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Loss of grazing by large mammalian herbivores can destabilize the soil carbon pool

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Grazing by mammalian herbivores can be a climate mitigation strategy as it influences the size and stability of a large soil carbon (soil-C) pool (more than 500 Pg C in the world's grasslands, steppes, and savannas). With continuing declines in the numbers of large mammalian herbivores, the resultant loss in grazer functions can be consequential for this soil-C pool and ultimately for the global carbon cycle. While herbivore effects on the size of the soil-C pool and the conditions under which they lead to gain or loss in soil-C are becoming increasingly clear, their effect on the equally important aspect of stability of soil-C remains unknown. We used a replicated long-term field experiment in the Trans-Himalayan grazing ecosystem to evaluate the consequences of herbivore exclusion on interannual fluctuations in soil-C (2006 to 2021). Interannual fluctuations in soil-C and soil-N were 30 to 40% higher after herbivore exclusion than under grazing. Structural equation modeling suggested that grazing appears to mediate the stabilizing versus destabilizing influences of nitrogen (N) on soil-C. This may explain why N addition stimulates soil-C loss in the absence of herbivores around the world. Herbivore loss, and the consequent decline in grazer functions, can therefore undermine the stability of soil-C. Soil-C is not inert but a very dynamic pool. It can provide nature-based climate solutions by conserving and restoring a functional role of large mammalian herbivores that extends to the stoichiometric coupling between soil-C and soil-N.

biogeochemistry | carbon cycle | phase-space analysis | path analysis | stoichiometry

Soils are a large pool (more than 1,800 Pg) of organic carbon (C) as they contain more C than vegetation and atmosphere combined (1). Additionally, soils also produce an annual efflux of 50 to 75 Pg C/y from land to the atmosphere through heterotrophic respiration, which if not balanced by influx from net primary production can deplete the soil-C pool (2, 3). This soil-C efflux not only is larger than human emissions but also is very sensitive to interannual variation in temperature and precipitation (2–4). The soil-C pool can be easily depleted in response to ongoing climate variability; this can in turn strengthen undesirable positive feedbacks with the global climate system (2, 5), and recovering lost soil-C can be frustratingly slow and uncertain (6). Alongside a growing focus on increasing the soil-C pool, an equally important climate mitigation solution lies in our ability to protect the preexisting soil-C pools, that is, stability (7). It is important to identify and manage various factors that favor the stability of soil-C because small changes in its stability can have large consequences for global climate (8). Moreover, factors that act locally on the stability of soil-C can be globally consequential (9, 10). Currently, our ability to address these aspects is limited by a lack of long-term time-resolved information on soil-C and how various factors influence its stability (11).

Grazing ecosystems play a major role in these land–atmosphere feedbacks because they cover about 40% of the world's terrestrial ice-free surface (nearly 50 million km^2) across grasslands, savannas, and shrub steppes, and they contain about a third of all soil-C (1, 12). On one hand, grazing ecosystems have a high potential to provide C-sinks via soil-C storage (6, 13–15), and the reliability of this potential C-sink is aided by climatic as well as edaphic factors (16). On the other hand, grazing ecosystems drive much of the interannual variability in the global C-cycle (17). Here, we are beginning to understand the role of large mammalian herbivores in the zoogeochemistry of the C-cycle (15, 18–20), and their potential for natural climate solutions is emerging (7). While considerable advances have been made in our understanding of how herbivores influence the size of the soil-C pool (15, 21–24), we know relatively little about whether they also influence its stability (25). Since inadequate grazing management can lead to rapid loss of soil-C, which cannot be easily recovered by improved management (6), how herbivores mediate the stability of soil-C remains important for C sequestration.

Various lines of evidence point to a strong influence of large mammalian herbivores on the size of the soil-C pool. For instance, herbivores consume plant biomass, altering

Significance

Grazing ecosystems store vast amounts of soil carbon (soil-C), and are experiencing a gradual loss of large mammalian herbivores that can affect ecosystem functions and services. While we increasingly understand how herbivores influence the size of the soil-C pool, their influence on the equally important aspect of temporal stability of soil-C remains unknown. We address this uncertainty in how large herbivores influence climate through soil-C. We find that while soil-C generally shows high interannual variability, grazing increases the temporal stability of soil-C, and grazing appears to mediate the influence of nitrogen on the stability of soil-C. Therefore, conserving large mammalian herbivores in grazing ecosystems remains a priority to ensure the persistence of soil-C to achieve nature-based climate solutions.

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the quantity as well as quality of C-input to the soil, which can influence belowground nutrient cycling (22, 26). They also modify vegetation composition (27), which can shift the underlying distribution of plant traits (e.g., C:N of leaf and litter) and in turn alter the quality and quantity of C input to soil (28, 29). Overgrazing depletes soil-C stocks; under moderate grazing intensities, these direct and indirect effects of herbivores can result in net soil-C gains under a wide range of environments, such as temperate grasslands (30), alpine steppes (15), subtropical shrub steppes (31), subtropical grasslands (32), and tropical savannas (33). Since this net positive effect on soil-C stocks can help decarbonize the atmosphere (21–23, 34), rewilding and conservation of large mammalian herbivores holds promise as a climate mitigation strategy (15, 35–37) given ongoing declines in their functional influence on ecosystems (38).

