Running Head: Can livestock be functional substitutes of native herbivores?

Functional substitutability of native herbivores by livestock for long-term soil carbon sequestration depends on microbial decomposers.

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Abstract

The influence of native mammalian herbivores on ecosystems is declining as livestock have displaced them to become the dominant grazers across the world. Alongside rewilding, restoration, and conservation, it is now important to address whether livestock can assume the functional roles of displaced native herbivores in grasslands, savannas, and steppes. If yes, this has implications for the future of biodiversity, ecosystem functions and services, food-security, and other social and environmental dimensions. We test competing alternative hypotheses with a replicated, long-term, landscape-level experiment to ask whether livestock in the Trans-Himalayan ecosystem can match decadal-scale (2006-17) soil-C sequestration under native herbivores. Despite comparable settings, livestock were imperfect substitutes and attained three-fourths of soil-C stocks under native herbivores. We evaluate multiple lines of evidence surrounding this difference with a grazing-exclusion experiment (nutrient-cycling, microbial metabolism, and e-DNA), and asses their inter-relationships. Livestock and native herbivores differed in their effects on several soil microbial variables. Structural equation models showed that livestock alter soil microbial communities towards lower fungal abundance, which is detrimental for microbial carbon use efficiency (CUE), and ultimately also for soil-C. Herbivore-identity influenced ecosystem functions even when total amount of herbivory was comparable. Lags in soil-C storage can be addressed through microbial restoration and rewilding of soil. Therefore, the idea of functional substitutability must be leveraged alongside microbial rewilding to restore and conserve ecosystem functions. Together they can reconcile conflicting demands from food-security and ecosystem services in ways that have eluded conventional approaches to become an integral part of nature-based climate solutions.

Significance Statement

Grazing by large mammalian herbivores affords an important natural climate solution via soil-C storage. But native grazers are progressively being replaced by domestic livestock to accommodate changing human diets. Even though livestock are now the dominant grazers around the world, it remains unclear whether they can be functional substitutes for native herbivores in sequestering soil-C. And if so, then to what extent? We find that livestock could only attain three-fourths of soil-C stocks under native herbivores, because they shift the microbial decomposer community in soil towards lower fungal abundance. This reduces microbial carbon use efficiency, and ultimately is detrimental for soil-C storage. So, microbial rewilding may help livestock production systems to emulate the ecosystem services provided by native herbivores.

Main text

Introduction

Large mammalian herbivores have undergone widespread declines in their abundance and distribution across the world's grasslands, steppes, and savannas. Pre-historic humans might have hunted many megafauna to extinction. More recently, humans have progressively replaced them with livestock across the world. Relatively intact assemblages of native herbivores are now increasingly confined to parks and reserves; elsewhere, livestock have not only become the most abundant herbivores, but they also represent the most geographically expansive land-use today (Asner et al. 2004, Bar-On et al. 2018). Further displacement of native herbivores seems inevitable due to steadily rising demands for livestock-products to accommodate evolving human-diets (Thornton 2010, Leclère et al. 2020). This severely restricts and alters the erstwhile influence of native herbivores on ecosystem structure and functions, their broad influence on global biogeochemical cycles, and their influence on soil-C in particular (Brodie et al. 2021, Roy and Bagchi 2021).

Increasingly, it is important to ask whether livestock can maintain the functional roles of the displaced native herbivores (Malhi et al. 2016, Cromsigt et al. 2018, Lundgren et al. 2020). If livestock can maintain ecological functions and services of their native counterparts, then such functional substitutability can have implications for the future of biodiversity, ecosystem functions and services, food-security, and other social and environmental dimensions (Sayer et al. 2013, Veblen et al. 2016, Willemen et al. 2020, Western et al. 2020, Delabre et al. 2021). The extent to which livestock can maintain ecosystem functions and services, particularly soil-C sequestration, becomes important to inform policies over sustainable use of grazing ecosystems and leverage their potential as natural climate solutions (Smith 2014, Cromsigt et al. 2018, Bossio et al. 2020).

Opportunities to restore and maintain ecosystem processes using livestock as surrogates of displaced native herbivores arise from the mutual similarity in their functional traits (Malhi et al. 2016, Lundgren et al. 2020, Schowanek et al. 2021). Indeed, traits are better predictors of ecosystem functions than taxonomic identity of species (Hooper et al. 2005). Analyses of key traits, such as body size, diet-choice, and fermentation type, hypothesize that livestock can be approximate surrogates for native herbivores, i.e., nearest neighbors in trait-space (Malhi et al. 2016, Lundgren et al. 2020, Schowanek et al. 2021). However, empirical evidence on ecosystem functions and services is needed before these ideas can be implemented toward ecosystem management, and such evidence is lacking (Reinhart et al. 2021).

From this functional-substitutability hypothesis one can ask (Betts et al. 2021) – if there is sufficient overlap in trait-space between livestock and native herbivores, then to what extent can they act as surrogates? It is challenging to address this question because many modern livestock-production systems no longer have native counterparts within comparable ecological settings that can allow a fair comparison. Unlike intensive livestock production systems that are often associated with various forms of environmental degradation, traditional pastoral production systems where both livestock and native herbivores occur in multiple-use landscapes use (Asner et al. 2004, du Toit et al. 2009, Veblen et al. 2016, Western et al. 2020) can be testbeds to assess whether livestock are good substitutes, or not. While this functional premise does not expect livestock to be perfect replacements for the displaced native herbivores (Bagchi and Ritchie 2010, Sitters et al. 2020), it is important to estimate the degree to which their ecological roles are matched to identify gaps and shortfalls as well as the scope for improved livestock production systems (Smith 2014, Western et al. 2020, Reinhart et al. 2021, Delabre et al. 2021).

