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Herbivores suppress soil microbes to influence carbon sequestration in the grazing ecosystem of the Trans-Himalaya

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ABSTRACT

Understanding factors that regulate carbon (C) pools is of high importance for offsetting greenhouse-gas emissions. Soils represent a vast C pool, whose size and stability are strongly influenced by land-use. Grazing, by native herbivores and livestock, is the predominant land-use across over 40% of the terrestrial surface and influences over 10^9 Mg of soil-C annually in the world's dry regions. The interactions between plants, grazers, and soil microbes, is of critical importance for this soil-C pool. However, soil microbial responses to grazing, and associated feedbacks, remain poorly understood. Grazing management policies are unable to adequately accommodate key interactions that are important for effective ecosystem stewardship. After 10-yr of experimental herbivore-exclusion in the semiarid Trans-Himalayan ecosystem, we measured grazer effects on soil microbial abundance in n = 30 herbivore exclosures, each paired with an adjacent control plot using substrate-induced respiration, microbial-carbon, and microbial-nitrogen (SIR, MBC, MBN). We found that grazing reduced soil microbial biomass by 13–16%, over the course of the vegetation growing season. But, the strength and direction of grazer effects varied through time at different points in the growing season. Grazing also shifted fungal:bacterial ratio towards dominance by fungi which were more tolerant of periodic dry-down and seasonal fluctuations in soil moisture than bacteria. So, grazer influence on microbial abundance and community composition may collectively play crucial roles in net soil-C dynamics. But, this effect is constrained by environmental factors, such as moisture availability. The projected climatic trend in the Trans-Himalaya is towards progressively wetter conditions, and this may counter grazer effect on microbes, alter microbial communities, and ultimately impact potential soil-C storage. So, accounting for projected changes in precipitation, in addition to managing stocking density of herbivores, may also be crucial for these large soil-C pools.

1. Introduction

Under ongoing and projected climate change, it is increasingly becoming important to identify and manage carbon (C) pools as C-sinks. Soils are the most important C-pool in the terrestrial realm; globally they store more C than the atmosphere (Chapin et al., 2009). As grazing ecosystems represent over 40% of the terrestrial realm, understanding and managing the impacts of herbivores becomes important for the size and stability of the soil-C pool (Bagchi and Ritchie, 2010a; Conant et al., 2002; Lal, 2004; Li et al., 2013; McSherry and Ritchie, 2013; Reid et al., 2004; Wang et al., 2014; Wen et al., 2013). Large mammalian herbivores (native grazers, and livestock), can exert both direct and indirect influence on soil-C (Conant et al., 2002; Derner et al., 2006; Derner and Schuman, 2007; Ganjegunte et al., 2005). Grazer effects on soil-C arise from a number of different inter-related pathways. These are conceptualized into three types (Cherif and Loreau, 2013; Hamilton and Frank, 2001; Hobbs, 1996): Type I – where grazing alters total plant production; Type II – where grazing alters the cycling of a limiting nutrient; Type III – where grazing induces plant physiological responses which further alter material and energy flow (e.g., root exudation). Their direct influence, on quantity of C-input to soil, is exerted by consuming plants and diverting C away from soils and toward secondary production, while releasing CO_2 and CH_4 in the process, and also altering vegetation composition. Their indirect influence, on quality of C-input to soil, determine how soil microbes respond to grazing, and represent feedbacks between producers, consumers and

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decomposers (Cherif and Loreau, 2013). Although this latter effect, mediated via feedbacks (Crowther et al., 2015; Jastrow et al., 2007; Yue et al., 2015), is likely very important, it remains relatively poorly understood. Key questions persist over the influence of herbivores on soil microbes, whether this interaction has implications for the size and stability of soil-C pools, and what underlying mechanisms could be involved (Tanentzap and Coomes, 2012).

Microbial responses to grazers is known to be highly variable, with positive, negative and neutral effects in different ecosystems (Tanentzap and Coomes, 2012). Since the different feedbacks involving microbes and grazers seldom act in the same direction, this gives rise to pluralistic results in different ecosystems (Cherif and Loreau, 2013). Grazer effects on soil-C generally shift from positive to negative with increasing precipitation in clayey soil, but this trend is reversed for sandy soils (McSherry and Ritchie, 2013); making grazing management very important for many arid and semi-arid regions. Existing theory posits that net effect of grazers on microbes can be explained by the balance between direct and indirect effects (Cherif and Loreau, 2013; Sankaran and Augustine, 2004), i.e., quantity of C-input vs. quality of C-input. While grazers reduce the quantity of C-input to soil through secondary production and respiration, they also alter the quality of C-input to soil by converting plant material to dung and urine and by altering species composition; this can change the fraction of labile substrates relative to more recalcitrant forms (Frank and Groffman, 1998). Simultaneous changes in quantity and quality can alter plant nutrient availability, plant production, and also grazing (Bagchi and Ritchie, 2010b; Cherif and Loreau, 2013; Ritchie et al., 1998; Wen et al., 2013). So, if the direct effect of grazing dominates, then reduction in quantity of C-input to soil will manifest as negative effects on soil microbes. Alternatively, if the indirect effect of grazing dominates, then improvement in quality of C-input to soil will manifest as a positive effect on soil microbes. Relative strengths of these pathways would also likely influence abundance of key microbial groups: bacteria and fungi, due to physiological differences between prokaryotes and eukaryotes (Barnard et al., 2013; Jastrow et al., 2007; Six et al., 2006; Strickland and Rousk, 2010; Waring et al., 2013). The net effect of grazing will arise from the balance between these two pathways, and will determine whether grazing increases or decreases soil microbial abundance, and ultimately influence the size and stability of soil-C pools.

