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Microbial metabolic response to winter warming stabilizes soil carbon

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Abstract

Current consensus on global climate change predicts warming trends with more pronounced temperature changes in winter than summer in the Northern Hemisphere at high latitudes. Moderate increases in soil temperature are generally related to faster rates of soil organic carbon (SOC) decomposition in Northern ecosystems, but there is evidence that SOC stocks have remained remarkably stable or even increased on the Tibetan Plateau under these conditions. This intriguing observation points to altered soil microbial mediation of carbon-cycling feedbacks in this region that might be related to seasonal warming. This study investigated the unexplained SOC stabilization observed on the Tibetan Plateau by quantifying microbial responses to experimental seasonal warming in a typical alpine meadow. Ecosystem respiration was reduced by 17%–38% under winter warming compared with year-round warming or no warming and coincided with decreased abundances of fungi and functional genes that control labile and stable organic carbon decomposition. Compared with year-round warming, winter warming slowed macroaggregate turnover rates by 1.6 times, increased fine intra-aggregate particulate organic matter content by 75%, and increased carbon stabilized in microaggregates within stable macroaggregates by 56%. Larger bacterial

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"necromass" (amino sugars) concentrations in soil under winter warming coincided with a 12% increase in carboxyl-C. These results indicate the enhanced physical preservation of SOC under winter warming and emphasize the role of soil microorganisms in aggregate life cycles. In summary, the divergent responses of SOC persistence in soils exposed to winter warming compared to year-round warming are explained by the slowing of microbial decomposition but increasing physical protection of microbially derived organic compounds. Consequently, the soil microbial response to winter warming on the Tibetan Plateau may cause negative feedbacks to global climate change and should be considered in Earth system models.

KEYWORDS
carbon degradation genes, microbial anabolism, microbial community, SOC stabilization, soil aggregate turnover, winter warming

1 | INTRODUCTION

Warming patterns caused by climate change are not equal across seasons (Piao et al., 2010; Suonan et al., 2017). Non-growing winter seasons experience larger temperature increases than summer growing seasons, especially in high-latitude, temperature-sensitive areas (Kreyling et al., 2019; Piao et al., 2010). This "asymmetric seasonal warming" (with the most significant temperature increase in winter) has been evident on the Tibetan Plateau over the last several decades (Li et al., 2010; Liu & Chen, 2000).

The Tibetan Plateau holds 30–40 Gt of soil organic carbon (SOC), which accounts for 2%–3% of the global SOC stock (Shang et al., 2016). Increased losses of SOC to the atmosphere as CO_2 across this vast area, caused by accelerated microbial decomposition driven by warming, could constitute an important positive feedback to further climate change. The average temperature increase in the region is approximately twice the average global warming rate (Chen et al., 2013) and winter temperatures have risen by 0.3°C per decade in the winter compared with 0.2°C during the summer (Li et al., 2010; Lu & Liu, 2010). Despite this tangible warming trend, there is evidence that topsoil SOC stocks have remained remarkably stable (Chen et al., 2020; Liu et al., 2018; Yang et al., 2009) or even increased (Ding et al., 2017). This suggests that the underlying processes controlling the capacity of Tibetan grasslands to maintain SOC stocks under global warming are not fully understood and require investigation.

The regulation of SOC turnover is increasingly conceptualized as part of a dynamic soil pore system, where the construction and destruction of pores control SOC availability to decomposing agents in the context of global change challenges (Franko & Schulz, 2020; Kravchenko et al., 2019). Long-term physical protection of SOC through aggregation afforded by the enmeshment of mineral particles by biological agents is widely recognized (Lehmann et al., 2017; Rillig & Mummey, 2006; Six et al., 2002; Tisdall & Oades, 1982). The association of microbial "necromass" (e.g., amino sugars from the residues of fungal and bacterial cell walls) and mineral-associated fractions (Glaser et al., 2006; Gunina et al., 2014; Zhang et al., 1998) represents a direct link between the stabilizing action of biota and microbial anabolism as a major contributor to the stable SOC pool (Kallenbach et al., 2016) that is incorporated into conceptual models and experiments (Gunina et al., 2014; Liang et al., 2017; Sokol et al., 2019). Nevertheless, despite the acknowledged stability of mineral-associated carbon, it is vulnerable to climate change.

Soil warming experiments, for example at the Harvard Forest (Pold et al., 2017), and an annual grassland in California (Liang & Balser, 2012; Rillig et al., 2002) revealed the depletion of mineral-associated organic carbon. This is because increased temperature shifts the sorption-desorption balance toward desorption (Conant et al., 2011) and decreases supplies of carbon as energy source and organic substances that bind soil particles together (Giovannini et al., 1988). However, the effects of soil warming in mesic regions may be different from that at high latitudes in (semi)arid alpine regions, where warming is often accompanied by severe summer droughts and frequent soil drying and rewetting, or freezing and thawing in winter and spring. Increasing intensity and duration of dry periods may progressively preserve SOC stocks (Borken & Matzner, 2009) through the formation of new and/or stronger organo-mineral interactions (Kaiser et al., 2015) and may stabilize aggregates rather than disrupt them (Denef et al., 2001; Najera et al., 2020). Continuous warming led to increase of mineral-associated carbon along with suppressed soil respiration in a semiarid grassland (Bai et al., 2020). Microorganisms have specific adaptations depending on the local climate; for example, fungi in arid grasslands are less sensitive to drought than those in temperate grasslands (Ochoa-Hueso et al., 2018), and this will influence the amount and stability of the microbial necromass (Ding et al., 2019; Liang & Balser, 2012). For instance, in cold, wet alpine ecosystems, plants may take up more nitrogen in the warmer growing season, while microbial nitrogen pools increase later in the year (Edwards & Jefferies, 2010; Jaeger III et al., 1999; Kuzyakov & Xu, 2013) potentially alleviating competition for nitrogen in the cooler non-growing season. Thus, the tendency toward SOC stabilization observed in the warming Tibetan Plateau may be a product of the effects of climate on soil physical, biogeochemical, and microbiological processes.

Manipulation experiments simulating climate change commonly apply uniform warming treatments (Jia et al., 2019; Ma et al., 2018),

but this does not allow the potential ecological impacts of the differential seasonal warming observed in high-latitude areas to be determined. To disentangle the processes caused by the observed temperature increases at different times of year on the Tibetan Plateau, a specific winter warming treatment was imposed in addition to a year-round warming treatment that would allow the identification and isolation of physical (soil aggregate size), chemical (labile and stable organic carbon pools), and biological (community structure and carbon degradation genes) processes specifically related to winter warming in this study. Specifically, we tested the hypothesis that SOC stabilization on the Tibetan Plateau is caused by the effect of winter warming on the carbon cycling functions of the soil microbial community.

2 | MATERIALS AND METHODS

2.1 | Experimental site and design

The study was conducted in an alpine meadow at the Damxung Grassland Station, located in the central, southern Tibetan Plateau (30°51′N and 90°05′E; 4333 m a.s.l.). The region has a semiarid continental climate (mean annual temperature 1.3°C, annual precipitation 477 mm) with 85% of precipitation occurring between June and August and the soil being frozen for 3 months, from November to January. The growing season is from late May to September. The shallow soil (0.3–0.5 m) is a Gelic Cambisol with 67% sand, 18% silt, 15% clay, and a pH of 6.95 (Shi et al., 2006). The vegetation cover is approximately 30%–50% *Kobresia pygmaea* C. B. Clarke var. *pygmaea*, Carex montis-everestii and *Stipa capillacea* Keng as dominant species (Table S1).

Field manipulations consisted of three warming treatments, including year-round warming (YW), winter-warming (WW) and no warming (control). The field experiment was established in 2010. In brief, 12 plots of three treatments with four replicates were laid out in a completely randomized block design. Each plot was 1 m × 1 m. Open-top chambers were used to generate artificially warmed conditions using the same methods as the International Tundra Experiment that was established to study the responses of alpine ecosystems to experimental warming (Birkemoe et al., 2016). The open-top chambers were constructed using 3 mm thick polycarbonate plastic (40 cm high ×140 cm diameter base with a 100-cm diameter aperture at the top). The open-top chambers were removed from the plots from late May to late September each year in the WW treatment plots. Air and soil (5 cm) temperature and soil moisture were recorded every 30 min (HOBO Weather Station, Onset Inc.).

2.2 | Ecosystem respiration

Ecosystem respiration (R_{eco}) was measured three times per month at 10-day intervals from June to September in 2015 using a LI-8100 (LI-COR Biosciences). PVC chambers (20 cm in diameter, 5 cm height) were inserted into the soil to a depth of 3 cm with plants intact. R_{eco} was measured from the linear rate of CO_2 accumulation within the sealed cylindrical headspaces. During the R_{eco} measurement process, PVC collars were covered by a removable lid that contained an opening with a CO_2 sensor. After closing the lid, CO_2 monitoring within the cylindrical headspace lasted for 1.5 min. Ecosystem CO_2 flux rates were calculated as a linear CO_2 increase using the 1-s readings during the 1.5-min closure time, with the initial 15 s mixing time after lid closure discarded in a LI-8100 file viewer application software (Zong et al., 2018).

2.3 | Soil sampling and analysis

Soil samples (0–20 cm depth) were taken using a soil corer (5 cm inner diameter) in June 2015. The soil samples were stored in airtight polypropylene bags and placed in a cool box at 4°C during transportation to the laboratory, where litter, roots, and gravel were carefully removed by hand, and the soil was divided into several subsamples for different analyses.

Subsamples of fresh soil for analysis of ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) concentration were stored at 4°C for no longer than 1 week before analysis using an autoanalyzer (TRAACS-2000, Bran+Luebbe) following 0.01 M KCL (1:10 w/v) extraction for 30 min. Subsamples for pH, SOC, and total nitrogen analyses were air dried at room temperature. Inorganic carbon was removed from the soil samples using an HCl-fumigation. The SOC and total nitrogen contents of bulk and aggregate size fractions were determined by combustion using a Vario EL III Elemental Analyzer (Elementar). Soil pH was measured with a pH meter after shaking the soil in deionized water (1:2.5 w/v) suspensions for 30 min. Subsamples for microbial community composition and functional gene (GeoChip) analysis were stored at −80°C.

2.4 | Soil aggregate size fractionation

Soil aggregate size separation was performed using a three-step fractionation method (Yan et al., 2012; Figure S1). Bulk soil was separated into three size fractions: macroaggregates (M, >0.25 mm), free microaggregates (Fm, 0.25–0.053 mm), and non-aggregated silt+clay (<0.053 mm). The macroaggregates were fractionated into coarse intra-aggregate particulate organic matter (Coarse iPOM, >0.25 mm), microaggregates-within-macroaggregates (mM), and silt+clay fractions using a microaggregate isolator (Six et al., 2000). The mM fraction was inverted in NaI solution (1.85 g cm^{-3}). The light fraction obtained by centrifugation was non-occluded intra-aggregate POM inside macroaggregates but outside microaggregates (Fine iPOM, 0.25–0.053 mm). The heavy fraction was dispersed by shaking in 0.5% sodium hexametaphosphate solution for 18 h with 10 glass beads. The dispersed samples were rinsed over

a 53-μm sieve to isolate POM inside mM (mM-POM) and silt+clay fractions inside mM (mM-silt+clay).