In comparison, there is scant evidence to assess whether herbivores also influence the stability of soil-C. However, previous studies allow us to envision the likely influence of herbivores on the stability of soil-C. For instance, globally distributed experiments across the world find that herbivore exclusion reduces the long-term stability of vegetation composition (39). A similar effect is also known for stability of plant production (40, 41), and these above-ground influences of herbivores should also be consequential below ground (42, 43). Furthermore, plants capture CO_2 from the atmosphere and nutrients from the soil, thereby coupling the carbon and nitrogen (N) cycles, and this coupling is maintained downstream in the soils through detritus and microbial processes (26, 44). Relatively long-term studies covering intervals of 5 to 10 y or longer (45–47) have found that N exerts a strong control on the dynamics of soil-C. Parallelly, studies have also found that herbivores exert a strong influence on N in soil (28, 33, 47). Therefore, herbivores and N, individually and in tandem, are likely to be consequential for the stability of below-ground C-pools (39), and herbivores may safeguard soil-C by diminishing perturbations in the below-ground pools (25). Yet, whether and how herbivores mediate the stability of soil-C and whether this is related to soil-N remain unknown because of near complete lack of long-term temporally resolved data to evaluate these questions (11). For instance, a review of soil functions from 17,186 locations around the world found temporal dynamics in soil-C are a major blind spot (11). Since changes to the soil-C pool can contribute to both local and global C cycling (8, 24), it is important to know how temporal variability translates to the stability of soil-C (17) where local factors are crucial for conservation of soil-C (9, 10).

Here we tested competing alternative hypotheses to evaluate the role of herbivores in the stability of the soil-C pool by using long-term data on interannual fluctuations in C and N in soil. We used a replicated field experiment (*Materials and Methods*) with grazed plots (herbivores-present and control) paired with fenced exclosures (herbivore exclusion and treatment). First, we estimated the interannual variability in soil-C and soil-N pools and whether it could be attributed to grazers after accounting for background spatiotemporal heterogeneity. Next, we evaluated metrics of temporal stability of soil-C jointly with that of soil-N, in the presence and absence of herbivores (*Materials and Methods*). Finally, we used structural equation models (SEMs) to quantify how herbivore effects on soil-C and soil-N pools could be related to stability of soil-C. (*Materials and Methods*). Specifically, we asked whether the stability of soil-C differs between grazed and fenced plots, and if so, what are the important paths that can favor the stability of soil-C.

Results

Soil-C (mean ± SD) across all plots and all years was 4.50 ± 2.33 kg C/m^2 (coefficient of variation [CV] = 51.7%) in the top 20 cm of the soil. Temporal variation in soil-C (CV of each plot through years, averaged for all plots) was 32.6%, and spatial variation in soil-C (CV of each year across all plots, averaged for all years) was 46.1%. Similarly, soil-N (mean ± SD) was 0.37 ± 0.16 kg N/m^2 (CV = 43.1%) with spatial and temporal variation of 34.1% and 37.5%, respectively. This variation in soil-C was explained by random effects of year and plot identity, alongside background correlation with soil-N (H_0: $F_{1,311} = 316.7$, $P < 0.0001$; Fig. 1). However, the alternative model with grazing as a fixed effect was a better explanation ($H_1 - H_0$: Δ root-mean-squared error [RMSE] = −0.015; Δ Akaike information criterion [AIC] = −4.31, likelihood-ratio [LR] = 8.31, $P = 0.016$). Importantly, there was a significant interaction between grazing and soil-N (H_1: $F_{1,309} = 6.93$, $P = 0.009$; *SI Appendix*, Table S2), indicating that N influences soil-C differently in the presence or absence of herbivores.

Univariate temporal indices (1/CV) indicated that soil-C and soil-N were more stable in grazed plots than under herbivore exclusion (*SI Appendix*, Fig. S6), and phase space analysis revealed different aspects of their joint dynamics (Fig. 2). All three phase space metrics showed soil-C and soil-N to be more stable under grazing compared to herbivore exclusion (Fig. 2). Edaphic factors as covariates—soil pH and soil texture—did not explain the observed variation in phase space stability metrics (*SI Appendix*, Table S3). Effect size (mean ± SE) for average distance between successive time intervals ($\bar{x}$) was log response-ratio [*LRR*] = 0.26 ± 0.09, or 30.1% higher in fenced plots than under grazing ($F_{1,26} = 18.3$, $P < 0.001$). Similarly, effect size for total distance covered was *LRR* = 0.26 ± 0.09, or 30.1% higher ($\sum x$, $F_{1,26} = 14.4$, $P < 0.001$); effect size for the area of the convex hull was *LRR* = 0.33 ± 0.12, or 39.2% greater ($F_{1,26} = 6.9$, $P = 0.014$) after herbivore exclusion (Fig. 2). Effect sizes in watersheds used primarily by livestock