Stoichiometry of litter and dung can regulate microbial responses to C-input and ultimately control soil-C (Cherif and Loreau, 2013). It is known that microbes can utilize the labile fraction of soil-C in 5–10 weeks, whereas it takes longer (20–50 weeks) to utilize the more recalcitrant forms (Frank and Groffman, 1998). So, distinguishing the relative roles of change in quantity and change in quality of C-input requires repeated sampling of microbial responses through time to account for this 4–5 fold difference in residence times. As previous studies have often depended on one-time sampling, usually during peak growing season, the purported feedbacks between producers, consumers, and decomposers remain inadequately resolved. Here we address this hypothesis over quantity and quality of C-input using a long-term herbivore-exclusion experiment (Bagchi and Ritchie, 2010a, b), by measuring changes in microbial biomass at regular intervals throughout the vegetation growing season in the Trans-Himalayan ecosystem of northern India. The specific questions addressed were: (1) do grazers suppress or enhance microbial biomass, and whether this varies across the growing season, and (2) how does grazing influence two key microbial groups, namely, bacteria and fungi. From these two inter-related aspects, one can draw inference over potential implications for the size and stability of soil-C pools (Derner and Schuman, 2007; Schuman et al., 2002).

2. Materials and methods

2.1. Experimental design

The Trans-Himalayas represent a vast high-altitude grazing ecosystem in Central Asia covering India (Spiti, Ladakh), China (Tibet) and Nepal (Mustang). Here, plant productivity is low. However, due to cold climate and arid conditions, the potential for soil-C sequestration is likely high (Bagchi and Ritchie, 2010a; Graham et al., 2012; Nikrad et al., 2016; Vincent, 2010). In 2005, we initiated a long-term study on grazing in Spiti region of northern India (32°N, 78°E). Twenty-four herbivore exclosures, each 100 m^2 (10 m × 10 m) and with a paired adjacent control plot, were established in 2005; another six exclosures were added in 2006. Elevation ranged between 4300 and 4500 m asl. Vegetation is a characterized by sedges (Cyperaceae) and grasses (Poaceae), with a few forbs and shrubs (mainly Fabaceae, Polygonaceae, Asteraceae, Chenopodiaceae). These rangelands are grazed by native herbivores (bharal, *Pseudois nayaur*; ibex, *Capra sibirica*; domesticated form of yak, *Bos grunniens*) and various non-native livestock (cattle, yak-cattle hybrids, donkey, horse, sheep, goat).

Here, grazers remove 55–68% of aboveground plant production during the growing season, and return about half of it as dung (Bagchi and Ritchie, 2010a). This prevailing grazing intensity falls in the range where grazing effects on soil-C can shift from positive to negative (McSherry and Ritchie, 2013; Zhang et al., 2015). This raises important concerns over degradation, which may call for pragmatic management interventions (Bagchi et al., 2012; Bagchi and Ritchie, 2010a). At the same time, C:N ratio of plant litter is between 55 and 60, and C:N ratio of dung is between 20 and 33 (Bagchi and Ritchie, 2011, 2010b). So, as expected (Cherif and Loreau, 2013; Frank and Groffman, 1998; Sankaran and Augustine, 2004), herbivores reduce quantity of C-input to soil, but improve the quality of C-input. Soils are slightly alkaline (pH between 7.6 and 8.0) and of sandy-loam texture. Average soil-C ranges between 1.5 and 2.0%, and soil-N between 0.1 and 0.2% (Bagchi et al., 2012; Bagchi and Ritchie, 2011, 2010a,b).

This ecosystem is highly seasonal. Vegetation growing season is short (May–August); temperatures drop below −30 °C during the winters (Fig. 1). Precipitation occurs as snow (100–200 cm, November-March) and rain (150–300 mm, July-August, Bagchi and Ritchie, 2010b). So, soil microbial abundance is expected to reflect these alternating wet/dry and cold/warm periods (Barnard et al., 2013; Sawicka et al., 2010).