2.5 | ^{13}C-CPMAS NMR

The composition of SOC was investigated by determining the relative abundances of functional groups using solid-state ^{13}C cross polarization with magic-angle-spinning (CPMAS) NMR. Soil samples were treated with 10% HF-HCL solution to concentrate the organic matter and to remove paramagnetic minerals. ^{13}C-CPMAS NMR spectra were acquired using an AVANCE III 400 WB spectrometer (Bruker BioSpin AG) at 100 MHz for ^{13}C and 400 MHz for ^{1}H with a spinning rate of 8 kHz, an acquisition of 20 ms, a recycle time of 3 s and contact time of 2 ms. The spectra were integrated into four chemical shift regions corresponding to alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm), carboxyl-C (160–190 ppm) functional groups (Mathers & Xu, 2003; Skjemstad et al., 1994). The NMR spectra were processed with MestReNova 5.3.1 software (Mestrelab Research S. L. Santiago de Compostela, Spain). The raw ^{13}C CPMAS NMR spectra are provided in Figure S2.

2.6 | Microbial residue analysis

Glucosamine, galactosamine, and muramic acid were used as biomarkers for microbial residues ("necromass"). The compounds were extracted from air-dried soil samples ground to <0.25 mm and analyzed as their aldononitrile derivatives following the methods of Zhang and Amelung (1996) using myo-inositol as an internal standard. The derivatized compounds were separated on a gas chromatograph equipped with an HP-5 column (30 m × 0.25 mm ×0.25 μm) and quantified using a flame ionization detector (Agilent 6890A, Agilent Technologies). Total microbial residues were estimated as the sum of glucosamine, galactosamine, and muramic acid. To calculate bacterial residues, we assumed that all muramic acid was derived from bacteria:

$$\text{Bacterial residue C} = \text{muramic acid} \times 45, \quad (1)$$

where 45 is the conversion value to bacterial residue (Appuhn et al., 2006; van Groenigen et al., 2010).

Fungal residue was calculated by subtracting bacterial-derived glucosamine from total glucosamine, assuming a 1:2 molar ratio for muramic acid and glucosamine in bacterial cells (Engelking et al., 2007):

$$\text{Fungal residue C} = (\text{mmol glucosamine} - 2 \times \text{mmol muramic acid}) \times 179.2 \times 9, \quad (2)$$

where 179.2 is the molecular weight of glucosamine and 9 is the conversion factor of fungal glucosamine to fungal residue (Joergensen & Wichern, 2008; van Groenigen et al., 2010). Total microbial residue was estimated as the sum of fungal and bacterial residue.

2.7 | DNA extraction, quantitative PCR, and amplicon sequencing

DNA was extracted from 0.25 g of well-mixed soil using the PowerSoil Isolation kit (MoBio Laboratories) according to the manufacturer's instructions. The quality of the purified DNA was assessed based on the 260/280 nm and 260/230 nm absorbance ratios obtained, using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc.). The DNA was stored at −80°C until use.

Bacterial and fungal abundances were determined by qPCR using the Power SYBR Green PCR Master MIX (Biosystems) on an ABI 7500 Real-Time PCR System (Applied Biosystems). The following primer sets were used: 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) for bacterial 16S rRNA gene abundance and fungal ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and 2043R (5′-GCTGCGTTCTTCATCGATGC-3′). The following program was used for 16S rRNA gene amplification: initial denaturation at 98°C for 2 min, followed by 34 cycles of 98°C for 10 s, 56°C for 60 s, and 72°C for 30 s; for analysis of ITS1: initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 5 s, 58°C for 30 s, and 72°C for 1 min.

The V4-V5 region of 16S rRNA and the internal transcribed spacer (ITS1) region of the rRNA, were then amplified in order to construct bacterial and fungal community profiles, respectively, using high-throughput sequencing. The primers F515 (5′-GTGCCAGCMGCCGCGC-3′) and R907 (5′-CCGTCAATTCMTTTRAGTTT-3′) were used to target the V4-V5 region of 16S rRNA because of the broad coverage to capture as wide diversity as possible (Yusoff et al., 2013). We used the ITS1 region for fungi due to its ability to discriminate against plant (Adams et al., 2013; Li et al., 2020). The following thermal program was used for amplification of 16S rRNA gene: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and single extension at 72°C for 10 min, and end at 4°C. The PCR amplification of ITS1 rRNA gene was performed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and single extension at 72°C for 10 min, and end at 4°C. PCR amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel ExtractionKit (Axygen Biosciences) according to the manufacturer's instructions. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd.

The raw sequences were subjected to quality control with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50-bp sliding window, and the truncated reads shorter than 50 bp were discarded; reads

containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) primers were exactly matched allowing two-nucleotide mismatching. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE version 7.1 (Edgar et al., 2011), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier Bayesian algorithm (Wang et al., 2007) against the Silva database (https://www.arb-silva.de/) and the UNITE database (https://unite.ut.ee/) using confidence threshold of 0.7. We used a randomly selected subset of 24,183 and 33,197 sequences per sample for subsequent bacterial and fungal communities' analysis.

2.8 | Microbial functional communities and intensities of carbon decomposition genes

Microbial functional communities and intensities of carbon degradation genes were determined using DNA hybridization performed using GeoChip 5.0 according to He et al. (2007) and Yang et al. (2013). Briefly, DNA samples were labeled with Cy-5 fluorescent dye using a random priming method and purified with the QIA quick purification kit (Qiagen). The DNA was dried in a SpeedVac (ThermoSavant) at 45°C for 45 min. GeoChip hybridization was carried out at 42°C for 16 h on a MAUI® hybridization station (BioMicro). After hybridization, GeoChips were scanned by a NimbleGenMS200 scanner (Roche) at 633 nm, using a laser power and photomultiplier tube gain of 100% and 75%, respectively. Raw GeoChip data were analyzed using a data analysis pipeline as described previously (He et al., 2007; Yang et al., 2013). The data were logarithmically transformed, and then divided by the mean value of each slide. Spots that were flagged or with a signal-to-noise ratio less than 2.0 were considered poor in quality and removed from statistical analysis (He et al., 2007; Yang et al., 2013).

2.9 | Statistical analysis

Soil organic carbon chemistry (functional groups), physical fractions, and other variables were analyzed using a one-way ANOVA with randomized block design by using SAS (SAS, 1996). Differences were considered significant at $p < 0.05$, and a post hoc least significant difference test was carried out to compare differences between warming treatments. The normal distribution of residues of the model was tested by using "shapiro.test" function of the *stats* package in R v.3.2.1 (R Core Team, 2018). The relationships between microbial residues and SOC physical and chemical fractions were assessed with linear regression analyses using the "lm" function of the *vegan* package in R v.3.2.1 (R Core Team, 2018).

Matrices of the pairwise taxonomic distance between bacterial, fungal, and functional microbial communities (Bray–Curtis) were constructed in R v.3.2.1 with the *vegan* package (R Core Team, 2018). Non-metric multidimensional scaling (NMDS) was used to assess changes in the bacterial, fungal, and microbial functional communities. Adonis analyses were further performed to confirm significant changes in community structures under influence of warming and in any pair of samples. NMDS and statistical analyses were performed in R v.3.2.1 with the *vegan* package (R Core Team, 2018).

Linear discriminant analysis effect size (LEfSe) was performed to identify significant differences in bacterial and fungal taxa among treatments (Segata et al., 2011). The significant differences were analyzed by using the nonparametric factorial Kruskal–Wallis (KW) sumrank test and then estimate the effect size of each feature of each differentially abundant feature with linear discriminant analysis (http://huttenhower.sph.harvard.edu/lefse/). A significance alpha of 0.05 and an effect size threshold of 2 were used for all of the biomarkers evaluated.

To further investigate the relationship between R_{eco} with environmental variables and biogeochemical processes, partial least squares path modeling (PLS-PM) analysis was performed. PLS-PM is a data analysis method for variables that can be summarized by using latent variables, and the fact that linear relationships exist between latent variables (Sanchez, 2013). Models with different structures are evaluated using the goodness of fit (GOF) statistics, a measure of their overall predictive power, with GOF > 0.7 considered acceptable values (Sanchez, 2013). The models were constructed using the "inner plot" function of the R package (*plspm*; R Core Team, 2018). The environmental drivers selected in the model were the main predictors according to their contribution for variation based on Random Forest analysis (% of increase of MSE). Based on the importance and the maximum explanation for variations of R_{eco}, the environmental drivers selected in the model include soil water and temperature, carbon degradation genes, fungal residue, aboveground biomass, bacterial/fungal ratio, and aggregate size fractions (non-aggregated silt and clay, mM, mM-silt and clay; Figure S3).

3 | RESULTS

3.1 | Soil temperature and moisture

Warming increased average topsoil temperature and decreased soil moisture compared with the ambient control soil (no warming; $p < 0.05$; Figure S4). The average change in soil temperature for the duration of the experiment was +1.3°C under YW and +1.0°C under WW. The average change in soil moisture for the duration of the experiment was −4.7% under YW and −3.3% under WW. During the growing season (June–September), soil temperature under YW increased by 1.2°C and soil moisture decreased by 4.5%. During the non-growing season, incorporating "winter" (October–May), the soil temperature increased by 1.3°C under YW and 1.4°C under WW, and average soil moisture was decreased by 4.9% in both YW and WW treatments.

3.2 | CO_2 fluxes (R_{eco}) and SOC chemistry

The soils contained 1.8% organic carbon and 0.19% total nitrogen (C:N ratio 9.5) with no significant differences between warming treatments (Figure 1a,b). Soil mineral nitrogen concentrations [NO_3^-N + NH_4^+-N] under YW and WW were 60% and 25% less, respectively, than in the control treatment ($p < 0.05$; Figure 1c). Compared to the control treatment, warming decreased the average R_{eco} during the growing season (June–September) by 17% and 38% under YW and WW, respectively ($p < 0.05$; Figure 1d). The CP-MAS NMR spectra of soils from all warming treatments had similar proportions of major functional groups, with O-alkyl-C comprising about 40% of the total in all treatments. Phenol-C was proportionately less in the YW plots than the control. Carboxyl-C was proportionately larger under WW than YW or control ($p < 0.05$; Figure 1e).

3.3 | SOC in aggregate size fractions

The warming treatments changed the percentage of aggregate-size fractions and their SOC content. YW and WW increased the proportion and carbon contents of the non-aggregated silt+clay fraction compared with control ($p < 0.05$; Table 1). Soils under YW had the smallest proportion of fine iPOM and associated carbon ($p < 0.05$; Table 1). The proportion of mM increased by 22% under WW compared to the control ($p < 0.05$; Table 1). The SOC in mM under WW was 1.57 times more than under YW ($p < 0.05$; Table 1). The proportions of mM-silt+clay and associated SOC were greater under WW than under YW ($p < 0.05$; Table 1). Based on the ratio of fine iPOM to coarse iPOM within macroaggregates, macroaggregate turnover rates were 1.6 and 1.2 times less in WW compared to YW and control, respectively.