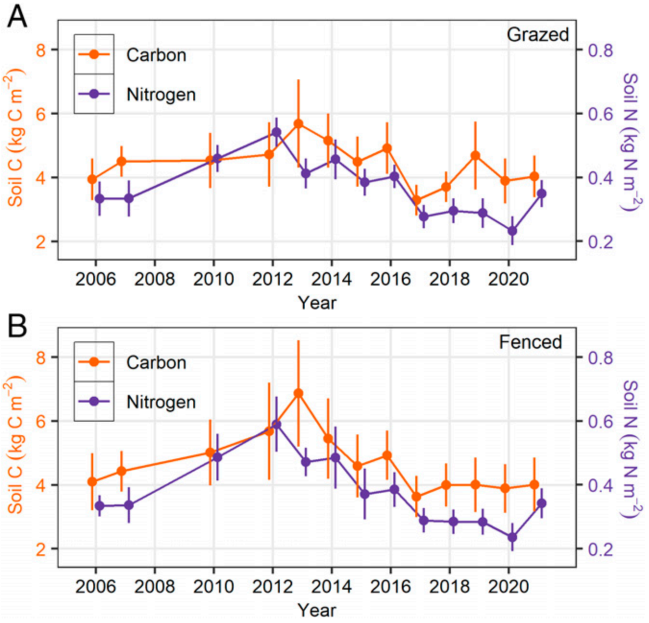

Fig. 1. Interannual fluctuations in average soil-C and soil-N show high variation in these pools (mean ± 95% CI) over decadal time scales (2006 to 2021). Data are from experimental herbivore exclusion for $n = 30$ paired (*A*) grazed and (*B*) fenced plots in the Spiti region of northern India (see *SI Appendix*, Fig. S1 for maps of the study area).

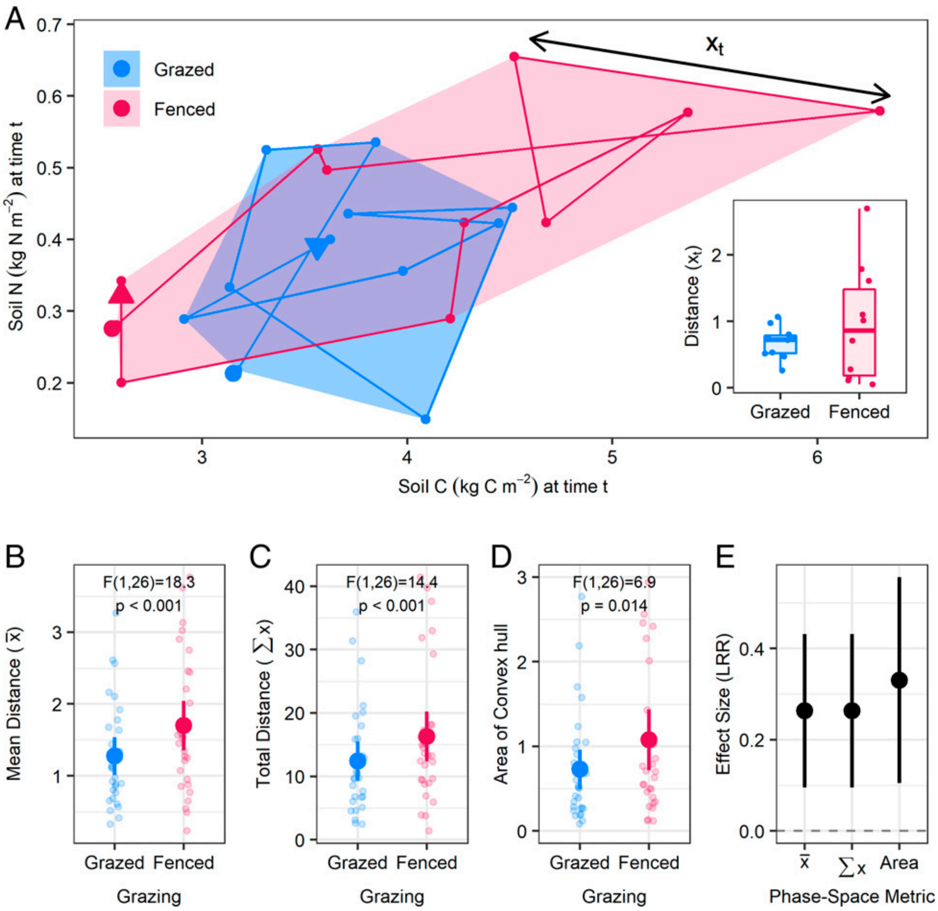

Fig. 2. Phase space analysis of joint fluctuations in soil-C and soil-N showed greater stability in the presence of herbivores than under herbivore exclusion. (*A*) Illustrative data from one representative paired and adjacent grazed and fenced plot over 16 y (2006 to 2021). See *SI Appendix*, Fig. S7 for all (n = 30) replicates. The lines represent the trajectories from 2006 (circle) to 2021 (arrow). Here "x_t" represents the distance between two consecutive measurements t_1 and t_2, with distribution of x_t shown as inset. The shaded region represents the area covered over 16 y. (*B*–*D*) Summary of three phase space metrics (mean ± 95% CI) for all plots and (*E*) overall effect size as $LRR = \ln\left(\frac{Fenced}{Grazed}\right)$.

were comparable to those in watersheds used primarily by native herbivores (*SI Appendix*, Fig. S8).