2.2. Sampling

In 2015, after c. 10 years of herbivore exclusion, we sampled soil with a 2.5 cm diameter and 20 cm depth corer five times at monthly intervals, to cover the entire growing season from prior to green-up till after senescence (Fig. 1, early-May to early-October), from the grazed and ungrazed plots. Soil microbial activity between November and April (i.e., the period not included in our study) is expected to be much less than during the growing season. Previous studies, in similar ecosystems, have found that microbial respiration is negligible when temperatures are below 5 °C, and increases sharply after 10 °C (e.g., Frank et al., 2002). So, any grazer impacts during the dormant period (Fig. 1) may have only a minor role in overall patterns. Plant rooting depth rarely exceeds 20 cm here, and the upper layers are most important for net soil-C dynamics (Bagchi and Ritchie, 2010a; Yue et al., 2015). Soils were sun-dried, then oven-dried at 40 °C, and transported for laboratory analysis. From monthly samples, we estimated soil microbial abundance with two methods: (1) substrate-induced respiration (SIR, Anderson and Domsch, 1978; Robertson et al., 1999), and (2) microbial biomass as carbon and nitrogen using

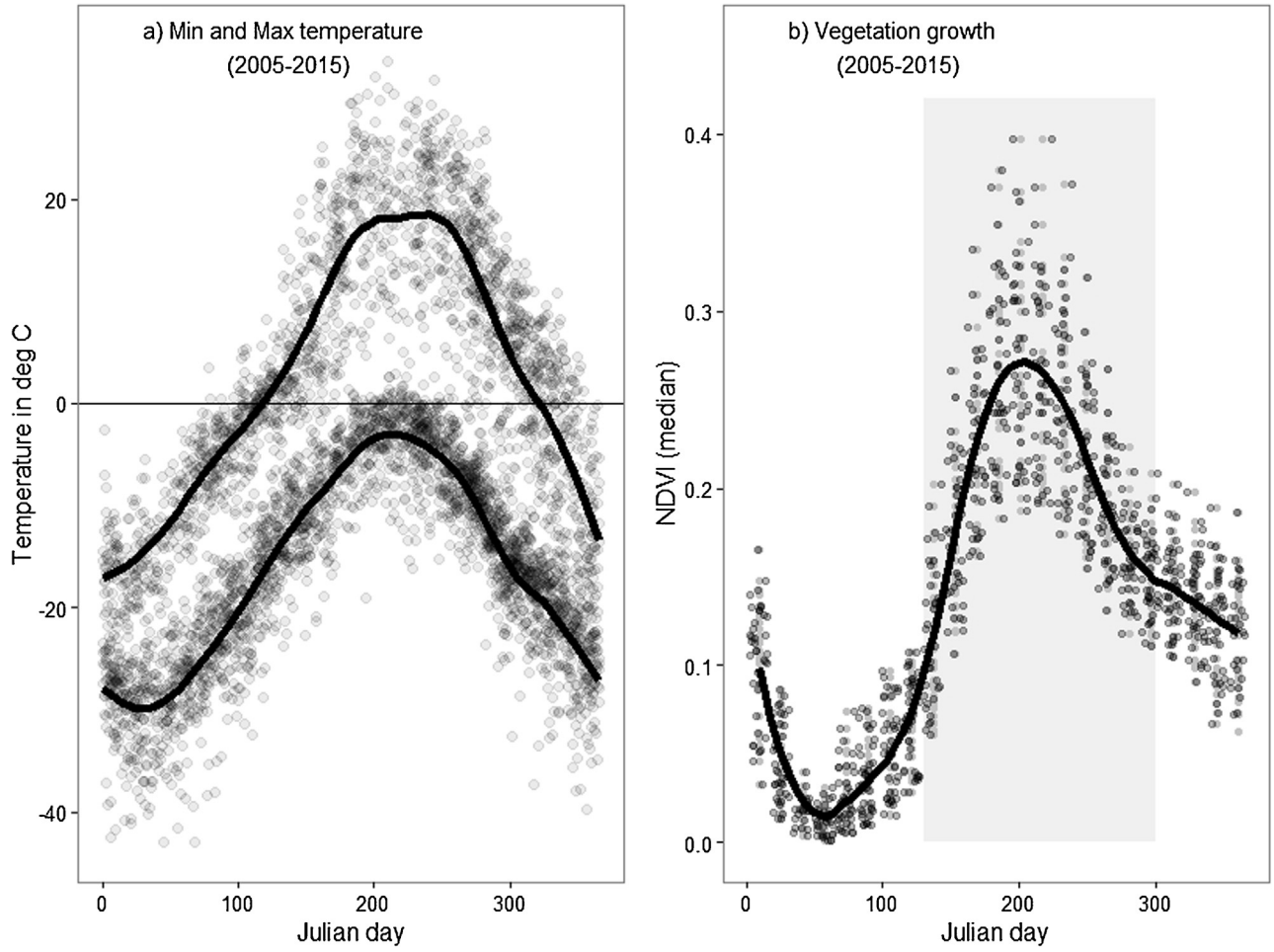

Fig. 1. Description of seasonality in temperature and vegetation growth in Spiti region of Trans-Himalaya . Average daily minimum (lower) and maximum (higher) temperature in the study area between 2005 and 2015 in (a), and average pattern of vegetation growth in (b). Vegetation growing season is between May and August (gray region). Temperature data are from MODIS 1-km resolution land surface temperature (LST) and vegetation growing season data are from 500-m resolution MODIS data for normalized difference vegetation index (NDVI).