Here we address impacts of livestock and native herbivores on soil-C sequestration as grazing by large mammals is an important component of the global carbon cycle (Schmitz et al. 2014, Roy and Bagchi 2021). We compare their ecological impacts in Spiti region of northern India, which is part of the greater Trans-Himalayan ecosystem spread across the Tibetan plateau and surrounding mountains. Soil-C sequestration in the Trans-Himalaya is particularly important under human land-use for livestock production and under ongoing climate change (Murthy and Bagchi 2018, Naidu and Bagchi 2021). We investigate long-term (decadal-scale) net soil-C sequestration under livestock and native herbivores, and their influence on key microbial decomposer functions related to the C-cycle (Falkowski et al. 2008, Sinsabaugh et al. 2016, Roy and Bagchi 2021). We take advantage of several key features in the Trans-Himalaya: (a) the livestock are a multi-species assemblage that show considerable trait-overlap with the native herbivores (Fig. 1), (b) livestock and native herbivores occupy distinct but juxtaposed watersheds with comparable environmental settings (Fig. S1-S4), (c) their abundances are comparable so that grazing pressure on vegetation is also similar (see Methods, Fig. S7). Absent major confounding influences, the livestock and native assemblages in Trans-Himalaya can help evaluate their functional substitutability (Malhi et al. 2016, Cromsigt et al. 2018, Lundgren et al. 2020, Schowanek et al. 2021). Specifically, we investigate (a) whether, and by how much, does grazing by livestock and native herbivores differ in its influence on long-term (decadal-scale) soil-C sequestration, and (b) whether any differences can be attributed to how each assemblage influences microbial decomposer functions in soil.

Results

LONG-TERM TREND IN SOIL-CARBON

Over a decade (2006-17), soils under native herbivores stored 8.12±1.3 SE more carbon (mg C cm^{-3}) than under livestock ($F_{1,27}$=9.86, P<0.01). Model comparison showed that spatio-temporal heterogeneity was not sufficient to explain long-term variation in soil-C, and it could instead be attributed to herbivore assemblage-type. There was stronger support for M_A^{soilC} than for M_0^{soilC} (difference in AIC=−34.3, and difference in RMSE=−0.66, and LR=36.2, P<0.001). Effectively, areas under livestock could realize up to 70% of the soil-C found under native herbivores (Fig. 2).

GRAZER-EFFECTS ON SOIL MICROBES

Analysis of grazer-effects indicated that the observed difference in soil-C (Fig. 2) was related to how soil microbes respond to grazing by livestock or native herbivores (Fig. 3). Livestock and native herbivores had comparable effects on B, 16s rDNA abundance, MBC, MBN, LC, LN, and qCO_2 (Fig. 3, Table S1). Grazing-intensity and grazer-effect on soil-C were also comparable between livestock and native herbivores. For these nine variables, the competing alternative models were not supported, and their variability could be attributed to spatio-temporal heterogeneity rather than to herbivore assemblage-type (Fig. 3, Table S1). But livestock and native herbivores differed in their influence on BR, SIR, F, ITS abundance, and CUE. For these five variables, there was stronger support for the competing models than the null models (Table S1), as assemblage-type was an important explanatory variable over and above background spatio-temporal heterogeneity (Fig. 3). Grazing by livestock increased both basal and potential microbial respiration (BR and SIR), compared to grazing by native herbivores (Fig. 3). But grazing by livestock reduced both the fungal fraction of microbial respiration and the abundance of fungal genetic markers (F and ITS). Respiration-based estimates of fungal and bacterial fractions were consistent with DNA-based estimates (Fig.

3). Importantly, grazing by livestock reduced CUE, whereas grazing by native herbivores increased it (Fig. 3).

INTER-RELATIONSHIPS BETWEEN SOIL MICROBIAL RESPONSES

To assess how differences in grazer-effects on one microbial variable may influence other variables, we evaluated two competing SEM models. The null model was supported by the data (SEM_0: Fisher's C=3.34, *P*=0.503, df=4, AIC=69.34). This showed, regardless of herbivore assemblage-type, grazing influences microbial community composition (fungal fraction) to alter CUE, and grazers also influence the labile-C pool via their effects on microbial biomass and labile-N (Fig. 4). The alternative competing model was also supported by the data (SEM_A: Fisher's C=2.108, *P*=0.716, df=4, AIC=88.11). It showed that change in assemblage-type can influence CUE via its effect on microbial community composition (Fig. 4). Specifically, change in herbivore-assemblage from native to livestock has negative influence on fungal abundance, which in turn is detrimental for CUE (Fig. 4). Both these SEMs indicate that direction and magnitude of grazer-effects are consistent with higher CUE under native herbivores improves net soil-C storage, compared to livestock (Fig. 2, Fig. 4).

LINKS BETWEEN MICROBIAL RESPONSES AND SOIL-C

We compared the role of herbivore assemblage-type ('identity') and grazing-intensity ('intensity') together, as a thought experiment to connect the inter-relationship between different microbial responses with soil-C (Fig. 5). This model showed that changing herbivore assemblage-type from native to livestock is detrimental to soil-C via the indirect effects on microbial community composition (fungal fraction) and microbial CUE (Fig. 4-5). In this way, it supported our expectation that effects arising from microbial processes at monthly scale (seen in Fig. 4) can indeed accumulate over years and decades to influence

soil-C (Fisher's C=12.82, P=0.118, df=8, AIC=124.82). The links between microbial community composition, CUE, and soil-C were not influenced by any background variation in grazing-intensity (Fig. 3). Since grazing-intensity was comparable between livestock and native herbivores, there were no paths connecting it to soil-C. In other words, herbivore identity was important even if total amount of herbivory is comparable (Fig. 5).