SEMs revealed that the data were consistent with the hypothesized paths where grazers influence the stability of soil-C via their direct and indirect effects on soil-N. First, in the full model, fluctuations in soil-C (ΔC) were affected directly by the sizes of the soil-C and soil-N pools and by interactive effects of herbivores (Fisher's C = 1.18, P = 0.55, degrees of freedom [df] = 2; AIC = 53.2; Fig. 3*A*). Expectedly, grazing influenced both the size and fluctuations of the soil-C pool (Fig. 3*A*). Since grazers influenced fluctuations in soil-C through N, both directly ($N_t \xrightarrow{\times grazing} \Delta C$; path coefficient = 0.20, P = 0.09) and indirectly ($N_t \xrightarrow{\times grazing} \Delta N \leftrightarrow \Delta C$; path coefficient = 0.32, P = 0.004; Fig. 3*A*), we explored SEMs for subsets of the data from grazed and fenced plots. When modeled separately, SEMs from grazed (Fisher's C = 0.68, P = 0.71, df = 2; AIC = 22.7) and fenced plots (Fisher's C = 1.45, P = 0.48, df = 2; AIC = 23.5) revealed differences in paths that influence fluctuations in soil-C (Fig. 3 *B* and *C*). Specifically, in the presence of grazers, soil-N was negatively related to ΔC, suggesting a stabilizing effect of soil-N on soil-C (path coefficient = −0.14, P = 0.02; Fig. 3*B*). This stabilizing effect was absent under herbivore exclusion (path coefficient = −0.002, P = 0.98; Fig. 3*C*). SEMs showed coupling between the sizes and between the interannual fluctuations of soil-C and soil-N, but this coupling was stronger under herbivore exclusion (Fig. 3 *B* and *C*). The alternative hypothesis where the directionality was reversed, showed no influence of soil-C on the stability of soil-N (*SI Appendix*, Fig. S9).

Discussion

Large mammalian herbivores are integral to the zoogeochemistry of the C-cycle (15, 18–20). From their influence on above- and below-ground C-pools, we anticipated that they should affect not only the size of the soil-C pool but also its stability. The data show that the soil-C pool is not inert but undergoes large interannual fluctuations (Fig. 1); grazing by herbivores favors stability of soil-C relative to herbivore exclusion (Fig. 2), and the favorable effect of herbivores appears to arise from a stabilizing effect of soil-N on soil-C (Fig. 3). Seen together, the functional role of large mammalian herbivores emerges as an important component of nature-based climate solutions that promotes the reliability of grazing ecosystems as C sinks (15, 25, 35–37).

Interannual fluctuations in soil-C are expected in subhumid regions (Fig. 1) since they experience successive wet and dry years that can alter C influx from net primary production, C efflux from soil microbial respiration, and the balance between these two opposing fluxes (2). Indeed, grazing ecosystems contribute substantially to the interannual variability of the global carbon cycle (17). In our analysis, we considered grazing as a binary variable (grazed vs. fenced), and this along with elements of our study design (fixed and random effects) explained about two-thirds of the variation in soil-C (Fig. 1). The remaining