chloroform fumigation and extraction (MBC and MBN, Jenkinson and Powlson, 1976; Robertson et al., 1999). SIR helps estimate the metabolically active pool of soil microbes, and can indicate potential soil respiration. Likewise, MBC and MBN help estimate standing biomass of microbes, similar to other methods such as lipid-based PLFA (Blagodatskaya and Kuzyakov, 2013; Insam, 1990; Stenström et al., 1998; Zhang and Zak, 1995). Since these two methods capture different but inter-related aspects, they can collectively offer detailed insights into soil microbial populations (Cheng and Virginia, 1993). Our laboratory assays do not incorporate local site-specific variation. In-situ measurements using gas-analyzers can be useful for capturing local site-specific variation, but these do not readily separate leaf-respiration, root-respiration, and microbial-respiration (Risch and Frank, 2006).

Briefly, for SIR, 4 g soil was pre-incubated at near 60% water holding capacity for 24 h at 27 °C, and trace amount of glucose was added (0.5% w/w). These were then placed in air-tight containers with 10 ml of 1N KOH as an alkali trap. After 15 h, the KOH was removed, and 15% $BaCl_2$ was added to precipitate the absorbed CO_2 as insoluble carbonate, and the supernatant was titrated with phenolphthalein indicator against 0.1N HCl to calculate CO_2 released from soil ($mg\,C\,g^{-1}$ soil day^{-1}), against corresponding controls. For MBC and MBN, 4 g soil was pre-incubated and kept in the dark in an air-tight container with ethanol-free chloroform for 24 h. These were aerated overnight at 27 °C to remove residual chloroform and extracted in 0.05 M K_2SO_4. C and N content in the extract was measured using a TOC-TN analyzer (Shimadzu LCPH/CPN, Japan). MBC and MBN was calculated as the difference in C and N between fumigated samples and corresponding controls (mg/g soil) with an extraction efficiency of 0.45 for MBC and 0.54 for MBN (Beck et al., 1997; Brookes et al., 1985). We used selective inhibition with anti-bacterial (Streptomycin) and anti-fungal agents (Cycloheximide) for estimating fungal:bacterial contribution to potential soil respiration (Anderson and Domsch, 1973; Beare et al., 1990). For fungal:bacterial respiration, we used samples from the middle months only (June, July, and August). Through initial trials, we determined that 8 mg/g soil of Streptomycin, and 8 mg/g soil of Cycloheximide achieved effective inhibition (Bailey et al., 2002), by comparing against samples where both agents were added, and none was added for controls. These samples were pre-incubated with their respective inhibitory agent for 24 h, and then analyzed in the way described above. The relative contribution of bacteria (Cycloheximide added) and fungi (Streptomycin added) was calculated as the log-ratio of the amount of CO_2 respired, $\log\left(\frac{\text{fungal } CO_2}{\text{bacterial } CO_2}\right)$. When this ratio is positive, it implies dominance of fungi; when it is negative, it implies dominance of bacteria.

In addition, we estimated soil moisture availability through the growing season (Volumetric Water Content, VWC) using a 20 cm Time Domain Reflectometry probe (TDR, Spectrum Technologies, USA). For this, we measured VWC at 6–8 random locations

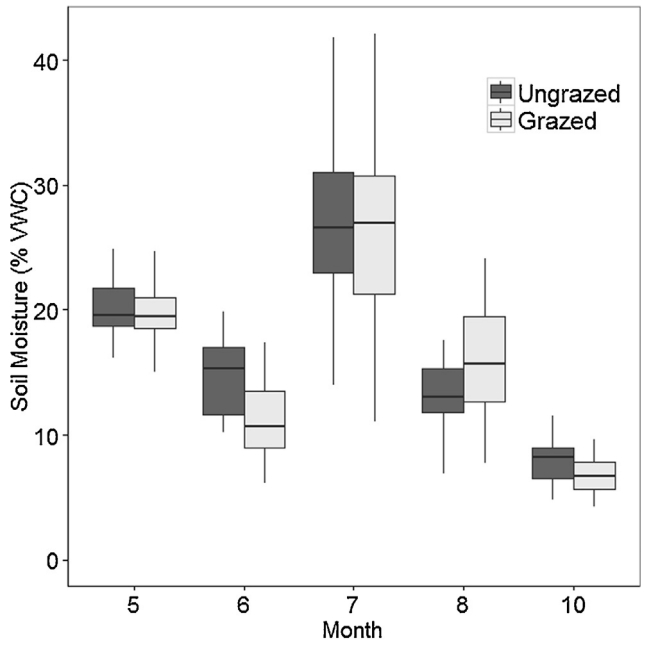

Fig. 2. Patterns in soil moisture availability across months (2015) in Spiti region of northern India. Soil moisture is higher during snowmelt (May) and during monsoon (July), which are quickly followed by dry-down conditions. Data are from n = 30 paired (grazed and ungrazed) plots, and show a significant interaction between grazing and time ($P < 0.05$, Table 1).