Discussion

Previous studies from around the world have emphasized that grazing by large mammalian herbivores leads to net soil-C storage (Conant and Paustian 2002, Witt et al. 2011, Abdalla et al. 2018, Derner et al. 2019, Liu et al. 2020), and grazing ecosystems should be integral to nature-based climate solutions (Smith 2014, Cromsigt et al. 2018, Willemen et al. 2020, Bossio et al. 2020, Roy and Bagchi 2021). In parallel, studies have also identified the limitations of nature-based climate solutions derived from soil, because soil-C tends to accrue at very slow rates but can be lost quite rapidly (Smith 2014, Reinhart et al. 2021). Therefore, preserving existing soil-C stocks is seen as an important and necessary step toward mitigating land-atmosphere feedbacks alongside more ambitious targets of gaining soil-C (Smith 2014, Naidu and Bagchi 2021). And we found that while soil-C has fluctuated, there has been no net loss over decadal timescales (Fig. 2). But it has remained unclear whether this service can be maintained when natural ecosystems are repurposed for livestock production (Reinhart et al. 2021). And if so, then to what extent?

Our results address several inter-related aspects of the functional ecology of native herbivores and livestock. We find that a multi-species herds of livestock can have remarkable functional similarities with the native assemblages they displace (Fig. 1). But livestock do not emerge as perfect substitutes since they achieve about three-fourths of the soil-C stock, compared to the native herbivores (Fig. 2). Nevertheless, consistent with the functional

substitutability hypothesis, a key ecosystem service provided by native herbivores can also be partly realized in livestock-production systems. This can have implications for how we manage environmental impacts of livestock (Sayer et al. 2013, Smith 2014, Veblen et al. 2016, Willemen et al. 2020, Western et al. 2020, Delabre et al. 2021), and meet the rising demand for livestock-products in the coming decades (Thornton 2010, Leclère et al. 2020).

To determine the scope of improved management, it is important to identify the potential drivers and mechanisms that explain why livestock lag native herbivores in maintaining soil-C. Prevailing management practices have largely focused on two aspects—overgrazing and vegetation change—which result in altered C-input to soil from plants (Bagchi and Ritchie 2010, Reinhart et al. 2021). However, this prevailing focus on C-input alone can be simplistic and inadequate since it does not account for subsequent metabolism of soil organic matter by microbes (Fontaine et al. 2004, Six et al. 2006, Falkowski et al. 2008, Sinsabaugh et al. 2013, Roy and Bagchi 2021). Our results from the grazer-exclusion experiment identify soil microbial responses as the proximate explanation for why livestock are not perfect substitutes for native herbivores (Fig. 3). Tests of the competing hypotheses address whether the observed patterns in soil-C reflects idiosyncratic and/or pre-existing differences between watersheds, or whether they are linked to the identity of the herbivores. We attribute this missing soil-C to how microbial processes respond to grazing when native herbivores are replaced by livestock (Fig. 4-5). However, this still leaves the possibility that livestock have been historically overgrazed their watersheds, and their soils have not yet recovered. Even though vegetation may recover from historical overgrazing, it may take much longer for soil microbial functions to recover (López Zieher et al. 2020). Seen together, these highlight the importance of soil microbial restoration in tandem with management of grazing intensity and stocking rates. CUE is a fundamental microbial trait that controls residence time of carbon in the soil, and it determines the flow of carbon through ecosystems

(Six et al. 2006, Falkowski et al. 2008, Sinsabaugh et al. 2013). The observed grazer-effects on fungi (Fig. 3) are also known from other ecosystems (Bardgett et al. 1997, Zhang and Fu 2021). Direction and magnitude of difference in the microbial community under livestock reduces CUE (Fig. 3-4), and this is consistent with lower soil-C (Fig. 5) because fungi are known to have higher CUE than bacteria (Fontaine et al. 2004, Six et al. 2006).

Several overlapping factors can alter microbial communities towards lower fungi, and their individual roles may not be easily disentangled from each other. For instance, variation in vegetation composition can result in differences in the quality of litter-input to soil, and this in turn can influence the rate and efficiency with which microbes metabolize soil organic matter (Fontaine et al. 2004, Six et al. 2006, Sinsabaugh et al. 2013). Next, as discussed above, microbial communities may have not yet recovered from any historical overgrazing in the past (López Zieher et al. 2020), even though vegetation biomass does not indicate overgrazing is currently a concern (Fig. 5). Additionally, veterinary care offered to the livestock leads to inadvertent release of antibiotics and other drugs into soil from their dung and urine, and this can alter microbial communities towards lower CUE (Wepking et al. 2019). Such overlapping factors need to be addressed together to improve soil-C sequestration, rather than in isolation.

Our results from alternative competing SEMs offer insights into how net soil-C sequestration can be improved (Fig. 4-5). Since replacing native herbivores with livestock is detrimental for CUE, promoting wildlife conservation over livestock-production would favor soil-C (Fig. 4-5). As that would compromise livelihoods and food-security, and it circles back to the inconsistencies and conflicts between the goals of conservation and human land-use. This conventional and exclusionary approach may no longer be tenable and is also increasingly difficult to justify (Adams and Mulligan 2002); it is timely to assess alternative ideas (Smith 2014). The null model (SEM_0, Fig. 4) can offer insights into ways to resolve

these conflicting demands on land-use. It suggests altered microbial communities compromise CUE regardless of whether the community shift is a fallout of land-use, or not. This opens opportunities for restoration of microbial communities to sustain ecosystem services under human land-use (Paluch et al. 2013, Wubs et al. 2016, Mills et al. 2017). Admittedly, there are many gaps in our current understanding of structure-function relationships for hyper-diverse microbial communities (Bier et al. 2015). Yet, there are encouraging signs that effective restoration can be achieved even with incomplete taxonomic knowledge (Paluch et al. 2013, Wubs et al. 2016, Mills et al. 2017). Since livestock already deliver 70% of the ecosystem service provided by native herbivores, further incorporating microbial rewilding of soils into livestock-production may offset the 30% lag against native herbivores—in the process, making livestock-production more environmentally responsible (Smith 2014, Cromsigt et al. 2018, Willemen et al. 2020, Bossio et al. 2020).