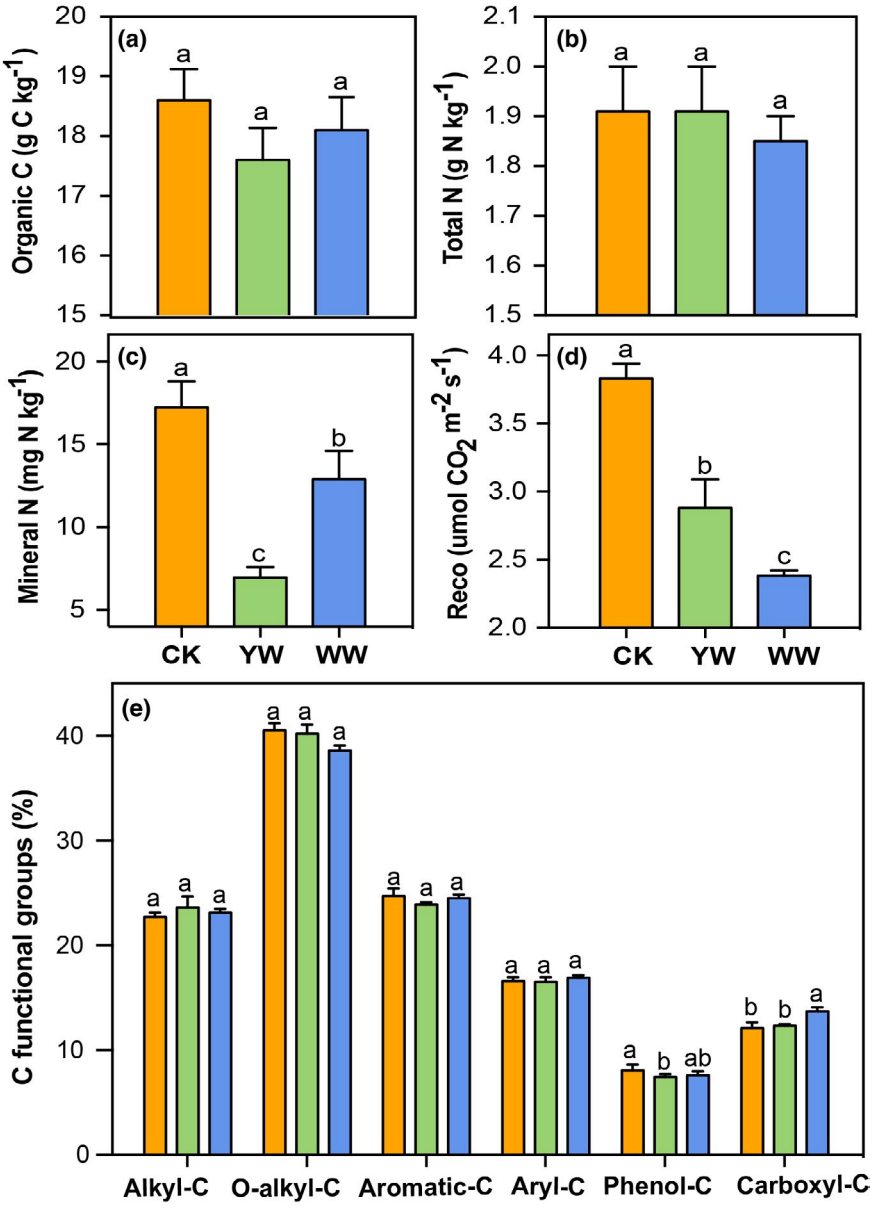

FIGURE 1 Effects of warming on soil chemistry (a–c), C flux (Reco) (d) and organic matter functional groups (e). Lowercase letters indicate significant differences between treatments; error bars indicate SEM ($n = 4$). CK, control; YW, year-round warming; WW, winter warming

TABLE 1 Effects of warming treatments on the proportion and soil organic carbon content of soil aggregate size fractions

	CK		YW		WW	
Soil aggregate size fraction	Size distribution (%)	C content (g C kg^{-1} soil)	Size distribution (%)	C content (g C kg^{-1} soil)	Size distribution (%)	C content (g C kg^{-1} soil)
Macroaggregates (M)	46.3 ± 2.56 a	7.45 ± 0.63 A	41.7 ± 2.47 a	6.36 ± 0.34 A	45.2 ± 4.51 a	6.65 ± 0.76 A
Free microaggregates (Fm)	40.2 ± 1.70 a	6.07 ± 0.28 A	37.4 ± 0.50 a	4.44 ± 0.07 C	38.9 ± 2.41 a	5.26 ± 0.31 B
Silt+clay	12.1 ± 0.60 b	3.22 ± 0.14 B	18.5 ± 0.95 a	4.62 ± 0.17 A	17.6 ± 0.39 a	5.10 ± 0.14 A
Coarse iPOM	10.2 ± 0.89 a	0.66 ± 0.11 A	8.30 ± 0.68 a	0.60 ± 0.07 A	9.10 ± 0.67 a	0.67 ± 0.07 A
mM	18.1 ± 0.84 ab	2.49 ± 0.13 AB	15.9 ± 1.39 b	2.00 ± 0.24 B	22.9 ± 2.45 a	3.13 ± 0.26 A
M-silt+clay	16.1 ± 1.45 ab	3.61 ± 0.37 A	12.1 ± 1.55 b	3.49 ± 0.11 A	16.6 ± 0.85 a	3.14 ± 0.34 A
Fine iPOM	0.29 ± 0.05 a	0.36 ± 0.07 AB	0.14 ± 0.01 b	0.24 ± 0.03 B	0.30 ± 0.08 a	0.42 ± 0.14 A
mM-POM	3.28 ± 0.22 a	0.26 ± 0.04 A	2.79 ± 0.15 a	0.29 ± 0.03 A	3.08 ± 0.34 a	0.33 ± 0.03 A
mM-silt+clay	14.8 ± 0.81 ab	1.86 ± 0.15 AB	13.4 ± 1.50 b	1.50 ± 0.20 B	19.9 ± 2.23 a	2.37 ± 0.29 A

Note: Values are means ± SEM (n = 4). Lowercase letters indicate significant differences in size distribution between treatments. Uppercase letters indicate significant differences in soil organic carbon contents between treatments.

Abbreviations: CK, control; Coarse iPOM, coarse intra-aggregate particulate organic matter (inside macroaggregates but outside microaggregates); fine iPOM, fine intra-aggregate particulate organic matter (inside macroaggregates but outside microaggregates); mM, microaggregates within macroaggregates; mM-POM, POM inside microaggregates within macroaggregates; mM-silt+clay, silt and clay-sized fractions inside mM; M-silt+clay, silt and clay-sized fractions inside macroaggregates; WW, winter warming; YW, year-round warming.

3.4 | Abundance and composition of soil bacteria and fungi

Fungal abundance was reduced under WW compared to the control ($p < 0.05$; Figure 2b). The abundance of bacteria did not change (Figure 2a). The bacterial/fungal ratio under WW was larger than YW ($p < 0.05$; Figure 2c). NMDS of the sequencing data followed by Adonis analysis indicated that warming altered bacterial and fungal community composition ($p < 0.05$; Figure S5). Further pairwise comparison indicated that the microbial community was changed in response to warming, and suggested that the difference was between the control and YW for the bacterial community, and control and YW, and YW and WW, for the fungal community ($p < 0.05$; Table S2).

The outcome of the LEfSe analysis indicated that the relative abundances of bacterial taxa from Acidobacteria (from phylum to class), Anaerolineae (from class to genus), and Longimicrobiaceae (from family to genus) were larger in the unwarmed control treatments relative to the warmed plots ($p < 0.05$; Figure 3). Further pairwise comparison revealed that the relative abundances of taxa from Acidobacteria, Fibrobacteres, and Gemmatimonadetes were smaller under YW treatment relative to control ($p < 0.05$; Figure S6). In contrast, abundant genera such as Microbacterium, Friedmanniella, Sciscionella, Flavobacterium, Pedobacter, Paenibacillus, Methylobacterium, Roseomonas, and Sphingomonas were enriched in YW plots ($p < 0.05$; Figure S6). Regarding soil fungal taxa, YW increased Cystobasidiomycetes abundance (from class to genus) as compared to WW and control treatments ($p < 0.05$; Figure 3). Mortierellomycota (from phyla to genus) were enriched under WW ($p < 0.05$; Figure 3).

3.5 | Microbial necromass carbon and linkage with soil aggregate turnover

Total residue carbon content was larger under WW than under YW ($p < 0.05$; Figure 4e). Fungal necromass content in all soils were more than twice greater than bacterial necromass under all warming treatments, and there were no differences in fungal necromass. The bacterial necromass was 30.8% larger under WW than under YW ($p < 0.05$; Figure 4d). Bacterial residue carbon increased with carbon associated with mM ($r = 0.53$; $p = 0.044$; Figure 5) and mM-silt+clay ($r = 0.51$; $p = 0.05$; Figure 5). Bacterial residue carbon had a positive relationship with total mineral-associated organic carbon ($r = 0.68$; $p = 0.008$; Figure 5), but a negative relationship with total POM ($r = 0.88$; $p = 0.001$; Figure 5).

3.6 | Microbial functional communities, carbon decomposition genes, and linkage with R_{eco}

Both WW and YW treatments changed soil functional microbial community structure relative to the control, as indicated by NMDS and Adonis tests (Figure 6a; Table S2; $p < 0.05$). Decreased abundances of total carbon degradation genes and specific genes associated with the decomposition of soluble storage compounds, that is starch, and structural compounds from plant cell walls (hemicellulose, cellulose, and lignin) and fungal cell walls (chitin) were observed in soils under WW ($p < 0.05$; Figure 6b).

Partial least squares path modeling of the water and temperature, aboveground biomass, edaphic (soil aggregate size fractions), and

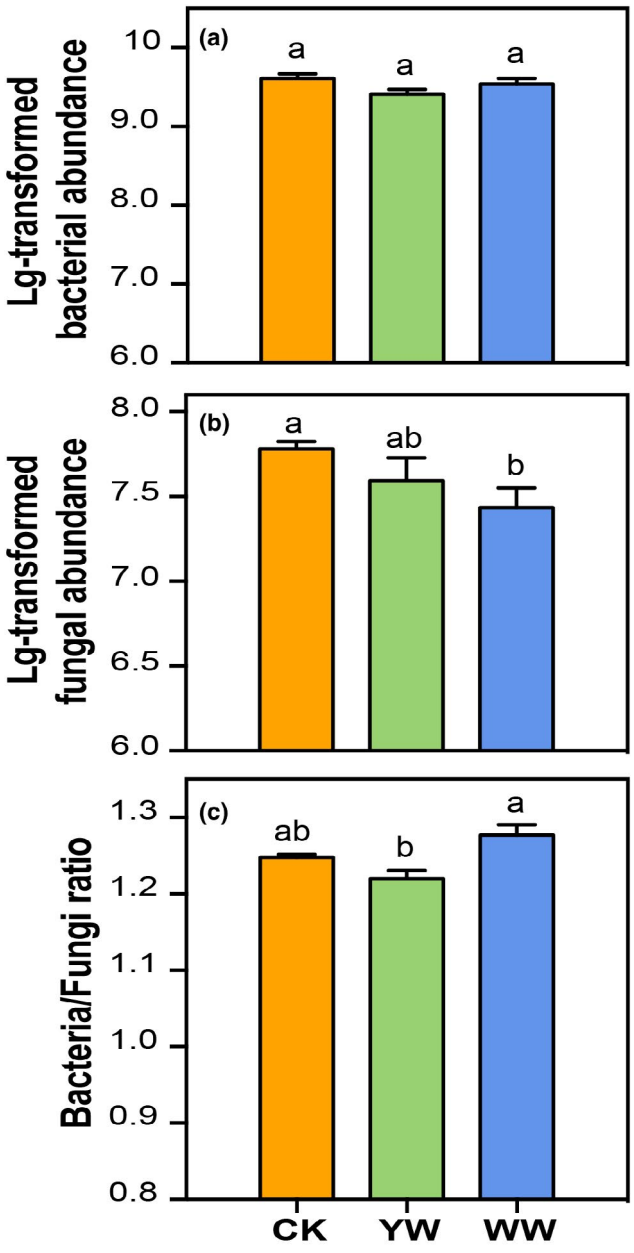

FIGURE 2 Effects of soil warming on (a) bacterial and (b) fungal abundances and (c) bacteria/fungi ratio. Lowercase letters indicate significant differences between treatments; error bars indicate SEM (n = 4). CK, control; YW, year-round warming; WW, winter warming

biological (fungal residues, bacterial/fungal ratio and carbon degradation genes) drivers of R_{eco} explained 96% of the R_{eco} variance, and provided the best fit of the data (GOF of 0.71; Figure 7). The soil water content and temperature showed the largest effect on R_{eco} variance via direct effect (path coefficient = −0.793). There were corresponding direct positive effects of carbon degradation genes on R_{eco} variance (path coefficient =0.687). The fungal residue and stable C fractions (silt+clay, mM, and mM-silt+clay) both resulted in a direct negative effect on R_{eco} variance (path coefficient = −0.426 and −0.240).