(averaged before statistical analysis) at each grazed and ungrazed plot at monthly intervals.

2.3. Data analysis

We used repeated-measures analysis of variance (ANOVA) to analyze grazer effect on SIR, MBC, MBN, microbial C:N ratio, and fungal:bacterial log-ratio. In these models, grazing (grazed and ungrazed) was considered as a categorical predictor, and time (month) was included for repeated measurements to investigate their main effects and interaction. We assessed co-variation between soil respiration and soil moisture availability using analysis of covariance (ANCOVA) with grazing and time (for repeated measures) as predictors. When grazing effects were significant in these statistical models, either as main effect or in interaction, we estimated the strength of grazer control (i.e., effect size) by comparing the variance explained by the full model against a baseline model with only time as predictor $\left(\text{i.e.,}\frac{\sum SS_{Treatments}}{SS_{Total}}\right)$. An alternative, but more complex approach, could involve analysis using mixed-effects models with grazing as a fixed effect, plot identity as a random effect, and an auto-covariance structure for time. The results of repeated-measures ANOVA and mixed-effects models were similar; we report results from the mixed-effects models in Appendix (Table S1). All analyses were performed in R 3.2.2.

3. Results

Variation in soil moisture (mean 17.24 ± 8.3 SD, CV = 48.2%) was explained by both grazing and time (Fig. 2), as there was a significant interaction (Table 1). Moisture availability indicated snowmelt (May) and monsoon (July), with rapid dry-down conditions at other times (Fig. 2). Expectedly, the statistical model with grazing and time explained 2.2% more variation in soil moisture than the baseline model with time alone, indicating that grazing has a significant but modest effect on soil moisture.

Variation in potential microbial respiration (SIR, 0.10 ± 0.07 SD, CV = 71.5%) was explained by both grazing and time (Fig. 3), as there was a significant interaction (Table 1). The statistical model with grazing and time explained 63.0% more variation than the baseline model with time alone, indicating a strong effect of grazing on potential microbial respiration. Overall, SIR in grazed plots was, on average, 13.7% lower than in ungrazed plots. While potential microbial respiration showed a unimodal pattern consistent with the growing season (Fig. 3), in grazed plots it reached a peak earlier (in June-July) than in the ungrazed plots (in August-September). Such lack of synchrony (Fig. 3) was consistent with the significant grazing × time interaction (Table 1).

Co-variation between potential microbial respiration and soil moisture, through time, was explained by grazing (Fig. 3), as there were significant grazing × moisture and grazing × time interactions (Table 1). The statistical model with grazing explained 86.8% more co-variation between potential microbial respiration and soil moisture than the baseline model, indicating a strong effect of grazing. As evident from the differences in temporal trends (Fig. 3), potential microbial respiration was correlated with soil moisture in the ungrazed plots, but not in the grazed plots, indicating that decomposers were less sensitive to fluctuations in soil moisture under grazing.

Variation in microbial biomass as MBC (0.13 ± 0.09 SD, CV = 70.9%), was explained by time (Table 1), but it did not follow the familiar unimodal pattern of the growing season (Fig. 4). Also, there were no significant effects of grazing, neither main effects, nor interaction (Table 1). Variation in microbial biomass as MBN (0.06 ± 0.03 SD, CV = 52.4%), was explained by time as well as by grazing, and also did not follow the familiar unimodal pattern of the growing season (Fig. 4). There were significant effects of grazing and time, but no significant interaction (Table 1). On average, MBN in grazed plots was 16% lower than in ungrazed plots (Fig. 4). The statistical model with grazing and time explained 32.6% more variation in the data than the model with time alone, indicating a strong effect of grazing on MBN. Variation in microbial biomass C:N ratio, as MBC/MBN (2.56 ± 2.59 SD, CV = 101.7%), was explained by both grazing and time (Fig. 4). There were significant effects of grazing and time, but no interaction (Table 1). On average microbial biomass C:N ratio was 29% higher in the grazed plots than in the ungrazed plots (Fig. 4). The statistical model with grazing and time explained 67.6% more variation in the data than the baseline model with time alone, indicating a strong effect of grazing on microbial C:N ratio.