In conclusion, our results highlight: (a) multi-species and functionally diverse assemblages of livestock can partly approximate the ecological roles of native herbivores, and (b) restoration and rewilding of soil microbial communities may enable livestock to emulate the services provided by native herbivores. Alongside continued management of stocking rates, functional substitutability and microbial restoration in-tandem may help reconcile rising demands for livestock-products with conservation of ecosystem functions and services.

Materials and methods

STUDY AREA

Traditional pastoral livestock production is the major land-use in Spiti region of the Trans-Himalayan grazing ecosystem. Watersheds near human settlements are used by a multi-species livestock assemblage, and these are juxtaposed with watersheds that retain native herbivores (Fig. 1, Fig. S1). Natural terrain and barriers (canyons, rivers, high ridges) that restrict frequent animal movement, maintain replicate watersheds under two alternative types of herbivore assemblages (Fig. S1, S2). Historical local extinctions of native herbivores and their replacement by livestock over the past decades remains a major conservation challenge in this region (Mishra et al. 2002, Namgail et al. 2013), and around the world (Veblen et al. 2016, Western et al. 2020). The extant native herbivores are ibex (*Capra sibirica*), bharal (*Pseudois nayaur*), and yak (*Bos grunniens grunniens*) that are related to wild yak (Bos grunniens mutus; also known as B. grunniens and B. mutus, respectively, 48). Currently, kiang (*Equus kiang*) are known from the fringes of the study area, and there are sporadic reports of argali (*Ovis ammon*). But Tibetan antelope (*Pantholops hodgsonii*) and Tibetan gazelle (*Procapra picticaudata*) are no longer found in the region. The livestock consist of goat, sheep, donkey, horse, cattle and yak-cattle hybrids (Fig. 1). The livestock and native herbivores (after including kiang and argali) show sufficiently high overlap in key traits (Lundgren et al. 2020), such as body mass, dietary guild and graminoid consumption, fermentation type, sexual-dimorphism, and limb morphology (Fig. 1). In recent decades, total animal biomass has remained comparable (6.9×104 kg for native and 7.2×104 kg for livestock; 23, 49), such that grazing pressure on vegetation has also remained similar for sufficiently long periods (Fig. S7).

The two types of watersheds are comparable in their abiotic conditions (Fig. S3–S4). Climate in Spiti is cold and semi-arid with temperature ranging from 25 °C in summer to

below -30 °C in winters (Fig. S5). Precipitation occurs as winter-snow (100-200 cm yr^{-1}) and summer-rain (150-300 mm yr^{-1}), and vegetation growth-season is short (May-August, Fig. S5). Vegetation in this treeless landscape consists of grasses (*Poa*, *Elymus*, *Festuca*), sedges (*Carex*, *Kobresia*), forbs and shrubs (*Artemisia*, *Lindelofia*, *Caragana*). Both types of watersheds have an overlapping pool of plant species, but show spatial variation in vegetation composition. Livestock watersheds have high abundance of sedges and grasses, have higher ground cover as well as higher aboveground plant production than grass-and-forb vegetation in the native watersheds (23).

SOIL CARBON

Between 2006 and 2017, we measured soil-C at regular intervals in four watersheds with native herbivores, and another four watersheds with livestock (Fig. S1, S2). In each watershed, we set up 3-4 replicated permanent plots (10×10 m^2), for a total of n=15 plots under native herbivores, and another n=15 plots under livestock. We collected soils with a 5 cm diameter and 20 cm deep cores, and measured carbon content (TruSpec, Leco, USA, and Elementar Analysensysteme, GmbH, Germany) to compare long-term soil-C stocks (mg C cm^{-3}) under livestock and native herbivores.

GRAZING-EXCLUSION EXPERIMENT

We distinguish whether any observed difference in soil-C between the watersheds is merely an artefact of pre-existing site-specific conditions, or it can be attributed to grazing by the alternative herbivore assemblages. For this, starting in 2005, we set up experimental grazer-exclusion with a paired-adjacent fence at each location (also 10×10 m^2, each). In 2017, after up to 12 years of herbivore-exclusion, we estimated grazer-effects on key soil microbial functions in the paired-adjacent fenced and control plots.

GRAZING INTENSITY

We measured standing above-belowground biomass at the end of the growing season in paired fenced and grazed plots as an indicator of grazing-intensity. We collected live shoot biomass from 0.5×0.5 m^2 quadrats in each paired plot. Biomass samples were sun-dried, and subsequently oven-dried to constant weight at 40 °C to obtain their dry-weight (g m^{-2}).

SOIL MICROBIAL FUNCTIONS

We estimated the response of key soil microbial functions to grazing, that are related to the carbon cycle and net soil-C sequestration: Basal respiration (BR), Potential, or substrate-induced respiration (SIR), Soil microbial community composition, broadly as fungal and bacterial fractions, Microbial biomass C and N (MBC and MBN), labile Carbon (LC) and labile Nitrogen (LN) fractions of soil organic matter, Metabolic quotient (qCO_2), and Microbial carbon use efficiency (CUE). Since these microbial processes have seasonal dynamics (Bagchi et al. 2017), we measured them at five time-points during the growing season, between May and September. We followed standard laboratory protocols to measure these microbial variables (Robertson et al. 1999, Bagchi et al. 2017, Roy and Bagchi 2021). Briefly, for BR we used an alkali-trap where we incubated 4 g soil at 60% water holding capacity and measured respired CO_2 (mg C g^{-1} soil day^{-1}). Similarly, we measured potential microbial respiration (mg C g^{-1} soil day^{-1}) using substrate-induced respiration (SIR) after adding 0.5% (w/v) of glucose to soil. We estimated broad microbial community composition as fungal and bacterial contributions to potential respiration under selective inhibition using Streptomycin (anti-bacterial) and Cycloheximide (anti-fungal). We used chloroform fumigation-extraction to estimate MBC (mg C g^{-1} soil), and MBN (mg N g^{-1} soil). We measured SOM from weight loss on ignition at 450 °C after 4 h. We measured labile