4 | DISCUSSION

The combination of physical (soil aggregate fractions), chemical (^{13}C NMR-CPMAS), and microbiological (R_{eco}, community composition, and carbon degradation genes) analysis allowed us to explore the effects of different seasonal warming (year-round or asymmetric winter warming) compared to ambient conditions on SOC dynamics and stabilization in this study. Using this multi-proxy approach, we determined divergent responses of the soil microbial community to differential seasonal warming that may explain the observed SOC stabilization in Tibetan steppe grasslands under different seasonal warming. Two major mechanisms emerged related to the effects of the treatments on microbial activity that affected the balance between SOC mineralization and stabilization in the alpine meadow topsoils (Figure 8): (1) increased physical protection of larger amounts of microbially derived SOC in soil stable aggregates and (2) decreased microbial decomposition caused by changes in the microbial community composition and the expression of carbon decomposition genes.

4.1 | Winter warming decreased microbial decomposition via changes in microbial function and community structure

R_{eco} in both year-round and asymmetric winter warming treatments was substantially depressed, and to a much greater extent under the winter warming treatment (Figure 1). This was related to the combination of increased soil temperature and decreased soil moisture over several years (Figure 7). We did not separate autotrophic and heterotrophic respiration as individual contributors to R_{eco} in this study, so cannot partition R_{eco} between plants and soil microorganisms. However, we determined that changing aboveground plant biomass in response to warming was not a strong determining factor of R_{eco} at the experimental site using PLS-PM analysis (Figure 7), which agrees with our previous report (Zong et al., 2018), and there was no difference in the much larger amounts of root biomass in response to warming (Table S4). A positive correlation between temperature increase and rates of soil respiration after warming is widely reported (Melillo et al., 2002; Rustad et al., 2001). In general, warming initially promotes microbial growth which stimulates decomposition, driving significant CO_2 losses in the early stages of many soil warming experiments (e.g., Garcia-Palacios et al., 2015; Karhu et al., 2014; Melillo et al., 2002; Romero-Olivares et al., 2017; Rustad et al., 2001). Soil moisture is an important factor regulating large-scale spatial patterns of R_{eco} in Tibetan alpine grasslands (Geng et al., 2012) and inconsistent effects of experimental warming on soil CO_2 flux that are related to interannual fluctuations in rainfall have been reported in semiarid steppe grasslands (Liu et al., 2009). Persistent moisture deficits in warmed soils may subsequently reduce soil CO_2 efflux and microbial biomass content (Liu et al., 2009; Quan et al., 2019). Thus, the reduction in R_{eco} with warming may be due

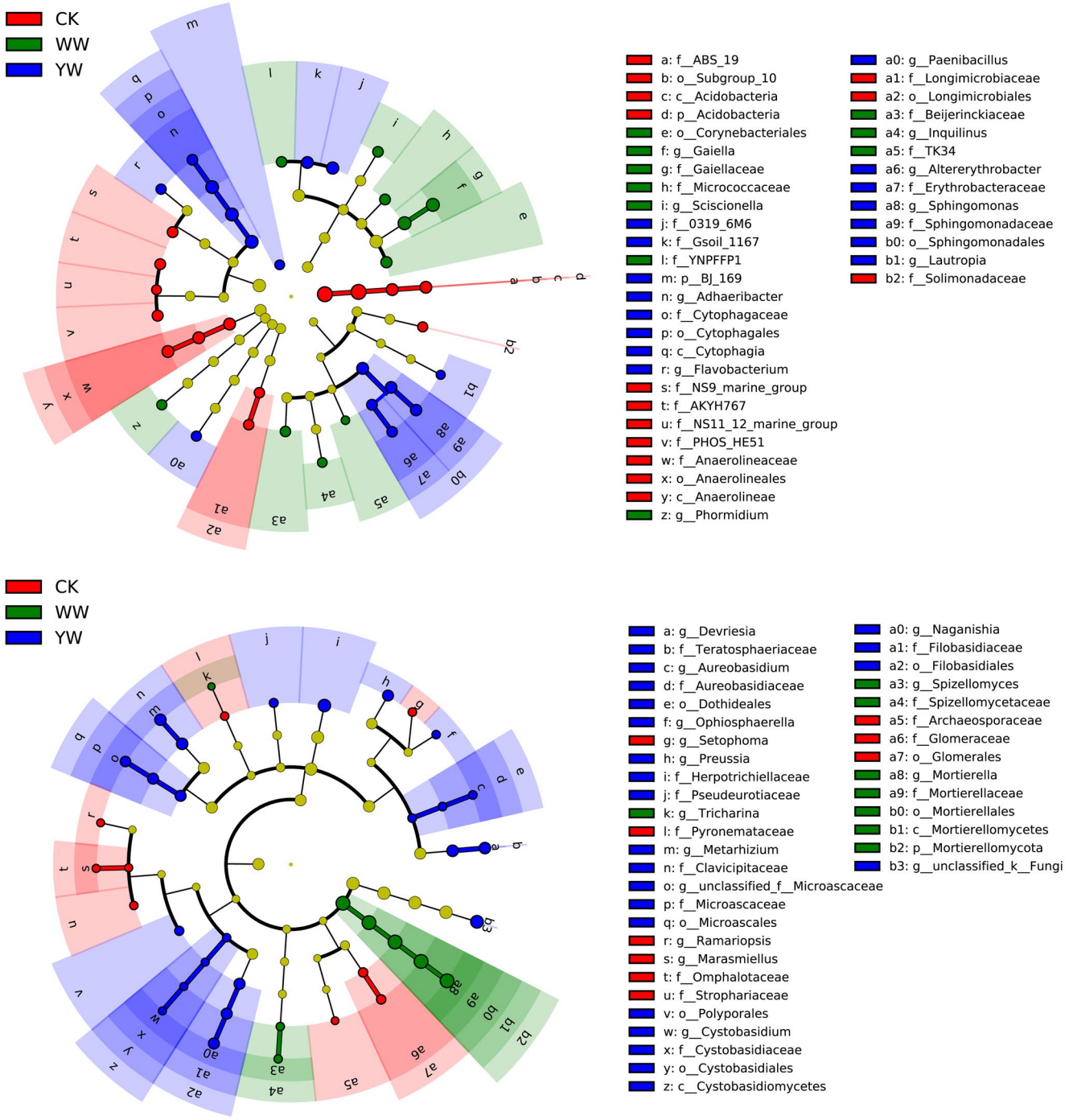

FIGURE 3 LEfSe analysis of significantly abundant taxa of bacteria (a) and fungi (b) in soil microbial communities under different treatments. In the evolutionary branch diagram, the circle radiating from inside to outside represents the classification level from the phylum to the genus. Each small circle at a different classification level represents a classification at that level. Differently colored nodes indicate the taxa that are significantly enriched in the corresponding group (red indicating control, green indicating winter warming, and blue indicating year-round warming), and yellow nodes indicate taxa that have no significant difference. The threshold on the logarithmic LDA score was 2.0

to a consequence of inhibition of microbial respiration or substrate supply as well as suppression of physiological activity by warming-induced soil water deficits (Niu et al., 2008).

Physiological stress and constraints on enzyme production due to reduced water availability (Tiemann & Billings, 2011) are likely to have contributed strongly to the decrease of carbon decomposition genes in warmed soils (Figure 6) leading to the subsequent positive direct effect on R_{eco} (Figure 7). This effect coincided with an increase in the intensities of stress response genes (Figure S7), suggesting a potential trade-off between stress tolerance versus

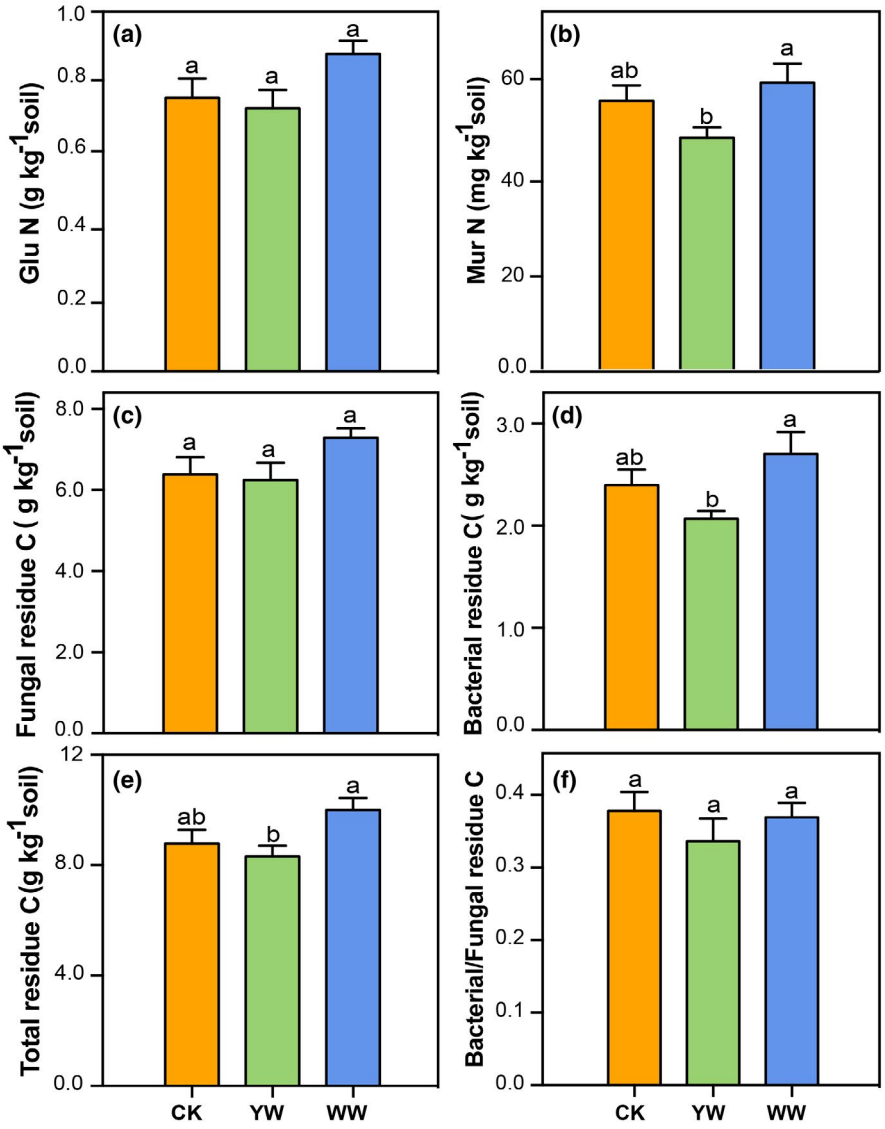

FIGURE 4 Effect of soil warming on amino sugar abundances (a, b), bacterial, fungal and total residue-derived carbon (c, d, e) and the ratio of bacterial to fungal residue C (f). Lowercase letters indicate significant differences among treatments; error bars indicate SEM (n = 4). CK, control; YW, year-round warming; WW: winter warming

resource acquisition as described by Malik et al. (2020). In support of this explanation, the abundance of functional genes involved in both labile and recalcitrant carbon cycling was significantly elevated by experimental increases in precipitation (Li et al., 2017). The larger reduction of carbon decomposition genes in the winter warming treatment (Figure 6) indicated that winter warming also inhibited the production of carbon decomposition enzymes through a decrease of soil fungal abundance and altered fungal community (Table S3). Decreases in genes for labile and recalcitrant carbon decomposition in response to soil drying caused by winter warming could indicate a negative feedback mechanism that reduces SOC losses. Warming also decreased the relative abundance of carbon decomposition genes in transplanted intact soil monoliths moved between sites at different altitudes in Tibet (Yue et al., 2015). The opposite was observed in irrigated arid soils where, for example, Li et al. (2017) determined an increase in the abundance of functional genes involved in both labile and recalcitrant carbon cycling after warming. This also contrasts with results from temperate prairies, where microbial genes for complex organic compound degradation were increased by warming (Feng et al., 2017). Our study therefore provides further evidence that changes in the water cycle caused by climate change have the potential to moderate warming-induced carbon losses and could even reverse the expected trend on the Tibetan Plateau. This implies a more prominent role for soil moisture in regulating SOC processing in the future than it has played in the past by enhancing the feedback between soil warming and global climate change (Werner et al., 2020).