Fungal respiration (under anti-bacterial agent Streptomycin) showed a significant interaction between grazing and time ($F_{2,132} = 8.20$, $P < 0.001$). Bacterial respiration (under anti-fungal agent Cycloheximide) showed no significant main effect of grazing and time. So, microbial respiration was dominated by fungi in the grazed plots, but by bacteria in the ungrazed plots, indicating a grazing-induced shift in the fungal:bacterial ratio. Variation in log-ratio of fungal:bacterial respiration (-0.18 ± 1.19 SD, CV = 659%) was explained by both grazing and time (Fig. 4), as there was a significant interaction (Table 1). Relative contributions of bacteria and fungi remained comparable through June and August in the ungrazed plots (Fig. 4). But, grazed plots showed that fungal contribution was higher in June, and gradually declined over time (Fig. 4) to be eventually dominated by bacteria in August. The statistical model with grazing and time explained 348.4% more variation in the data than the baseline model with time alone, indicating a strong effect of grazing on fungal: bacterial ratio.

Table 1
Summary of ANOVA and ANCOVA results for different variables measured through time in grazed and ungrazed plots in Spiti region of northern India. Results are from repeated-measures ANOVA for soil moisture, potential microbial respiration, microbial C, microbial N, microbial C:N, and fungal:bacterial ratio.

Variable	Effect	F	P
Soil moisture (ANOVA)	Grazing	$F_{1,215} = 0.06$	0.79
	Time	$F_{4,215} = 155.90$	<0.001
	Interaction	$F_{4,215} = 3.45$	0.01
Microbial respiration (ANOVA)	Grazing	$F_{1,261} = 5.29$	0.02
	Time	$F_{4,261} = 19.25$	<0.001
	Interaction	$F_{4,261} = 11.27$	<0.001
Microbial respiration (ANCOVA)	Soil moisture	$F_{1,226} = 17.29$	<0.001
	Grazing	$F_{1,226} = 5.03$	0.02
	Time	$F_{4,226} = 12.86$	<0.001
	Soil moisture × Grazing	$F_{1,226} = 32.24$	<0.001
	Soil moisture × Time	$F_{4,226} = 0.27$	0.89
	Grazing × Time	$F_{4,226} = 5.68$	<0.001
	3-way interaction	$F_{4,226} = 1.63$	0.16
Microbial C (ANOVA)	Grazing	$F_{4,216} = 0.47$	0.49
	Time	$F_{4,216} = 5.89$	<0.001
	Interaction	$F_{4,216} = 1.06$	0.37
Microbial N (ANOVA)	Grazing	$F_{1,256} = 10.66$	0.001
	Time	$F_{4,256} = 10.51$	<0.001
	Interaction	$F_{1,256} = 0.99$	0.41
Microbial C:N ratio (ANOVA)	Grazing	$F_{1,213} = 9.47$	0.002
	Time	$F_{4,213} = 10.43$	<0.001
	Interaction	$F_{4,213} = 1.37$	0.24
Fungus:Bacteria ratio (ANOVA)	Grazing	$F_{1,128} = 0.08$	0.78
	Time	$F_{2,128} = 0.92$	0.39
	Interaction	$F_{2,128} = 6.32$	0.002