fractions (mg g^{-1} soil) of SOM using two-step hydrolysis with H_2SO_4. We estimated metabolic quotient as the ratio of BR and MBC (qCO_2, respiration per unit biomass, hr^{-1}). We estimated CUE from established relationships between key microbial extracellular enzymes (carbon-acquiring Beta-glucosidase, alongside nitrogen-acquiring Leucine aminopeptidase and *N*-acetyl glucosaminidase), MBC and MBN, and labile fractions of C and N in SOM (Sinsabaugh et al. 2013, 2016). We measured activity of the three enzymes using their specific fluorogenic substrates (4-MUB beta-D-glucopyranoside for Beta-glucosidase, L-Leucine-7-amido-4-methylcoumarin hydrochloride for Leucine aminopeptidase, and 4-MUB N-acetyl-beta-D-glucosaminide for N-acetylglucosamindase) (German et al. 2011) in soils from growth season of 2017. These parameters influence microbial metabolism to determine their investment in growth (G) and respiration (R), so that $\mathrm{CUE} = \frac{\mathrm{G}}{\mathrm{G+R}}$ (Sinsabaugh et al. 2013, 2016). Theoretically CUE can range between 0 and 1, i.e., nil to perfect efficiency, with higher values indicating greater potential for C-sequestration. But, CUE in nature generally does not exceed 0.6 (Sinsabaugh et al. 2013, 2016).

SOIL MICROBIAL DNA

Based on patterns seen in fungal and bacterial contribution to soil respiration, we evaluated community composition in more detail using their DNA markers in 2019 (Fierer et al. 2005, Maestre et al. 2015). We collected soils from ten paired-adjacent plots at four time points during the growing season and extracted microbial DNA using MOBIO Power Soil DNA Extraction Kit following manufacturer's protocols. Next, we quantified the Internal Transcribed Spacer (ITS-1 and ITS-2) regions of rDNA for fungal abundance, and region of 16s rDNA for bacterial abundance with qPCR (Fierer et al. 2005, Maestre et al. 2015). We used these genetic data to confirm whether DNA-based estimates align with respiration-based

estimates of fungal and bacterial abundance. Additional details of laboratory analyses are in Appendix.

DATA ANALYSIS

We evaluated competing hypotheses (Tredennick et al. 2021, Betts et al. 2021) to determine whether the observed variation in the data merely represent site-specific and time-specific heterogeneity between replicates, or it is necessary to invoke herbivore assemblage-type (livestock or native) as an explanatory variable. We used a linear mixed-effects model to evaluate long term trends in soil-C and grazer-exclusion effects on each microbial variable. For soil-C, we used a null-model $\left(M_0^{soilC}\right)$ with time (years, 2006-17) and plot identity as random-effects to account for background variation due to sampling locations and times. This model was evaluated against the alternative competing model $\left(M_A^{soilC}\right)$ which included assemblage-type as a fixed-effect, alongside both random-effects. We compared these models based on parsimony, goodness-of-fit, and likelihood-ratio test (i.e., AIC, RMSE, and LR, (Tredennick et al. 2021)).

We estimated grazer-effect on each microbial variable as the log-ratio of values in the fenced and paired-adjacent control plots i.e., $\Delta = ln\left(\frac{\mathrm{Grazing_i}}{\mathrm{Fenced_i}}\right)$ for the ith pair, and calculated the mean and 95% CI of the log-ratio (Roy and Bagchi 2021). As earlier, we first evaluated a null-model to assess whether the observed variation in grazer-effects can simply be attributed to heterogeneity across sampling locations and times (months, May-September). As the null-model for the jth microbial variable $\left(M_0^j\right)$, we used plot identity and month as random-effects. In the competing alternative model for each variable $\left(M_A^j\right)$, we included herbivore assemblage-type as a fixed-effect alongside the random-effects. As before, we used AIC, RMSE and LR for model comparison to judge whether herbivore assemblage-type is a

necessary explanatory variable for the data (Tredennick et al. 2021). We assessed whether the data met statistical assumptions from observed and theoretical quantiles of residuals; the variables required no further transformation (Δ is already in log-scale). We used 'nlme' library in R (Pinheiro et al. 2020) to perform these analyses.

We assessed the inter-relationships between grazer-effects (Δ) on different microbial variables with structural equations models (SEM). SEM help evaluate hypothesized pathways over how change in one variable can influence another (Grace 2006). We identified SEM paths from *a-priori* examples in the literature (Fig. 4-5). To avoid multicollinearity in SEM arising from redundancy among variables, we removed those variables which were either highly correlated with each other ($|r| > 0.49$), or were derived from each other (Fig. S8). So, ΔSIR, ΔB, ΔMBN, and ΔqCO_2 were not included in this step, and we built SEM using ΔMBC, ΔLN, ΔLC, ΔF, and ΔCUE. We did not use genetic data from ITS and 16s rDNA in the SEM because these were collected from a subset of plots, were also not from the same year as the other microbial variables, and because estimates from e-DNA broadly matched respiration-based estimates of community composition. As earlier, we evaluated two competing SEMs – with and without herbivore assemblage-type as an explanatory variable, i.e., SEM_0 and SEM_A. We incorporated SEM paths as mixed-effects models for each variable as piece-wise structural equations (Lefcheck 2016). We assessed goodness of fit with Fisher's C statistic (Shipley 2009, Roy and Bagchi 2021) and report standardised path coefficients alongside their statistical significance (α=0.05). When SEM paths are supported by data, then they offer a plausible explanation for an underlying process. When SEM paths are not supported, the hypothesized process may not give rise to the observed data.