4.2 | Stronger physical protection of SOC under winter warming

Soil organic carbon associated with minerals is considered as one of the most fundamental long-term, stable carbon pools (Cotrufo et al., 2015; Six & Paustian, 2014). The mM fraction is suggested as a robust indicator for management-induced SOC changes over decadal time scales (Six & Paustian, 2014). In this study, as with bulk SOC, there was no difference between warming treatments in coarse

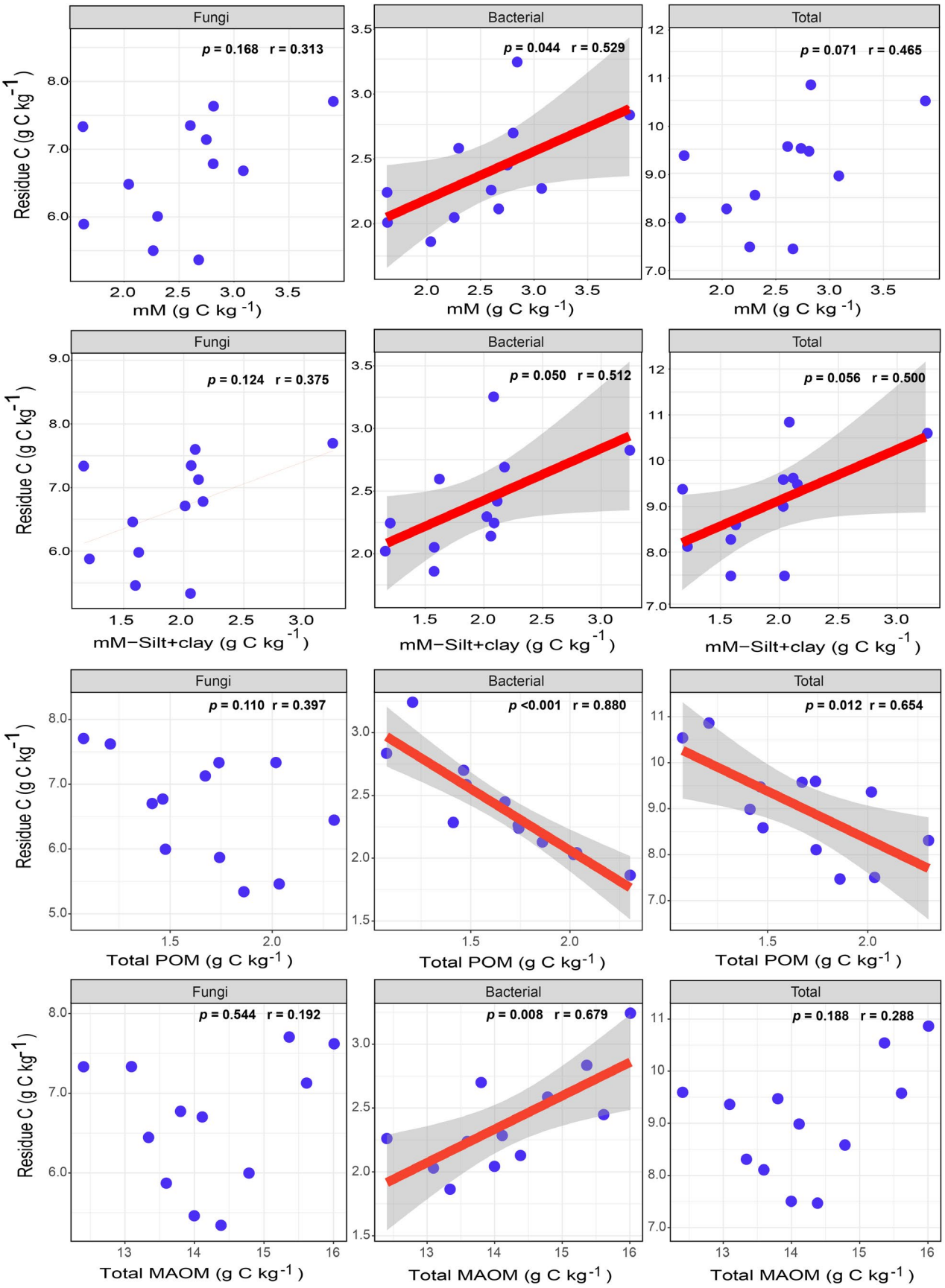

FIGURE 5 Relationships between bacterial and fungal residue carbon and soil aggregate fractions. The red fitted lines are from linear regression. Only significant fitted lines are displayed on the graphs. Shaded areas show 95% confidence interval of the fit

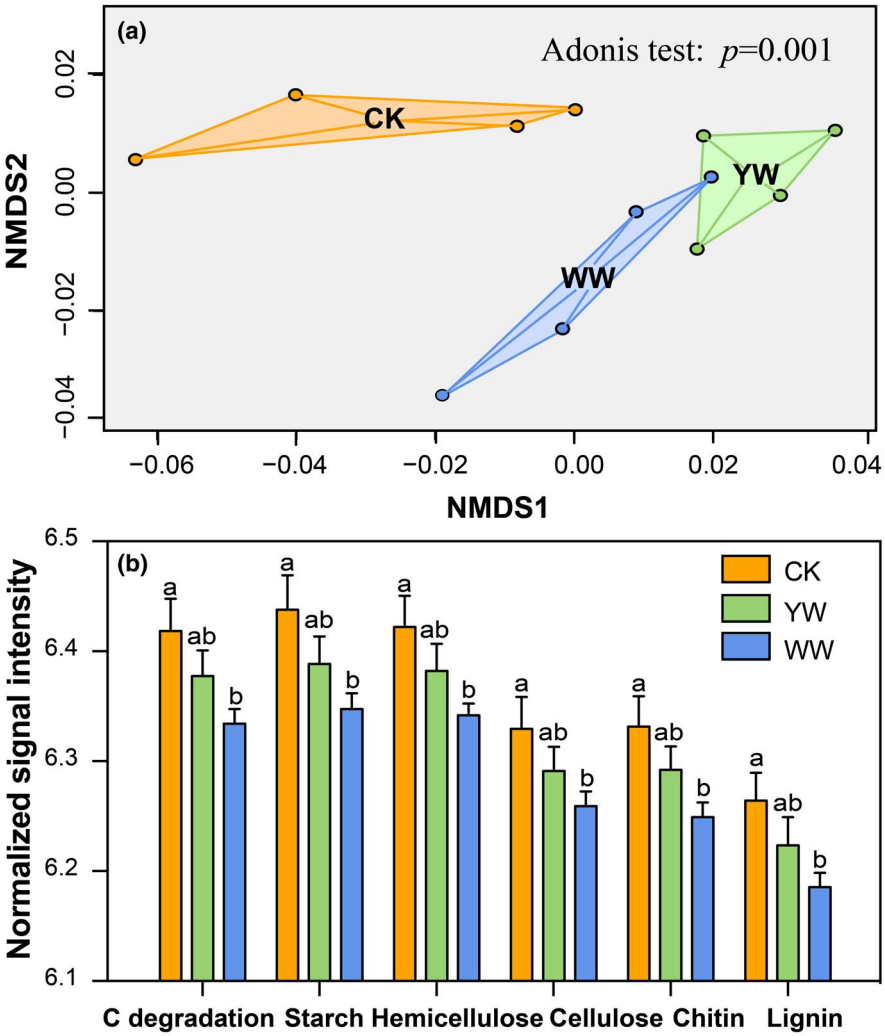

FIGURE 6 Effects of soil warming on functional microbial communities (a) and carbon degradation genes (b). Lowercase letters indicate significant differences between treatments; error bars indicate SEM (n = 4). CK, control; YW, year-round warming; WW, winter warming. The functional microbial communities were ordinated by non-metric multidimensional scaling (NMDS) analysis. The stress value for the plot was <0.06, which indicates that these data were well represented by the two-dimensional representation

iPOM-C concentrations or in the size distribution of macroaggregates (Table 1). However, macroaggregate turnover rates (based on the ratio of fine iPOM to coarse iPOM within macroaggregates; Six et al., 2000) were up to 1.6 times less in the winter warming treatments compared to the control. So, in contrast to year-round warming which decreased soil aggregate stability, winter warming had slower macroaggregate turnover and increased carbon contents in smaller aggregate size fractions, indicating increased carbon stabilization via organo-mineral interactions and microaggregation (Table 1).

The enmeshing action of fungal mycelia in the formation of soil aggregates is widely recognized (Lehmann et al., 2017; Rillig & Mummey, 2006). Rillig et al. (2002) reported that warming of an annual grassland in the United States decreased the water stability of soil aggregate and abundance of arbuscular mycorrhizal (AMF) hyphae (*Glomus* sp.). In this study, AMF were not measured directly, but a reduction in phylum Glomeromycotina abundance was determined under year-round warming (Figure S6) indicating a mechanism for the reduction in macroaggregate stability under this treatment. The changes in the AMF fungi response to warming and their relationship to the soil aggregation represent a key focus for further study.

Microbial residue ("necromass") is an important constituent of stable SOC due to its tendency for sorption to mineral surfaces or protection within stable aggregates (Kuzyakov & Mason-Jones, 2018; Liang et al., 2017; Miltner et al., 2012), described as the "entombing effect" by Liang et al. (2017). Soil water content was up to ~5% less in the warmed treatments in this study (Figure S4). Soil drying can promote the formation of new and/or stronger organo-mineral interactions with microbial by-products (e.g., amino sugars; Bai et al., 2020; Kaiser et al., 2015). The importance of the contribution of microbial necromass to stable SOC pools has recently been reassessed (Liang et al., 2019). This was confirmed in our study where up to half of total SOC was derived from amino sugars, and ~60% were of fungal origin (Figure 5). Fungi tend to be more resistant to drought, while bacteria are more resilient (de Vries et al., 2012) and can return close to control conditions upon rewetting (Canarini et al., 2017). In our study, soils under winter warming contained more of the mM and mM-silt+clay fraction than year-round warmed soils. Bacterial residue carbon content was positively related to the carbon content of the mM and mM-silt+clay fractions (Figure 5) revealing the enhanced physical preservation of SOC under winter warming.