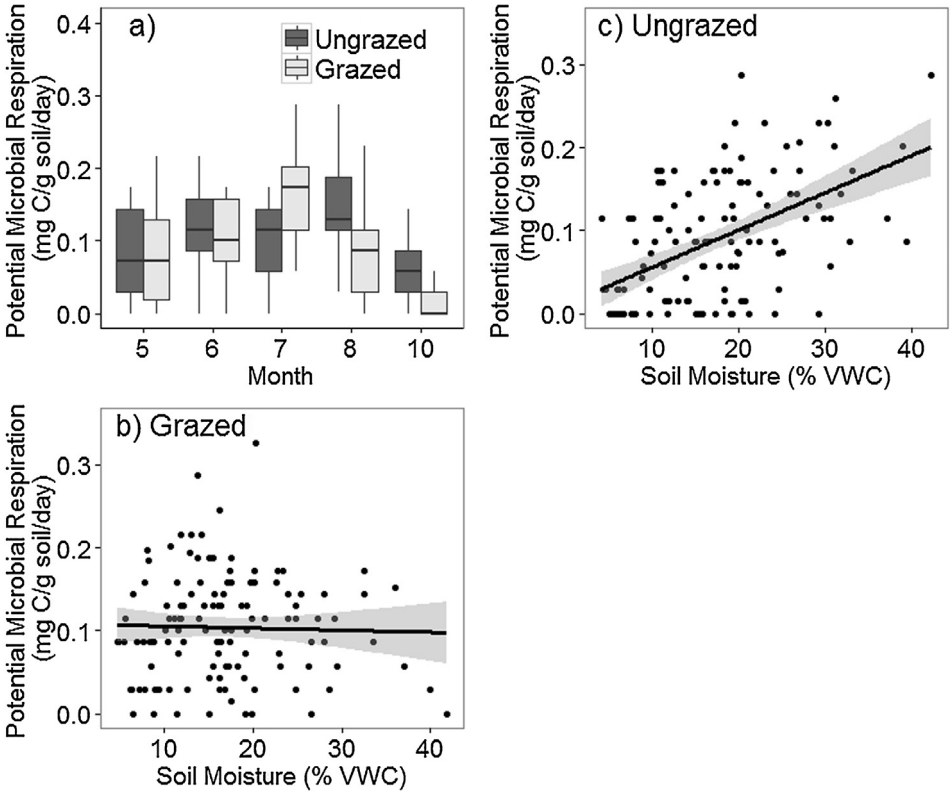

Fig. 3. Patterns in potential microbial respiration over time in Spiti region of northern India in (a). Correlation between potential microbial respiration and soil moisture in grazed in (b), and in ungrazed plots in (c). Data are from n = 30 paired (grazed and ungrazed) plots, and show a significant interaction between grazing and time ($P < 0.05$, Table 1), and between grazing and soil moisture ($P < 0.05$, Table 1).

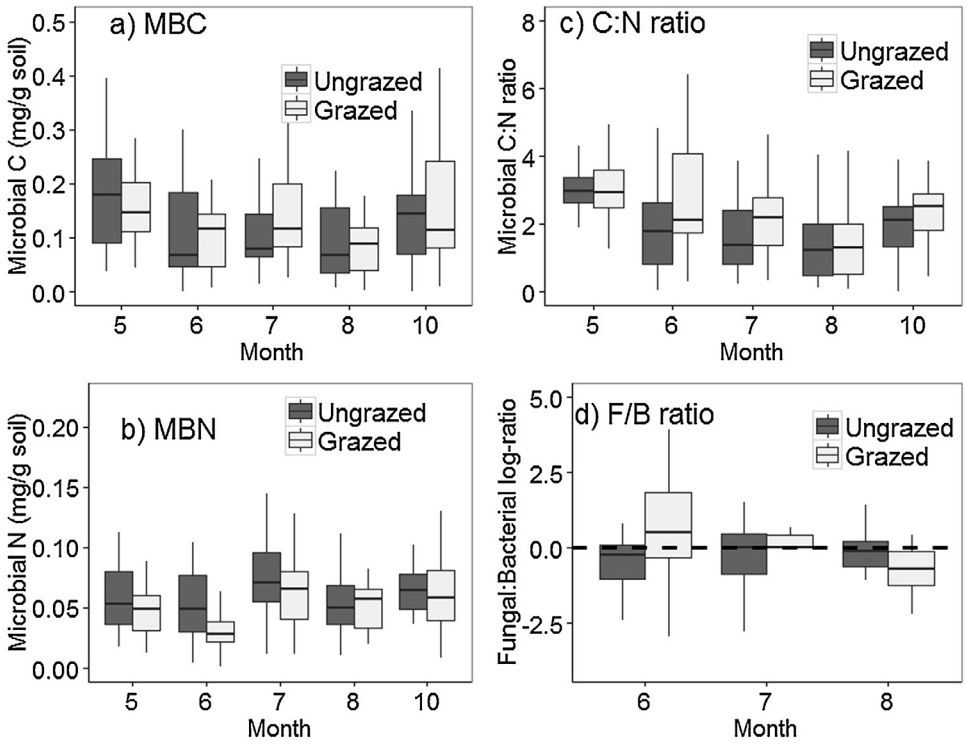

Fig. 4. Patterns of microbial biomass carbon (MBC) in (a), microbial biomass nitrogen (MBN) in (b), and microbial C:N ratio in (c), and fungal:bacterial log-ratio in (d) through time, in Spiti region of northern India. Fungal:Bacterial log-ratio is from selective inhibition using anti-bacterial (Streptomycin) and anti-fungal agents (Cycloheximide). Values above zero (dashed line) represents greater relative contribution of fungi, while values below zero represent greater relative contribution of bacteria. Ungrazed plots showed relatively greater contributions from bacteria through time, whereas fungi are dominant in June and July under grazing. Data are from n = 30 paired (grazed and ungrazed) plots, and show a significant effect of grazing and/or time ($P < 0.05$, Table 1).

4. Discussion

Overall, our results for SIR, MBC and MBN, show negative effect of herbivores on potential microbial respiration as well as biomass (Figs. 3–4). This is consistent with the hypothesis that reductions in quantity of C-input are not compensated by simultaneous improvements in quality of C-input (Cherif and Loreau, 2013; Sankaran and Augustine, 2004). But, grazers had comparatively stronger effects on potential microbial respiration (SIR), than on standing biomass (MBC, MBN). So, grazer effects on potential microbial activity (Insam, 1990; Stenström et al., 1998; Zhang and Zak, 1995), rather than their abundance (which includes dormant microbial biomass), may be more important for the soil-C pool.