Since the temporal scale and span of the microbial variables (monthly dynamics) are different from soil-C (decadal scale dynamics), SEM_0 and SEM_A by themselves cannot directly evaluate how grazer-effects ultimately influence soil-C via microbes. While these

data have a common spatial dimension (n=30 replicate pairs), they differ in their temporal dimensions. So, we evaluated their connections under the expectation that effects seen on microbial processes (fast variables) can accumulate over years and eventually affect soil-C (slow variables). For this, we first calculated the average (mean effect on j^{th} variable, $\overline{\Delta^{J}}$) across all the months for each microbial variable, and across years for soil-C and grazing intensity, for all replicates. Next, we evaluated SEM paths using these average values to assess whether herbivore assemblage type and grazing intensity explain variation in soil-C via grazer-effects on microbes. Here, plot identity was a random-effect in the SEM paths as this allows the intercept to vary between samples that may have background difference in $\overline{\Delta^{J}}$ due to other factors such as grazing-intensity. Although grazing-intensity did not vary systematically between the native herbivores and livestock (Fig. S7), we evaluated SEM with both identity and intensity as explanatory variables to test for the relative influence of herbivore identity and amount of herbivory.

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Figures

Fig. 1

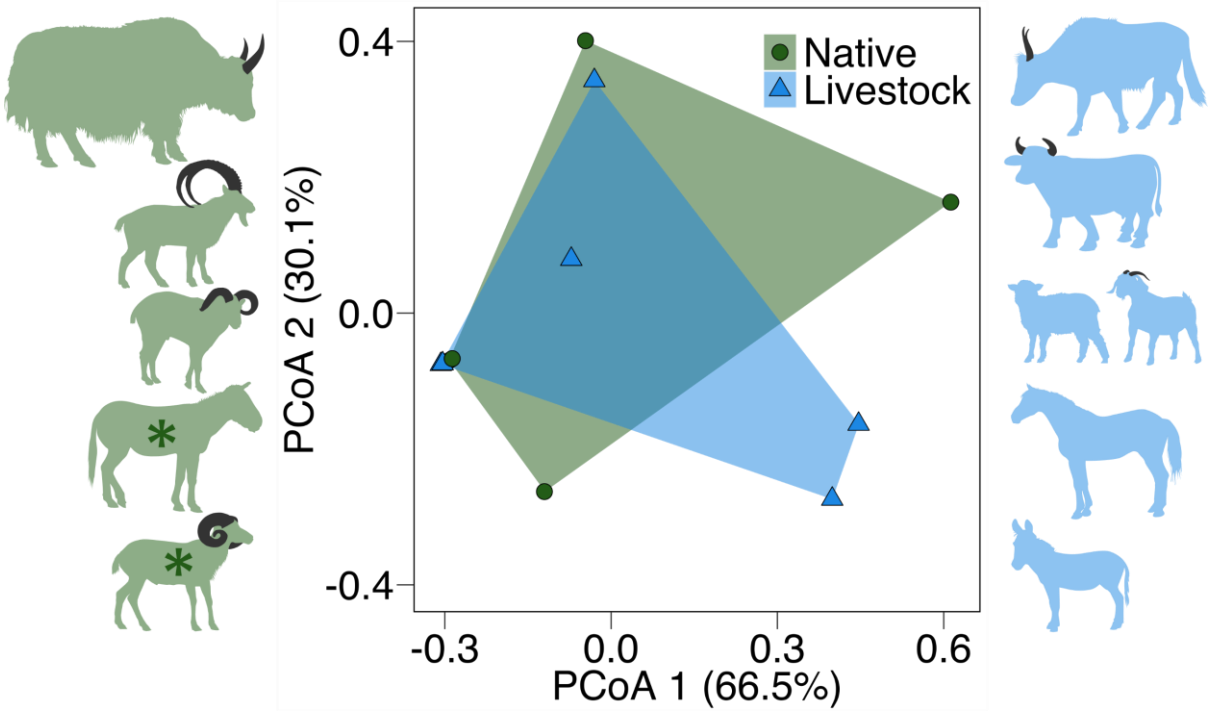

Figure 1. Trait space of livestock and native herbivores in Spiti, India. We find there is considerable overlap in multidimensional trait space between native herbivores (green) and livestock (blue). Native herbivores in the study area are ibex, bharal, yak; asterix (kiang, argali) indicates that these animals are found in nearby regions. The livestock consist of goat, sheep, donkey, horse, cattle and yak-cattle hybrids. Six traits were used to construct the trait-space: body mass, dietary guild, fermentation type, sexual-dimorphism, graminoid, and limb morphology (Lundgren et al. 2020) with Principal Coordinates Analysis on the Gower's distance matrix.