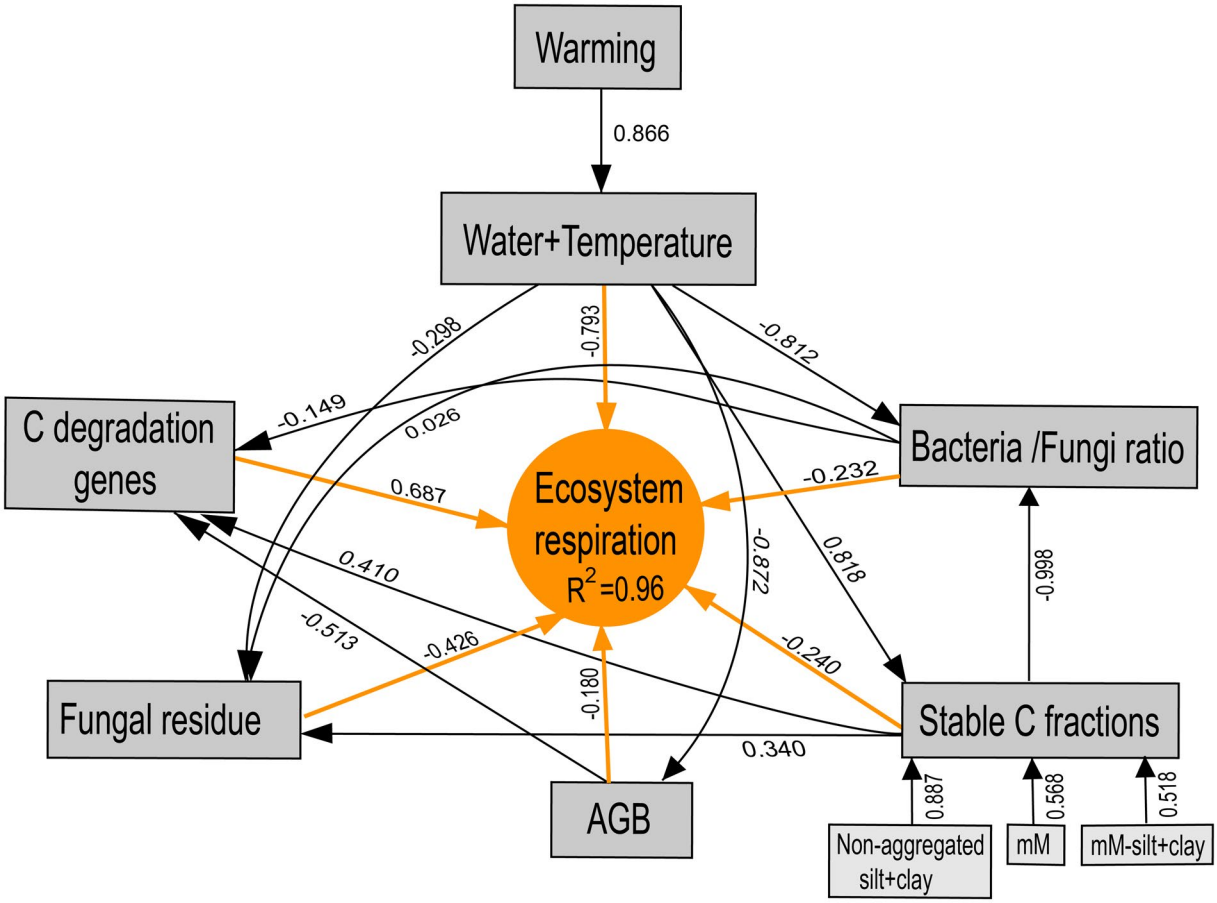

FIGURE 7 Partial least squares path analysis for ecosystem respiration, showing the relationship between selected biogeochemical processes, microbial community composition, and functional gene abundance. The orange arrows are the direct effect of environmental and microbial variables on ecosystem respiration, and the black arrows indicate the indirect path. The numbers listed within arrows are the standardized path coefficient. AGB, aboveground biomass; mM, microaggregates within macroaggregates

4.3 | Effects of winter warming on soil chemistry

Soil organic carbon dynamics associated with specific organic compounds can be sensitive indicators of effects of warming because the temperature sensitivity of recalcitrant organic matter increases with warming over time as the labile pool is depleted more rapidly by increasing enzyme activity (Hartley & Ineson, 2008). Like Jia et al. (2019), we determined a decrease in plant-derived phenols from lignin and suberin (identified herein as the phenol-C functional group by ^{13}C NMR) in topsoils after 5 years of continuous warming experiment (YW treatment). A reduction in lignin content of plant inputs was previously identified in warmed temperate grasslands suggesting a potential mechanism (Henry et al., 2005; Sanaullah et al., 2014). An increased abundance of carboxyl-C was unique to winter-warmed soils (Figure 1). Although carboxylic (fatty) acids are susceptible to rapid oxidation by soil microorganisms, they have the potential for sequestration within the soil by complexing with clay minerals and other forms of organic matter (Bull et al., 2000) suggesting a route to enhanced physical protection (Figure 5; Table 1) of plant and microbial derived carbon. The analysis of lipid biomarkers has the potential to assign source of the carboxyl-C in future analysis of warmed soils to complement the analysis of amino sugars as indicators of soil microbial contributions to SOC.

Unlike winter warming, year-round warming caused a decline in total microbial residues and bacterial residue carbon (Figure 4). This observation is similar to the observed reduction of total microbial residues in soils in an annual grassland after 9 years of continuous warming in the United States (Liang & Balser, 2012). Inorganic nitrogen content was significantly less under year-round warming (Figure 1c) indicating nitrogen limitation due to climate change in Tibetan alpine meadow ecosystems previously recognized by Ding et al. (2019) that may be exacerbated by drought stress. A recent large-scale field investigation along a 1000-km transect in the Tibetan Plateau revealed that gross rates of N mineralization were positively associated with soil moisture (Mao et al., 2020). Microbial residues are comparatively rich in nitrogen (Cotrufo et al., 2015) and fungal residues (derived from chitin) are considered to be more persistent than bacterial residues (derived from peptidoglycan; Ding et al., 2019; Six et al., 2006) making the latter more susceptible to nitrogen mining during the growing

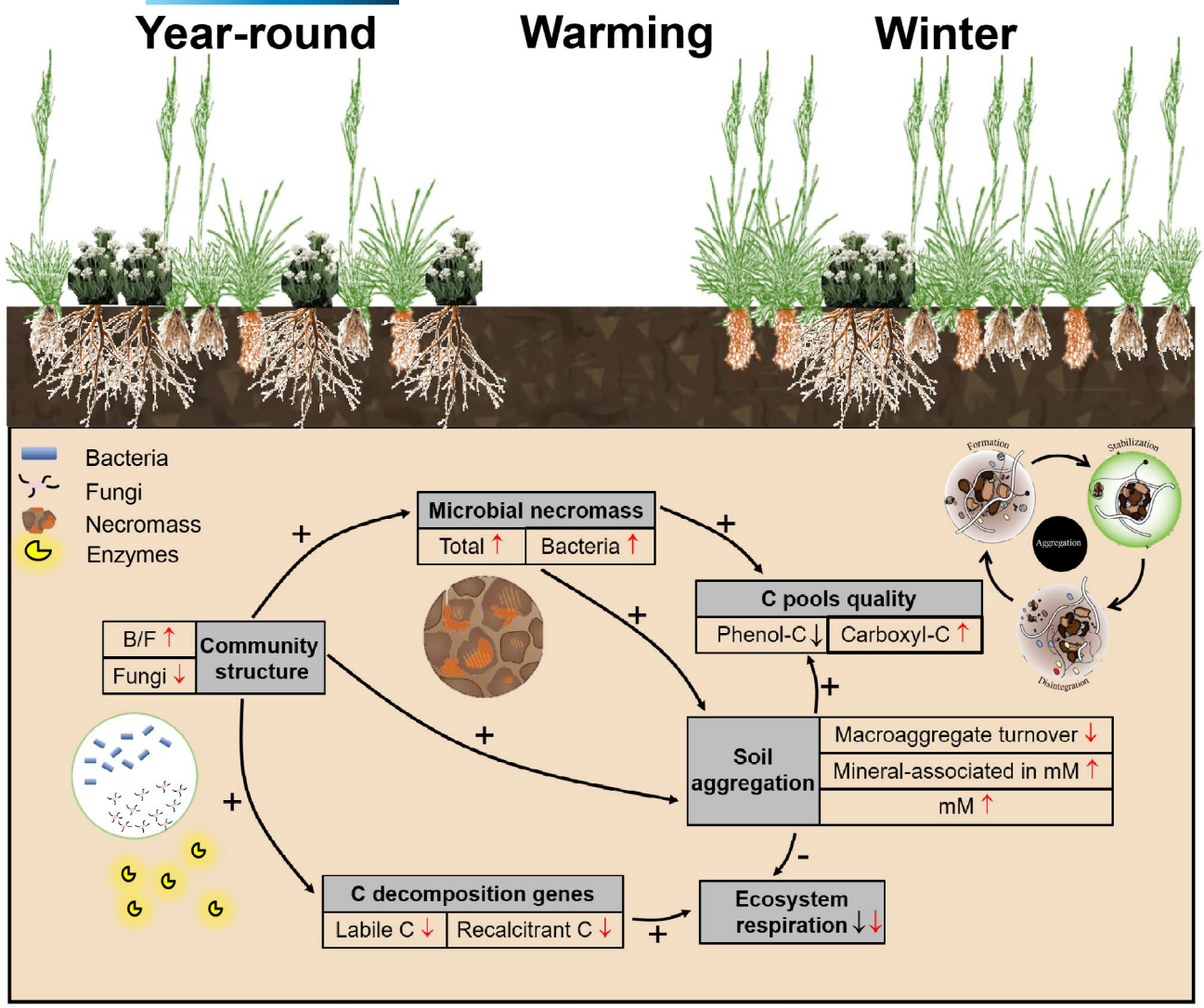

FIGURE 8 A conceptual diagram illustrating the increasing or decreasing impact of warming on aggregate turnover, microbial community, necromass, and the activity of microbial functional genes that control carbon cycling after year-round or winter warming as compared to control. Red and black arrows indicate significant trends in winter and year-round warmed soil as compared to control. The (+) indicate positive interactions between aggregate life cycle, microbial community, necromass, and the potential activity of microbial functional carbon genes in winter warming condition

season. Jaeger III et al. (1999) reported the transfer of microbial nitrogen to plant-available pools was observed in the early growing season in an alpine ecosystem. Most organic nitrogen is associated with clay-sized particles where physicochemical interactions may limit the accessibility of N-containing compounds (Jilling et al., 2018). From an alternative perspective, the greater aggregate turnover under year-round warming may result in potential destabilization of bacterial residues from clay-silt fractions (Table 1; Figure 5) due to microbial exploitation of organic nitrogen sources under conditions of low mineral-N availability (Figure 1).

5 | CONCLUSIONS

With respect to other grassland ecosystems, alpine grassland systems account for more than 40% of the Tibetan Plateau area and are considered to be particularly sensitive to climatic change. Compared to year-round warming, winter warming reduced macroaggregate turnover, increased bacterial residues (necromass), and increased mM, an aggregate size fraction that is closely related to the long-term physical protection of SOC of microbial origin. Winter warming decreased the activity of carbon decomposition genes to a greater extent than year-round warming.

The deliberate isolation of winter warming from year-round warming in our field experiment allowed us to disentangle its effects in situ, to reveal potential mechanisms for observed SOC stabilization as a climate-C feedback. The fact that this was different from the year-round warming treatments suggests that summer can weaken the effects of winter warming, and further study is required to determine the relative importance of winter versus summer warming in controlling SOC protection mechanisms. In this context, it would be valuable to establish experiments in which year-round

warming treatments also reflect the actual and predicted differences in the magnitudes of climate warming in different seasons, with greater absolute warming in winter on the Tibetan Plateau.