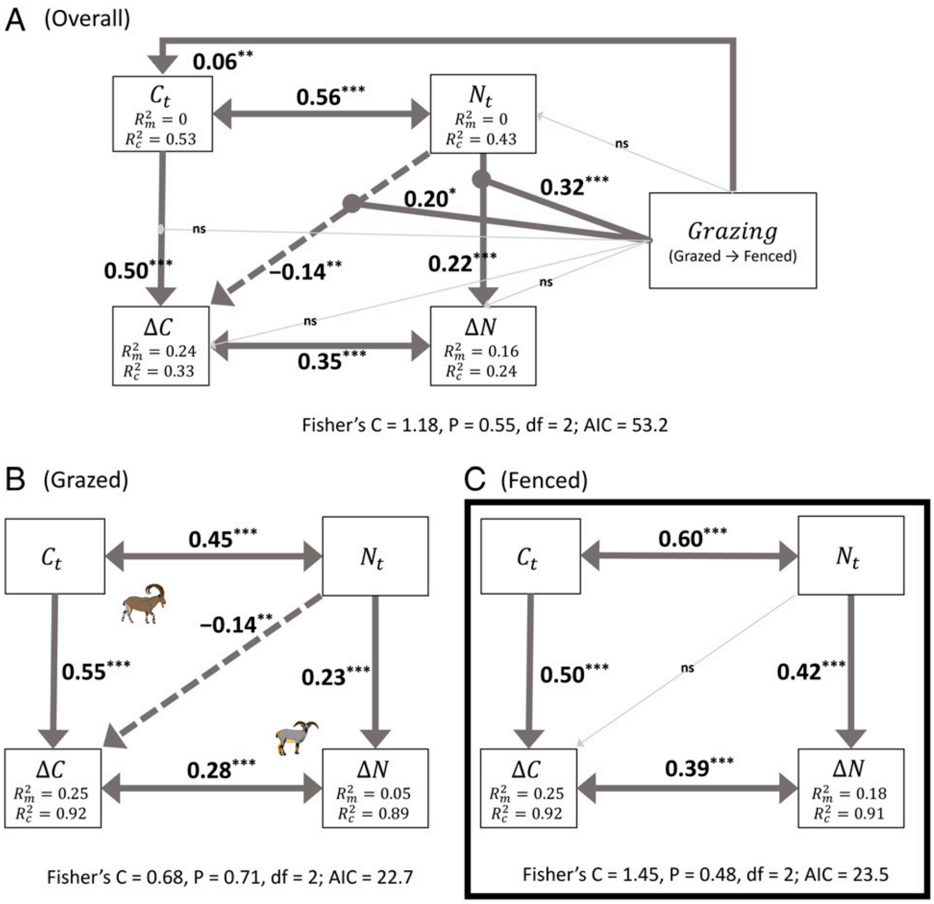

Fig. 3. Structural equation models summarizing the paths that can stabilize (negative coefficient, dashed arrow) or destabilize (positive coefficient, solid arrow) soil-C. (*A*) SEMs for the full model show herbivores have an interactive effect on how N affects the stability of soil-C. (*B*) SEM for grazed plots shows a stabilizing effect of N on soil-C in the presence of herbivores. (*C*) This stabilizing effect is absent under experimental herbivore exclusion. Unidirectional arrows represent hypothesized causal paths, and bidirectional arrows represent correlated paths. Each path is estimated from linear mixed-effects models, and data indicate good support for the hypothesized paths in each SEM. Rounded arrowheads in *A* represent interactive effects of grazing in the modeled SEM paths. Asterisks represent statistical significance of path coefficients ($^{***}P \le 0.005$, $^{**}P \le 0.05$, and $^{*}P \le 0.1$), and nonsignificant (ns) paths are shown with thin gray lines. Overall, herbivores show a strong effect on the size and stability of the soil-C pool through their effects on soil-N.

unexplained variation could be related to how other unmeasured factors can cause the degree of grazing to vary across space and time. The extent to which this variability is mediated by large mammalian herbivores is an important dimension of how they impact climate, particularly through stability of soil-C. Indeed, we find that grazing increases the stability of soil-C (Fig. 2). Since neither livestock nor native herbivores had a dominant impact on the results (*SI Appendix*, Fig. S8), this suggests that domesticated animals can, at least partially, approximate some of the functional roles of native wildlife (25). Grazing impacts on the size of the soil-C pool vary substantially along geographic and geologic gradients of edaphic factors, and they have variable effects between different ecoregions. For instance, grazing can favor soil-C in sandy soils more than in soils with finer texture (22). However, we did not find any corresponding systematic covariation between grazer effects on stability of soil-C and background edaphic factors in our data (*SI Appendix*, Table S3). This does not imply that grazer effects are independent of edaphic conditions, and one should not expect them to be invariant across different ecosystems. For instance, if soil-C preferentially enters the less reactive mineral-associated pool of soil organic matter instead of the more reactive pool of particulate matter, then its stability will probably vary between ecosystems (16, 25). This aspect remains an active area of research (16, 25), and more detailed investigations are needed to identify how it plays a role in stabilizing soil-C. The paths from SEM (Fig. 3) also suggest candidate mechanisms for how grazing influences the stability of soil-C. While SEM paths do not necessarily represent causal connections, they point toward testable hypotheses about underlying mechanisms that receive support from the variance and covariance in the data. Below we discuss the potential implications of these hypothesized paths (Fig. 3) as three interrelated questions, and potential explanations for the purported underlying mechanisms that can be subjects for future studies.

How Can the Size of the Soil-C Pool Negatively Influence Its Own Stability? We find that greater soil-C stocks at time t_1 increase the fluctuations in soil-C at the next time step t_2 ($C_t \rightarrow \Delta C$; Fig. 3). This relationship, though counterintuitive, is well known. Carbon input from plants, a major source of energy for microbial decomposers, stimulates microbial activity through priming, increasing the decomposition of soil-C and its release as C efflux (48), thereby destabilizing the soil-C pool. The effect of priming is present in both grazed and fenced plots (Fig. 3 *B* and *C*), because soil microbes can be inherently C limited (49) in both the presence and absence of grazers. Signatures of such priming effects are seen in controlled laboratory experiments (48) and in global meta-analyses of field studies (50).