While potential microbial respiration followed a unimodal pattern through time, consistent with vegetation growth, temperature, and soil moisture (Figs. 1–3), microbial standing biomass did not show a similar trend. In fact, microbial biomass, MBC (Fig. 4), was higher at the beginning of the growing season (May) than in the middle of the growing season (July). This is consistent with other studies that have also found higher microbial standing biomass early in the growing season than in the middle of the growing season in Central Asian highlands (Fu et al., 2012), which reiterates that grazer effects on potential microbial activity rather than biomass may exert a greater influence on stability of soil-C pools.

Our understanding of soil C dynamics in dryland grazing ecosystems is largely based on studies where seasonal patterns have remained unaccounted. This limits inference on broader questions over interactions between herbivores and soil microbes. For example, a study conducted in June-July would likely reach a different conclusion than one conducted in August-September (Figs. 3–4). Perhaps this explains why different studies find positive, negative, and neutral effect of grazers on soil-C dynamics, in a seemingly idiosyncratic manner, even though the effects should be predictable over a broad range of environmental conditions (Cherif and Loreau, 2013; Sankaran and Augustine, 2004). Given the anticipated difference in residence time between labile and recalcitrant fractions of C-input (Frank and Groffman, 1998), it is necessary to accommodate variation, at least, over the duration of a growing season (Figs. 3–4).

Grazing also shifted microbial community towards fungi. Since fungi are more efficient at decomposing cellulose than bacteria, one would expect grazing-induced change in C-input to favour bacteria (Eriksson et al., 1990). But, we found the opposite pattern; grazing favoured fungi. Though it may seem counter-intuitive, this is in fact a consistent and recurring pattern across many grazing ecosystems (Chen et al., 2015). Fungi are also known to have higher C:N ratio (between 7 and 25) than bacteria (between 5 and 7), and this pattern (Fig. 4) is consistent with other studies (de Vries et al., 2006; Jastrow et al., 2007; Waring et al., 2013). In general, higher fungal:bacterial ratios have a favourable impact on a number of other ecosystem processes, e.g., nutrient retention (de Vries et al., 2006). Importantly, fungal dominance in soil, as opposed to bacterial dominance, is also attributable to slower soil-C turnover and higher net C-storage (Jastrow et al., 2007; Kallenbach et al., 2016; Six et al., 2006; Strickland and Rousk, 2010). So, grazers could potentially influence net soil-C storage via their joint effects on microbial abundance and composition (McSherry and Ritchie, 2013; Sankaran and Augustine, 2004).

The changes in microbial community could be explained by seasonal changes in soil moisture. Fungi are likely to be more tolerant of fluctuations in soil moisture than bacteria (Barnard et al., 2013), and this is reflected by continued activity during the dry-down period in June under grazing (Figs. 2–3). But, potential respiration declined during this dry-down period in the ungrazed plots, and increased only after July-August (Fig. 3). However, since

the quantity of C-input is lower under grazing, overall potential microbial activity declines by August even though moisture from July-monsoon is still available. Whereas, potential microbial activity peaks later in the growing season in the ungrazed plots, and is also more sensitive to moisture availability (Figs. 3–4). This pattern is consistent with expected shorter residence time for labile-C under grazing (only 5–6 weeks, Frank and Groffman, 1998). So, differential microbial activity under labile/recalcitrant C substrates is constrained by moisture availability.

Grazer-microbe interactions can influence the size and stability of the soil-C pool, but this effect is mediated by existing climatic constraints over soil moisture availability. Future climate scenarios that alter water availability may influence net C storage vs loss through the indirect interactions between grazers and microbes (Bradford et al., 2016; Zhang et al., 2016). The prevailing climatic trend in these Central Asian highlands, though locally variable, is toward increased precipitation (Roxy et al., 2015; Shaohong et al., 2007; Shenbin et al., 2006). This may likely alter microbial growth patterns by changing the dry-down early in the growing season (May–June), and may also have an impact later in the growing season (July-August). So, anticipated changes in precipitation could potentially lead to higher soil respiration (Figs. 3–4) by removing water-limitation for soil microbes, particularly bacteria. These changes could impact the size and stability of a large C-sink in Central Asia (Bradford et al., 2016; Chen et al., 2015; Crowther et al., 2015). Hence, while controlling grazing intensity is a critical step (Bagchi and Ritchie, 2010a), accounting for altered feedbacks should also become a key feature managing soil-C in grazing ecosystems. Understanding how the interactions between plants, herbivores, and decomposers are constrained by climatic conditions is an important step. We find (Figs. 3–4), these effects are predictable (Cherif and Loreau, 2013). This can help in the search for better management of the stability of potential C-sinks (Bradford et al., 2016; Crowther et al., 2015) in grazing ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2017.01.033.

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