Fig. 2

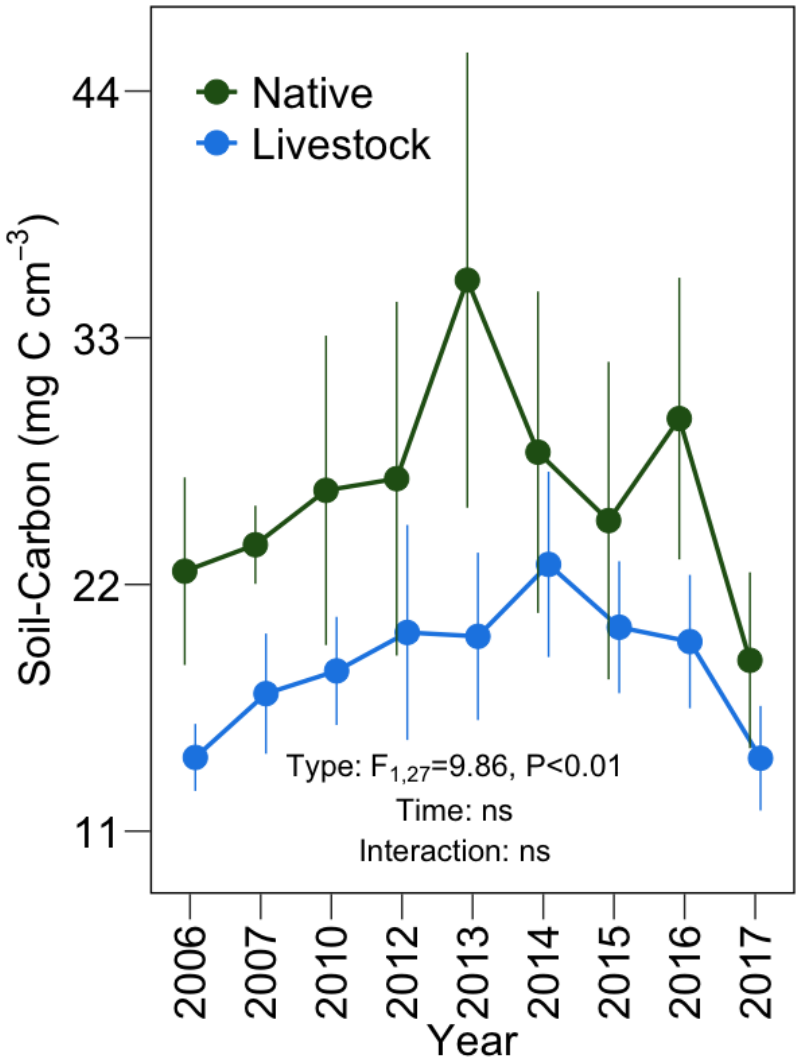

Figure 2. Long-term change in soil-C (mean ± 95CI) under grazing by native herbivores and livestock in Spiti, India. Areas under livestock attained 70% of soil-C compared to areas under native herbivores. This difference (two-thirds) was consistent across more than a decade. Data are from n=30 permanent plots in Spiti region of northern India.

Fig. 3

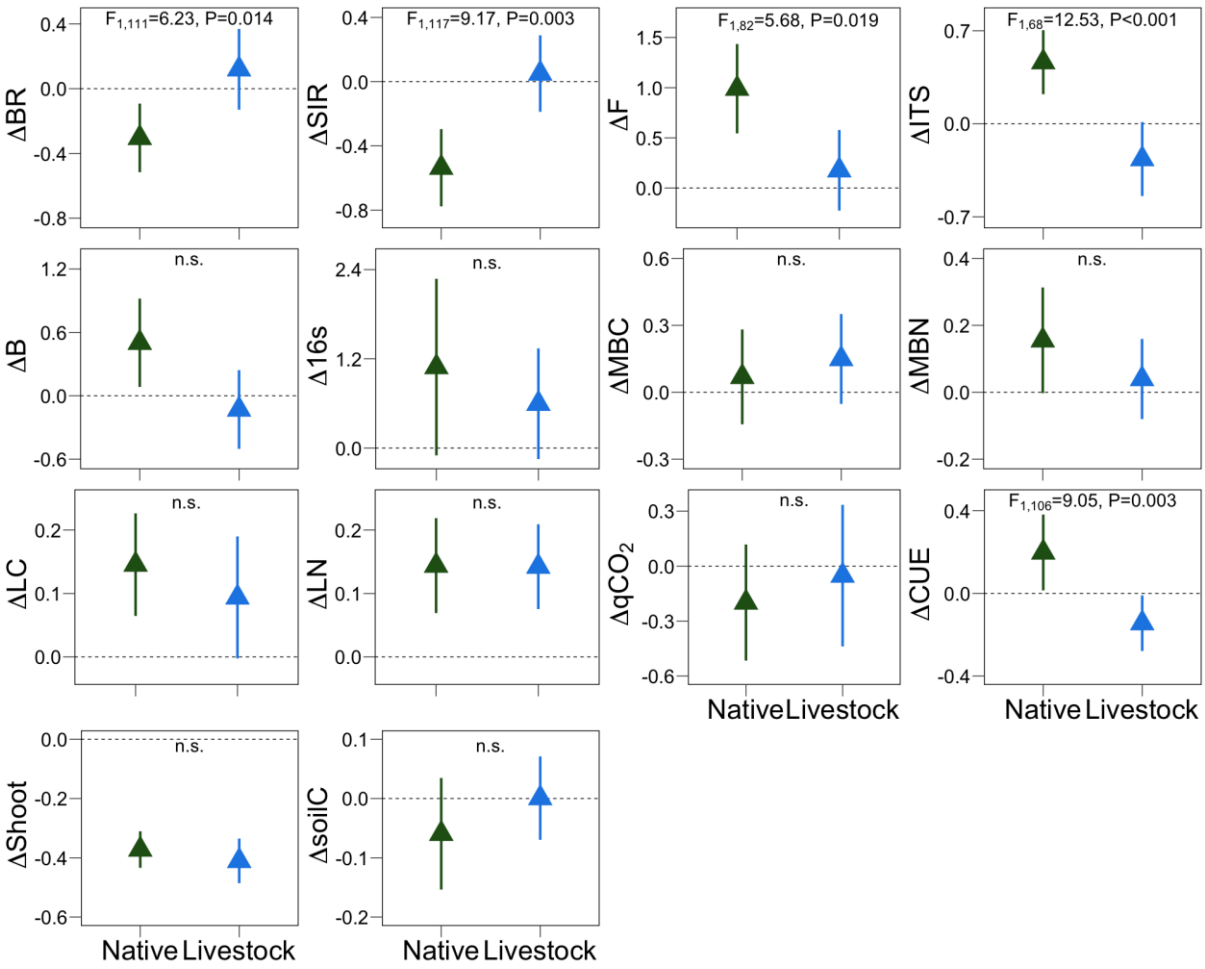

Figure 3. Grazer-effect ($\Delta = ln\left(\frac{grazed}{fenced}\right)$), as mean ± 95CI) of livestock and native herbivores on twelve soil microbial variables, alongside grazer-effects on aboveground biomass and soil-C, in Spiti, India. These variables are basal microbial respiration (BR), potential microbial respiration (SIR), Fungal fraction (F), ITS DNA-amplicon abundance (ITS), Bacterial fraction (B), 16s DNA-amplicon abundance (16s), Microbial Biomass Carbon (MBC), Microbial Biomass Nitrogen (MBN), Labile-C (LC), Labile-N (LN), Metabolic quotient (qCO_2), and microbial carbon use efficiency (CUE) which were measured at monthly intervals during the growth season. Grazing-intensity (ΔShoot: from difference in peak aboveground biomass) and grazer-effect on soil-C (ΔsoilC) are from long-term decadal measurements. Direction and magnitude of differences in BR, SIR, F, ITS abundance, and

CUE varied with herbivore-assemblage type. But the remaining variables were not influenced by herbivore-assemblage type. Data are from n=30 permanent plots in Spiti region of northern India (except e-DNA data, which are from a subset of 20 plots).