How Can Soil-N Influence the Stability of Soil-C? We find that there is a direct ($N_t \rightarrow \Delta C$) and an indirect effect of soil-N ($N_t \rightarrow \Delta N \leftrightarrow \Delta C$) on the fluctuations in soil-C (Fig. 3). This allows N to have highly variable (positive, negative, or neutral) effects on soil-C (51), due to multiple overlapping mechanisms. In a similar high-elevation and dry ecosystem, N increased the decomposition of labile soil organic matter (51). As this would release readily usable carbon sources for microbes to metabolize, it effectively mimics the priming effect and therefore can also destabilize soil-C (48). In parallel, N also reduced the decomposition of recalcitrant fractions of soil organic matter, thereby stabilizing soil-C (51). Thus, the net effect is the balance between these opposing influences of N on soil-C. These stabilizing and destabilizing effects have been encountered in the synthesis of globally distributed field experiments on how N affects soil-C (52).

How Can Grazer Effects of N Influence Soil-C? We find that herbivores have interactive effects on how N influences the fluctuations in soil-C directly ($N_t \xrightarrow{\times grazing} \Delta C$), and indirectly through fluctuations in soil-N ($N_t \xrightarrow{\times grazing} \Delta N \leftrightarrow \Delta C$) (Fig. 3). Specifically, a potentially stabilizing effect of N on soil-C is absent under herbivore exclusion (Fig. 3). This indicates a shift in the balance between stabilizing and destabilizing effects of N on soil-C (51, 52), which can be due to multiple overlapping mechanisms. First, grazers digest plant biomass (high C:N) and return dung and urine (low C:N), thereby altering the relative proportion of labile and recalcitrant organic matter input to soil as compared to fenced plots. Second, this shift also modifies how microbes forage, using extracellular enzymes to degrade soil organic matter (15, 48). Third, this shift can also change microbial community composition, which in turn alters the rate of decomposition (53). Fourth, this can also influence soil aggregate formation (54, 55) into mineral-associated organic matter and particulate organic matter fractions that differ in their susceptibility to perturbations (16, 25). These processes are not mutually exclusive and may act simultaneously in the same direction, and their relative importance can vary between ecosystems and along environmental gradients. Together, these processes may allow herbivores to have a favorable effect on soil-C in many ecosystems. From Fig. 3, one expects N can destabilize soil-C under herbivore exclusion. Indeed, N addition leads to greater soil-C loss in the absence of herbivores in globally distributed field experiments (33). These experiments encompassed a wide range of herbivore species, soil types, and climate, and documented 10% loss in soil-C within 5 y of herbivore exclusion and N addition (33). Such losses could be a consequence of reduced stability in absence of herbivores, and they suggest that our result—herbivores increase the stability of soil-C through their effects on N—may be general pattern driven by a handful of overlapping mechanisms across different ecosystems (15, 16, 25, 48, 51, 56).

While global consensus on estimating the annual fluxes of soil-C has gained importance over the past two decades (2–4), the interannual changes in the soil-C stocks remain understudied (11). Previous studies on temporal dynamics of soil-C generally used long time intervals (5 to 10 y apart) and assume that the accrual or loss of soil-C is slow (45–47). Importantly, faster processes at interannual scales can be consequential for global terrestrial C-fluxes (3), particularly in dry ecoregions (17). As a thought experiment, we resampled our time series data to obtain datasets with different time intervals (e.g., t_1 and t_2 are 5 y apart), to assess how the length of time intervals influenced the interpretation of dynamics in soil-C. We repeated this thought experiment for different time intervals (from 1 y to 7 y; *SI Appendix*, Fig. S10). Not surprisingly, we found that the effects of grazers on soil-C dynamics became undetectable at long time intervals, and one can easily reach a potentially erroneous conclusion that herbivores do not influence ecosystem functions, or they have inconsistent effects (*SI Appendix*, Fig. S11). This reiterates that soil-C (also soil-N) is a dynamic and reactive pool, and therefore estimating these pools at appropriate time scales is necessary for our understanding of the global C-cycle (1, 9, 12, 17, 45). Since climate variability is expected to increase over both space and time (57, 58), this in turn can potentially increase the interannual fluctuations in soil-C pools globally, in the near future. This highlights the importance of identifying and managing the factors that control the stability of soil-C, such as large mammalian herbivores (Figs. 2 and 3). Since the functional influence of large mammalian herbivores is declining (38) alongside a simultaneous increase in N deposition (33), we are at risk of losing a safeguard of soil-C and a natural climate solution in grazing ecosystems.

Overall, we conclude that herbivores exert strong influence on both the stability and the size of the soil-C pool, and their persistence is essential for decarbonization services derived from grazing ecosystems. Their local extinctions can also exacerbate the consequences of the global N deposition challenge. Conservation and rewilding of large mammalian herbivores should remain a priority to protect the soil-C pool and achieve natural climate solutions.