Overall, our findings demonstrate that understanding the effects of warming at different times of year on SOM protection mechanisms is critically important for predicting whether SOM will be lost or gained in response to climate change.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Adams, R. I., Miletto, M., Taylor, J. W., & Bruns, T. D. (2013). Dispersal in microbes: Fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *The ISME Journal*, *7*(7), 1262–1273. https://doi.org/10.1038/ismej.2013.28

Appuhn, A., Scheller, E., & Joergensen, R. G. (2006). Relationships between microbial indices in roots and silt loam soils forming a gradient in soil organic matter. *Soil Biology and Biochemistry*, *38*, 2557–2564. https://doi.org/10.1016/j.soilbio.2006.03.011

Bai, T., Wang, P., Hall, S. J., Wang, F., Ye, C., Li, Z., Li, S., Zhou, L., Qiu, Y., Guo, J., Guo, H., Wang, Y. I., & Hu, S. (2020). Interactive global change factors mitigate soil aggregation and carbon change in a semi-arid grassland. *Global Change Biology*, *26*, 5320–5332. https://doi.org/10.1111/gcb.15220

Birkemoe, T., Bergmann, S., Hasle, T. E., & Klanderud, Y. (2016). Experimental warming increases herbivory by leaf-chewing insects in an alpine plant community. *Ecology and Evolution*, *6*(19), 6955–6962. https://doi.org/10.1002/ece3.2398

Borken, W., & Matzner, E. (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology*, *15*, 808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x

Bull, I. D., van Bergen, P. F., Nott, C. J., Poulton, P. R., & Evershed, R. P. (2000). Organic geochemical studies of soils from the Rothamsted classical experiments – V. The fate of lipids in different long-term experiments. *Organic Geochemistry*, *31*, 389–408. https://doi.org/10.1016/S0146-6380(00)00008-5

Canarini, A., Kiær, L. P., & Dijkstra, F. A. (2017). Soil carbon loss regulated by drought intensity and available substrate: A meta-analysis. *Soil Biology and Biochemistry*, *112*, 90–99. https://doi.org/10.1016/j.soilbio.2017.04.020

Chen, H., Zhu, Q., Peng, C., Wu, N., Wang, Y., Fang, X., Gao, Y., Zhu, D., Yang, G., Tian, J., Kang, X., Piao, S., Ouyang, H., Xiang, W., Luo, Z., Jiang, H., Song, X., Zhang, Y., Yu, G., … Wu, J. (2013). The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. *Global Change Biology*, *19*, 2940–2955.

Chen, Y., Feng, J., Yuan, X., & Biao, Z. (2020). Effects of warming on carbon and nitrogen cycling in alpine grassland ecosystems in the Tibetan Plateau: A meta-analysis. *Geoderma*, *370*, 114363. https://doi.org/10.1016/j.geoderma.2020.114363

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., Hopkins, F. M., Hyvönen, R., Kirschbaum, M. U. F., Lavallee, J. M., Leifeld, J., Parton, W. J., Megan Steinweg, J., Wallenstein, M. D., Martin Wetterstedt, J. Å., & Bradford, M. A. (2011). Temperature and soil organic matter decomposition rates-synthesis of current knowledge and a way forward. *Global Change Biology*, *17*, 3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, A. J. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, *8*, 776–779. https://doi.org/10.1038/NGEO2520

de Vries, F. T., Manning, P., Tallowin, J. R. B., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., Hobbs, P. J., Quirk, H., Shipley, B., Cornelissen, J. H. C., Kattge, J., & Bardgett, R. D. (2012). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters*, *15*, 1230–1239. https://doi.org/10.1111/j.1461-0248.2012.01844.x

Denef, K., Six, J., Bossuyt, H., Frey, S. D., Elliott, E. T., Merckx, R., & Paustian, K. (2001). Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biology and Biochemistry*, *33*, 1599–2161. https://doi.org/10.1016/S0038-0717(01)00076-1

Ding, J., Chen, L., Ji, C., Hugelius, G., Li, Y., Liu, L. I., Qin, S., Zhang, B., Yang, G., Li, F., Fang, K., Chen, Y., Peng, Y., Zhao, X., He, H., Smith, P., Fang, J., & Yang, Y. (2017). Decadal soil carbon accumulation across Tibetan permafrost regions. *Nature Geoscience*, *10*, 420. https://doi.org/10.1038/NGEO2945

Ding, X. L., Chen, S. Y., Zhang, B., Liang, C., He, H. B., & Horwath, W. R. (2019). Warming increases microbial residue contribution to soil organic carbon in an alpine meadow. *Soil Biology and Biochemistry*, *135*, 13–19. https://doi.org/10.1016/j.soilbio.2019.04.004

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Edwards, K. A., & Jefferies, R. L. (2010). Nitrogen uptake by *Carex aquatilis* during the winter–spring transition in a low Arctic wet meadow. *Journal of Ecology*, *98*, 737–744. https://doi.org/10.1111/j.1365-2745.2010.01675.x

Engelking, B., Flessa, H., & Joergensen, R. G. (2007). Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. *Soil Biology and Biochemistry*, *39*, 2111–2118. https://doi.org/10.1016/j.soilbio.2007.03.020

Feng, W., Liang, J., Hale, L. E., Jung, C. G., Chen, J. I., Zhou, J., Xu, M., Yuan, M., Wu, L., Bracho, R., Pegoraro, E., Schuur, E. A. G., & Luo, Y. (2017). Enhanced decomposition of stable soil organic carbon and microbial catabolic potentials by long-term field warming. *Global Change Biology*, *23*, 4765–4776. https://doi.org/10.1111/gcb.13755

Franko, U., & Schulz, E. (2020). Carbon accumulation in a bare fallow Chernozem soil with high carbon input rates. *European Journal of Soil Science*, *72*(1), 265–273. https://doi.org/10.1111/ejss.12937

Garcia-Palacios, P., Vandegehuchte, M. L., Shaw, E. A., Dam, M., Post, K. H., Ramirez, K. S., Sylvain, Z. A., de Tomasel, C. M., & Wall, D. H.

(2015). Are there links between responses of soil microbes and ecosystem functioning to elevated CO_2, N deposition and warming? A global perspective. *Global Change Biology*, *21*, 1590–1600. https://doi.org/10.1111/gcb.12788

Geng, Y., Wang, Y., Yang, K., Wang, S., Zeng, H., Baumann, F., Kuehn, P., Scholten, T., & He, J.-S. (2012). Soil respiration in Tibetan alpine grasslands: Belowground biomass and soil moisture, but not soil temperature, best explain the large-scale patterns. *PLoS ONE*, *7*, e34968.

Giovannini, G., Lucchesi, S., & Giachetti, M. (1988). Effect of heating on some physical and chemical-parameters-parameters related to soil aggregation and erodibility. *Soil Science*, *146*, 255–261. https://doi.org/10.1007/BF03403404

Glaser, B., Millar, N., & Blum, H. (2006). Sequestration and turnover of bacterial- and fungal-derived carbon in a temperate grassland soil under long-term elevated atmospheric pCO(2). *Global Change Biology*, *12*, 1521–1531. https://doi.org/10.1111/j.1365-2486.2006.01186.x

Gunina, A., Dippold, M. A., Glaser, B., & Kuzyakov, Y. (2014). Fate of low molecular weight organic substances in an arable soil: From microbial uptake to utilisation and stabilisation. *Soil Biology and Biochemistry*, *77*, 304–313. https://doi.org/10.1016/j.soilbio.2014.06.029

Hartley, I. P., & Ineson, P. (2008). Substrate quality and the temperature sensitivity of soil organic matter decomposition. *Soil Biology and Biochemistry*, *40*, 1567–1574. https://doi.org/10.1016/j.soilbio.2008.01.007

He, Z., Gentry, T. J., Schadt, C. W., Wu, L., Liebich, J., Chong, S. C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., & Zhou, J. (2007). GeoChip: A comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *The ISME Journal*, *1*, 67–77.

Henry, H. A., Cleland, E. E., Field, C. B., & Vitousek, P. M. (2005). Interactive effects of elevated CO_2, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia*, *142*, 465–473. https://doi.org/10.1007/s00442-004-1713-1

Jaeger III, C. H., Monson, R. K., Fisk, M. C., & Schmidt, S. K. (1999). Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. *Ecology*, *80*, 1883–1891. https://doi.org/10.1890/0012-9658(1999)080[1883:SPONBP]2.0.CO;2

Jia, J., Cao, Z., Liu, C., Zhang, Z., Lin, L. I., Wang, Y., Haghipour, N., Wacker, L., Bao, H., Dittmar, T., Simpson, M. J., Yang, H., Crowther, T. W., Eglinton, T. I., He, J.-S., & Feng, X. (2019). Climate warming alters subsoil but not topsoil carbon dynamics in alpine grassland. *Global Change Biology*, *25*, 4383–4393. https://doi.org/10.1111/gcb.14823

Jilling, A., Keiluweit, M., Contosta, A. R., Frey, S., Schimel, J., Schnecker, J., Smith, R. G., Tiemann, L., & Grandy, A. S. (2018). Minerals in the rhizosphere: Overlooked mediators of soil nitrogen availability to plants and microbes. *Biogeochemistry*, *139*(2), 103–122.

Joergensen, R. G., & Wichern, F. (2008). Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry*, *40*, 2977–2991. https://doi.org/10.1016/j.soilbio.2008.08.017

Kaiser, M., Kleber, M., & Berhe, A. A. (2015). How air-drying and rewetting modify soil organic matter characteristics: An assessment to improve data interpretation and inference. *Soil Biology and Biochemistry*, *80*, 324–340. https://doi.org/10.1016/j.soilbio.2014.10.018

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communication*, *7*, 13630. https://doi.org/10.1038/ncomms13630

Karhu, K., Auffret, M. D., Dungait, J. A. J., Hopkins, D. W., Prosser, J. I., Singh, B. K., Subke, J.-A., Wookey, P. A., Ågren, G. I., Sebastià, M.-T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A. T., Salinas, N., & Hartley, I. P. (2014). Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature*, *513*, 81–84. https://doi.org/10.1038/nature13604

Kravchenko, A., Guber, A., Razavi, B. S., Koestel, J., Quigley, M. Y., Robertson, G. P., & Kuzyakov, Y. (2019). Microbial spatial footprint as a driver of soil carbon stabilization. *Nature Communications*, *10*, 3121. https://doi.org/10.1038/s41467-019-11057-4

Kreyling, J., Grant, K., Hammerl, V., Arfin-Khan, M. A. S., Malyshev, A. V., Peñuelas, J., Pritsch, K., Sardans, J., Schloter, M., Schuerings, J., Jentsch, A., & Beierkuhnlein, C. (2019). Winter warming is ecologically more relevant than summer warming in a cool-temperate grassland. *Scientific Report*, *9*, 14632. https://doi.org/10.1038/s41598-019-51221-w

Kuzyakov, Y., & Mason-Jones, K. (2018). Viruses in soil: Nano-scale undead drivers of microbial life, biogeochemical turnover and ecosystem functions. *Soil Biology and Biochemistry*, *127*, 305–317. https://doi.org/10.1016/j.soilbio.2018.09.032

Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, *198*, 656–669. https://doi.org/10.1111/nph.12235

Lehmann, A., Zheng, W. S., & Rillig, M. C. (2017). Soil biota contributions to soil aggregation. *Nature Ecology & Evolution*, *1*, 1828–1835. https://doi.org/10.1038/s41559-017-0344-y

Li, H., Yang, S., Xu, Z., Yan, Q., Li, X., van Nostrand, J. D., He, Z., Yao, F., Han, X., Zhou, J., Deng, Y. E., & Jiang, Y. (2017). Responses of soil microbial functional genes to global changes are indirectly influenced by aboveground plant biomass variation. *Soil Biology and Biochemistry*, *104*, 18–29. https://doi.org/10.1016/j.soilbio.2016.10.009

Li, L., Yang, S., Wang, Z., Zhu, X., & Tang, H. (2010). Evidence of warming and wetting climate over the Qinghai-Tibet Plateau. *Arctic Antarctic and Alpine Research*, *42*, 449–457. https://doi.org/10.1657/1938-4246-42.4.449

