microarray/.

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