Materials and Methods

Study Area. We quantified long-term effects of herbivores on soil functions in the Spiti region of the Trans-Himalayan ecosystem in northern India (32° N, 78° E; *SI Appendix*, Fig. S1). This is a high-altitude grazing ecosystem spread across the Tibetan highlands and Central Asian mountains (3,800 to 4,500 m asl). The climate of this region is cold and semiarid, with high seasonality in temperature and precipitation (*SI Appendix*, Fig. S2). Temperatures drop to −30 °C during the winter between November and March, and the growth season extends between May and August (peak in July and August) with temperatures near 20 °C (*SI Appendix*, Fig. S2). Precipitation occurs as snow in winter (200 to 300 mm/y) and as rain during the summer monsoons (50 to 200 mm/y, *SI Appendix*, Fig. S2). In addition, this region also experiences high interannual variation in water availability (e.g., dry years and wet years), which affects plant production (*SI Appendix*, Figs. S3 and S4). Vegetation consists of perennial grasses, sedges, forbs, and shrubs, with above-ground plant production between 40 and 50 $g/m^2/y$ (29). The soils are sandy loam with near-neutral pH (7.3 ± 0.2; mean ± SD), bulk density of 1.1 ± 0.1 g/cm^3, and water holding capacity of 1.0 ± 0.1 mL/g. The grazers are native herbivores (bharal, *Pseudois nayaur*; ibex, *Capra sibirica*; yaks, *Bos grunniens*), and livestock include cattle, yak–cattle hybrids, horses, donkeys, sheep, and goats. Their body sizes range between 35 and 40 kg (goats and sheep) and 400 kg (yaks). The livestock and native herbivores show little overlap in their spatial distribution (29), and they occupy distinct watersheds demarcated by natural barriers such as steep escarpments, deep gorges, and high ridges that constrain how easily and frequently animals can move across the mountainous terrain (*SI Appendix*, Fig. S1). In the past two decades, native herbivore biomass has been 1.0 to 1.2×10^5 kg and livestock biomass has been 1.3 to 1.5×10^5 kg over an area of 40 to 50 km^2 in the watersheds (*SI Appendix*, Fig. S1) surrounding village Kibber (29, 59).

Field Experiment. We established $n = 30$ herbivore exclosures (fenced plots, 10 m × 10 m) starting in 2005, each with a paired-adjacent grazed plot (control). These were distributed across eight watersheds around village Kibber (*SI Appendix*, Fig. S1), with three or four paired plots per watershed. Four watersheds were used primarily by native herbivores and the remaining four by the livestock (29). We collected soil samples from these paired plots during the peak growth season (July to August) 13 times during 2006 to 2021 with a corer (5 cm diameter and 20 cm depth). We determined soil-C and soil-N content (TruSpec, Leco, USA till 2019; thereafter Elementar Analysensysteme, Germany) to estimate their stocks up to 20 cm depth in kg C/m^2 and kg N/m^2, respectively. This long-term experimental record at decadal scales provides us an opportunity to understand how interannual fluctuations in soil-C can help address various outstanding questions on the role of herbivores in ecosystem functioning (11). Turnover times for soil-C in subhumid ecoregions is 10 to 30 y, whereas they can be 100 to 500 y in more mesic conditions such as tundra and swamps (1, 12), so the duration of our study should capture the dynamics of soil-C in this ecosystem (9, 17). We expect temporal dynamics in soil-C, rather than an inert pool, because of interannual variability in plant production (17). For instance, the last two decades have experienced substantial variation in the form of wet and dry years, alongside variation in plant biomass that was evident in our plots and in the appearance of vegetation from outer space across the general region (*SI Appendix*, Figs. S3 and S4). Together, these can influence C influx to soil from vegetation and C efflux from soil heterotrophic respiration (2–5), which result in interannual variations in soil-C stocks (1, 12). Next, we expect temporal variability in soil-C to be related to grazing because previous studies in this ecosystem have found herbivores influence both the above- and below-ground compartments (15, 29, 53, 60). For instance, herbivore exclusion is known to increase shoot, root, and litter biomass and also increase soil microbial biomass (*SI Appendix*, Fig. S5). Simultaneously, herbivore exclusion also alters C:N stoichiometry of plants, litter, and soil microbes (*SI Appendix*, Fig. S5). From these grazer effects in this ecosystem (*SI Appendix*, Fig. S5), and in other ecosystems [e.g., temperate grasslands (30), alpine steppes (15), subtropical shrub steppes (31), subtropical grasslands (32), and tropical savannas (33)], we expect that above-ground impacts of grazers should also influence below-ground processes (15).

Our objective is to estimate the temporal variability in soil-C and soil-N and assess whether it can be attributed to the experimental treatment (herbivore exclusion). From this we address one important uncertainty in the zoogeochemistry of large herbivores. Soil-C and soil-N are of biotic origins but can vary with natural gradients of edaphic factors such as pH, soil texture, and mineralogy (16, 22). Since these edaphic factors are geologically determined, they can vary between ecosystems depending on the parent material of the soil and can change over millennial time scales (e.g., mineral weathering) rather than over years to decades. As our herbivore exclusion experiment used paired and adjacent treatment and control plots, we did not observe large and systematic differences in several edaphic variables due to herbivore exclusion (*SI Appendix*, Table S1). However, there were small differences in soil pH and texture (*SI Appendix*, Table S1), and we assessed their potential influence as covariates alongside that of herbivore exclusion (see below).