Fig. 4

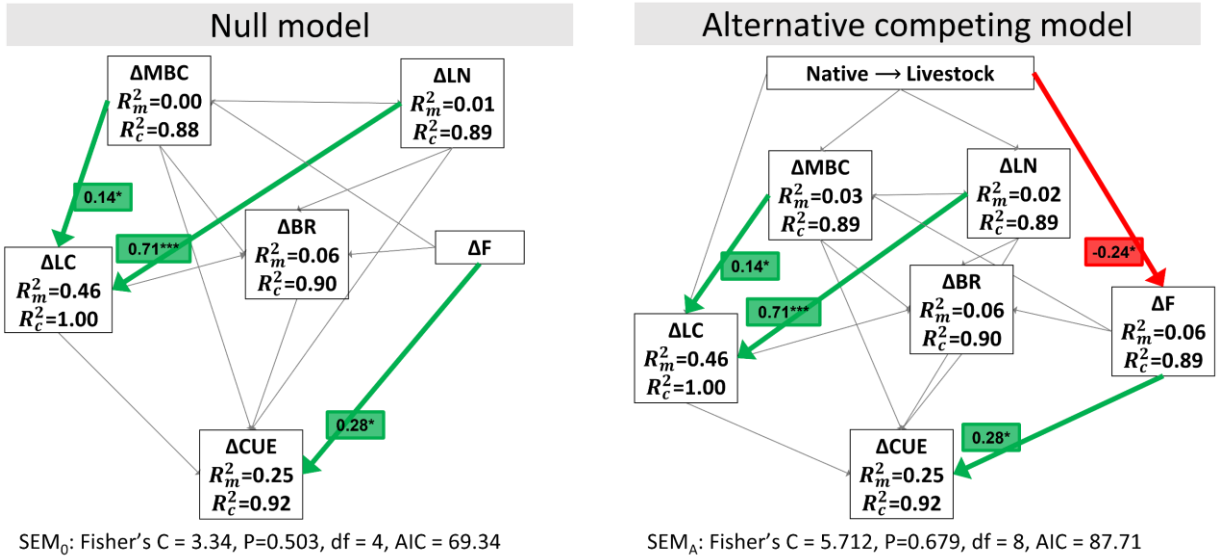

Figure 4. Mixed-effects structural equation models to evaluate two competing hypotheses – with and without herbivore assemblage-type as an explanatory variable, i.e., SEM_A and SEM_0. For each variable, grazer-effect ($\Delta = ln\left(\frac{grazed}{fenced}\right)$) was calculated from grazed and ungrazed paired plots. The variables are ΔBR: basal respiration, ΔMBC: microbial biomass-C, ΔLC: labile-C, ΔLN: labile-N, ΔF: fungal biomass, ΔCUE: carbon use efficiency. In SEM_A, as herbivore-assemblage goes from native to livestock, a decrease in ΔF leads to a decrease in ΔCUE. Alternatively, in SEM_0, increase in ΔF increases ΔCUE, independent of herbivore-assemblage type. Green and red arrows represent significant positive and negative standardized path coefficients respectively, while grey arrows represent non-significant paths (α=0.05). Marginal and conditional R^2 are reported, and asterix represent statistical significance (*** P≤0.001, ** P≤0.01, and * P≤0.05).

Fig. 5.

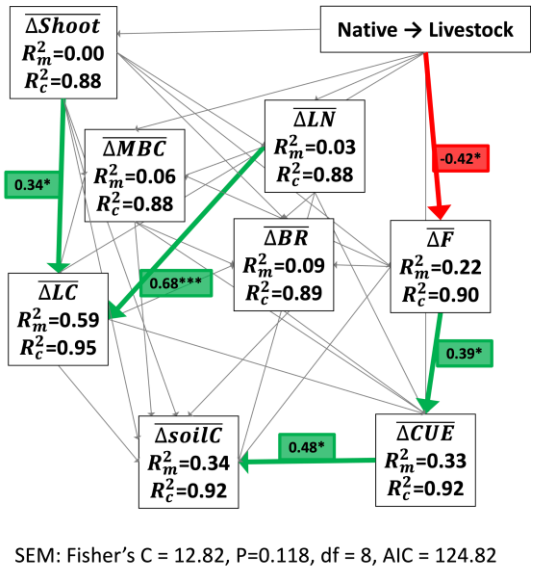

Fig. 5. Mixed-effects structural equation models to evaluate whether grazer-effects on microbial responses explain variation in soil-C. Unlike Fig. 4, here $\bar{\Delta}$ is the mean of grazer-effect averaged across time (months for microbial variables and years for soil-C and grazing-intensity). The helps us evaluates the relative influence of herbivore-identity (assemblage type), and total amount of herbivory (grazing-intensity, $\overline{\Delta Shoot}$) on variation in soil-C. This model shows that replacing native herbivores with livestock alters soil microbial communities to reduce their CUE, and this is ultimately also detrimental for soil-C storage. Green and red arrows represent significant positive and negative standardized path coefficients respectively, while grey arrows represent non-significant paths (α=0.05). Marginal and conditional R^2 are reported, and asterix represent statistical significance (*** P≤0.001, ** P≤0.01, and * P≤0.05).