Li, S., Deng, Y. E., Wang, Z., Zhang, Z., Kong, X., Zhou, W., Yi, Y., & Qu, Y. (2020). Exploring the accuracy of amplicon-based internal transcribed spacer markers for a fungal community. *Molecular Ecology Resources*, *20*(1), 170–184. https://doi.org/10.1111/1755-0998.13097

Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, *25*, 3578–3590. https://doi.org/10.1111/gcb.14781

Liang, C., & Balser, T. C. (2012). Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool. *Nature Communication*, *3*, 1222. https://doi.org/10.1038/ncomms2224

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*, 17105. https://doi.org/10.1038/nmicrobiol.2017.105

Liu, H., Mi, Z., Lin, L. I., Wang, Y., Zhang, Z., Zhang, F., Wang, H., Liu, L., Zhu, B., Cao, G., Zhao, X., Sanders, N. J., Classen, A. T., Reich, P. B., & He, J.-S. (2018). Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 4051–4056. https://doi.org/10.1073/pnas.1700299114

Liu, W., Zhang, Z., & Wan, S. (2009). Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biology*, *15*, 184–195. https://doi.org/10.1111/j.1365-2486.2008.01728.x

Liu, X., & Chen, B. (2000). Climatic warming in the Tibetan Plateau during recent decades. *International Journal of Climatology*, *20*(14), 1729–1742. https://doi.org/10.1002/1097-0088(20001130)20:14<1729:AID-JOC556>3.0.CO;2-Y

Lu, H., & Liu, G. (2010). Trends in temperature and precipitation on the Tibetan Plateau, 1961–2005. *Climate Research*, *43*(3), 179–190. https://doi.org/10.3354/cr00909

Ma, Z., Zhao, W., Liu, M., & Liu, Q. (2018). Responses of soil respiration and its components to experimental warming in an alpine scrub

ecosystem on the eastern Qinghai-Tibet Plateau. *Science of The Total Environment*, *643*, 1427–1435. https://doi.org/10.1016/j.scitotenv.2018.06.243

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal*, *14*(1), 1–9. https://doi.org/10.1038/s41396-019-0510-0

Mao, C., Kou, D., Chen, L., Qin, S., Zhang, D., Peng, Y., & Yang, Y. (2020). Permafrost nitrogen status and its determinants on the Tibetan Plateau. *Global Change Biology*, *26*, 5290–5302. https://doi.org/10.1111/gcb.15205

Mathers, N. J., & Xu, Z. (2003). Solid-state 13C NMR spectroscopy: Characterization of soil organic matter under two contrasting residue management regimes in a 2-year-old pine plantation of subtropical Australia. *Geoderma*, *114*(1–2), 19–31. https://doi.org/10.1016/S0016-7061(02)00339-7

Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., Catricala, C., Magill, A., Ahrens, T., & Morrisseau, S. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, *298*, 2173–2176. https://doi.org/10.1126/science.1074153

Miltner, A., Bombach, P., Schmidt-Bruecken, B., & Kaestner, M. (2012). SOM genesis: Microbial biomass as a significant source. *Biogeochemistry*, *111*, 41–55. https://doi.org/10.1007/s10533-011-9658-z

Najera, F., Dippold, M., Boy, J., Seguel, O., Köster, M., Stock, S., Merino, C., Kuzyakov, Y., & Matus, F. (2020). Effect of drying/rewetting cycles on carbon sequestration in forest soil. *Biology and Fertility of Soils*, *56*, 893–905. https://doi.org/10.1007/s00374-020-01469-6

Niu, S., Wu, M., Han, Y., Xia, J., Li, L., & Wan, S. (2008). Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. *New Phytologist*, *177*, 209–219. https://doi.org/10.1111/j.1469-8137.2007.02237.x

Ochoa-Hueso, R., Collins, S. L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W. T., Sinsabaugh, R. L., Smith, M. D., Knapp, A. K., & Power, S. A. (2018). Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. *Global Change Biology*, *24*, 2818–2827. https://doi.org/10.1111/gcb.14113

Piao, S., Ciais, P., Huang, Y., Shen, Z., Peng, S., Li, J., Zhou, L., Liu, H., Ma, Y., Ding, Y., Friedlingstein, P., Liu, C., Tan, K., Yu, Y., Zhang, T., & Fang, J. (2010). The impacts of climate change on water resources and agriculture in China. *Nature*, *467*, 43–51. https://doi.org/10.1038/nature09364

Pold, G., Grandy, A. S., Melillo, J. M., & DeAngelis, K. M. (2017). Changes in substrate availability drive carbon cycle response to chronic warming. *Soil Biology and Biochemistry*, *110*, 68–78. https://doi.org/10.1016/j.soilbio.2017.03.002

Quan, Q., Tian, D., Luo, Y., Zhang, F., Crowther, T. W., Zhu, K., Chen, H. Y. H., Zhou, Q., & Niu, S. (2019). Water scaling of ecosystem carbon cycle feedback to climate warming. *Science Advances*, *5*, 1131. https://doi.org/10.1126/sciadv.aav1131

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*, 41–53. https://doi.org/10.2307/3694482

Rillig, M. C., Wright, S. F., Shaw, M. R., & Field, C. B. (2002). Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos*, *97*, 52–58. https://doi.org/10.1034/j.1600-0706.2002.970105.x

Romero-Olivares, A. L., Allison, S. D., & Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: A meta-analysis of field studies. *Soil Biology and Biochemistry*, *107*, 32–40. https://doi.org/10.1016/j.soilbio.2016.12.026

Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., & Gurevitch, J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*, 543–562. https://doi.org/10.1007/s004420000544

Sanaullah, M., Chabbi, A., Girardin, C., Durand, J. L., Poirier, M., & Rumpel, C. (2014). Effects of drought and elevated temperature on biochemical composition of forage plants and their impact on carbon storage in grassland soil. *Plant and Soil*, *374*(1–2), 767–778. https://doi.org/10.1007/s11104-013-1890-y

Sanchez, G. (2013). *PLS path modeling with R*. Trowchez Editions, 383 pp. http://creativecommons.org/licenses/by-nc-sa/3.0/

SAS. (1996). *Statistical analysis system. SAS system for windows version 6.12*. SAS Institute Inc.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), 1–18. https://doi.org/10.1186/gb-2011-12-6-r60

Shang, W., Wu, X. D., Zhao, L., Yue, G. Y., Zhao, Y. H., Qiao, Y. P., & Li, Y. Q. (2016). Seasonal variations in labile soil organic matter fractions in permafrost soils with different vegetation types in the central Qinghai-Tibet Plateau. *CATENA*, *137*, 670–678. https://doi.org/10.1016/j.catena.2015.07.012

Shi, P. L., Sun, X. M., Xu, L. L., Zhang, X. Z., He, Y. T., Zhang, D. Q., & Yu, G. R. (2006). Net ecosystem CO_2 exchange and controlling factors in a steppe – Kobresia meadow on the Tibetan Plateau. *Science in China Series D-Earth Sciences*, *49*, 207–218. https://doi.org/10.1007/s11430-006-8207-4

Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*, *241*, 155–176. https://doi.org/10.1023/A:1016125726789

Six, J., Elliott, E. T., & Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, *32*, 2099–2103. https://doi.org/10.1016/s0038-0717(00)00179-6

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal*, *70*, 555–569. https://doi.org/10.2136/sssaj2004.0347

Six, J., & Paustian, K. (2014). Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry*, *68*, A4–A9. https://doi.org/10.1016/j.soilbio.2013.06.014

Skjemstad, J. O., Clarke, P., Taylor, J. A., Oades, J. M., & Newman, R. H. (1994). The removal of magnetic-materials from surface soils – A solid state 13C CP/MAS NMR study. *Australian Journal of Soil Research*, *32*, 1215–1229.

Sokol, N. W., Sanderman, J., & Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology*, *25*, 12–24. https://doi.org/10.1111/gcb.14482

Suonan, J., Classen, A. T., Zhang, Z., & He, J. S. (2017). Asymmetric winter warming advanced plant phenology to a greater extent than symmetric warming in an alpine meadow. *Functional Ecology*, *3*, 2147–2156. https://doi.org/10.1111/1365-2435.12909

Tiemann, L. K., & Billings, S. A. (2011). Changes in variability of soil moisture alter microbial community C and N resource use. *Soil Biology and Biochemistry*, *43*(9), 1837–1847. https://doi.org/10.1016/j.soilbio.2011.04.020

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *European Journal of Soil Science*, *33*, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

van Groenigen, K.-J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., & Jones, M. B. (2010). Abundance, production

and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biology and Biochemistry*, *42*, 48–55. https://doi.org/10.1016/j.soilbio.2009.09.023

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Werner, W. J., Sanderman, J., & Melillo, J. M. (2020). Decreased soil organic matter in a long-term soil warming experiment lowers soil water holding capacity and affects soil thermal and hydrological buffering. *Journal of Geophysical Research: Biogeosciences*, *125*(4), https://doi.org/10.1029/2019JG005158

Yan, Y., Tian, J., Fan, M., Zhang, F., Li, X., Christie, P., Chen, H., Lee, J., Kuzyakov, Y., & Six, J. (2012). Soil organic carbon and total nitrogen in intensively managed arable soils. *Agriculture, Ecosystems & Environment*, *150*, 102–110. https://doi.org/10.1016/j.agee.2012.01.024

Yang, Y., Fang, J., Smith, P., Tang, Y., Chen, A., Ji, C., Hu, H., Rao, S., Tan, K., & He, J.-S. (2009). Changes in topsoil carbon stock in the Tibetan grasslands between the 1980s and 2004. *Global Change Biology*, *15*, 2723–2729. https://doi.org/10.1111/j.1365-2486.2009.01924.x

Yang, Y., Wu, L., Lin, Q., Yuan, M., Xu, D., Yu, H., Hu, Y., Duan, J., Li, X., He, Z., Xue, K., van Nostrand, J., Wang, S., & Zhou, J. (2013). Responses of the functional structure of soil microbial community to livestock grazing in the Tibetan alpine grassland. *Global Change Biology*, *19*, 637–648. https://doi.org/10.1111/gcb.12065

Yue, H., Wang, M., Wang, S., Gilbert, J. A., Sun, X., Wu, L., Lin, Q., Hu, Y., Li, X., He, Z., Zhou, J., & Yang, Y. (2015). The microbe-mediated mechanisms affecting topsoil carbon stock in Tibetan grasslands. *ISME Journal*, *9*, 2012–2020. https://doi.org/10.1038/ismej.2015.19

Yusoff, M. Z. M., Hu, A. Y., Feng, C. J., Maeda, T., Shirai, Y., Hassan, M. A., & Yu, C.-P. (2013). Influence of pretreated activated sludge for electricity generation in microbial fuel cell application. *Bioresource Technology*, *145*, 90–96. https://doi.org/10.1016/j.biortech.2013.03.003

Zhang, X. D., & Amelung, W. (1996). Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. *Soil Biology and Biochemistry*, *28*, 1201–1206. https://doi.org/10.1016/0038-0717(96)00117-4

Zhang, X., Amelung, W., Yuan, Y., & Zech, W. (1998). Amino sugar signature of particle-size fractions in soils of the native prairie as affected by climate. *Soil Science*, *163*, 220–229. https://doi.org/10.1097/00010694-199803000-00007

Zong, N., Geng, S. B., Duan, C., Shi, P. L., Chai, X., & Zhang, X. Z. (2018). The effects of warming and nitrogen addition on ecosystem respiration in a Tibetan alpine meadow: The significance of winter warming. *Ecology and Evolution*, *8*, 10113–10125. https://doi.org/10.1002/ece3.4484

SUPPORTING INFORMATION

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