Data Analysis.

Linear models. We estimated variation in soil-C and soil-N as their respective coefficients of variation CVs, defined as the ratio of the SD and mean. Temporal variation was the CV of a plot across all years, averaged across all plots. Similarly, spatial variation was the CV for each year across all plots, averaged across all years. We used linear mixed-effects models to evaluate alternative competing hypotheses on the explanatory variables for long-term variation in soil-C. First, we considered that variation in soil-C merely reflects spatiotemporal heterogeneity and background correlation with soil-N (i.e., H_0). For this H_0, we used time (year, 2006 to 2021) and plot identity (30 replicates) as nested random effects, and soil-N as a covariate. Next, we evaluated whether grazing (grazed vs. fenced) is a necessary explanatory variable (H_1) by including it as a fixed effect, in addition to covariation with soil-N and spatiotemporal heterogeneity. We compared these competing explanations based on goodness-of-fit, parsimony, and likelihood ratio tests (i.e., root-mean-square error, AIC, and LR) in the "nlme" package in R. From theoretical and observed quantiles of the residuals, we found that the data did not require any transformations to meet the assumptions of the linear models.

Temporal stability. An intuitive and widely used approach to quantify stability is the inverse of the CV. However, this may not be adequate because of the nonindependence between the estimates of mean and variance, and because its univariate characteristic does not account for any interactive effects of soil-N on soil-C (*SI Appendix*, Fig. S6). We use phase space analysis to overcome these shortcomings to quantify joint variation in soil-C and soil-N. Phase space represents simultaneous changes in more than one variable over time (e.g., soil-C and soil-N) and can quantify various aspects of stability in dynamic systems (61). Phase space analyses have a wide variety of applications for ecological systems where, for example, the contraction or expansion of phase space indicates gain or loss of stability (62, 63), as these geometric properties reflect changes in the temporal dynamics. We built two-dimensional phase spaces of soil-C and soil-N for each paired and adjacent treatment and control plot through time to derive three phase space metrics (*SI Appendix*, Fig. S7). First, we estimated the distance traveled between two successive time points (t_1 and t_2) as $x_t = \sqrt{(C_{t1} - C_{t2})^2 + (N_{t1} - N_{t2})^2}$. Next, across the duration of our study, we estimated mean distance traveled ($\overline{x}$) and the total distance traveled ($\sum x$). Lastly, we estimated the area of the polygon (convex hull) traversed over the duration of our study. We used linear mixed-effects models with grazing (grazed vs. fenced) and edaphic covariates as fixed effects and plot identity as a random effect to evaluate whether the phase space metrics differ in the presence and absence of herbivores (*SI Appendix*, Table S1). As covariates, we included soil pH and texture. For texture, we included clay and sand content; we did not include silt to avoid multicollinearity since the three fractions add up to unity. We estimated the overall effect size in each phase space metric for the *i*th paired plot as log response-ratio $LRR_i = \ln\left(\frac{Fenced_i}{Grazed_i}\right)$. For each metric, $LRR > 0$ indicates increase, $LRR < 0$ represents decrease, and $LRR = 0$ suggests no change, respectively, under herbivore exclusion as compared to the presence of herbivores. To evaluate whether livestock or native herbivores had a dominant effect on overall soil-C stability, we compared the *LRR* of the three phase space metrics between their respective watersheds.

Structural equation models. We used structural equation models (SEMs) to evaluate potential interactive effects of soil-N and herbivores on the stability of the soil-C pool. SEMs can test competing alternative hypotheses based on paths linking predictor and response variables (64). Statistical significance of SEM paths suggests whether an underlying process envisioned as paths can give rise to the observed data (64). We built SEM paths by using linear mixed-effects models in "piecewiseSEM" package in R (65). The predictor variables were grazing (grazed vs. fenced plots) and the sizes of soil-C and soil-N pools. The response variable was absolute change in interannual soil-C fluctuations between consecutive time points ($\Delta C = |C_{t1} - C_{t2}|$, and similarly for ΔN). We used linear mixed-effects models for each hypothesized path with time (t; year identity) nested within plot identity as a random effect (65, 66).

The hypothesized paths for the SEMs were motivated by evidence from previous studies. a) Herbivores alter the size and fluctuations of both soil-C (Grazing $\longrightarrow C_t$, Grazing $\longrightarrow \Delta C$) and soil-N (Grazing $\longrightarrow N_t$, Grazing $\longrightarrow \Delta N$) (33). b) The size of the soil-C pool influences its own fluctuations ($C_t \longrightarrow \Delta C$) (48), and similarly for soil-N ($N_t \longrightarrow \Delta N$) (51). c) N affects fluctuations in soil-C both directly ($N_t \longrightarrow \Delta C$) and indirectly ($N_t \longrightarrow \Delta N \longleftrightarrow \Delta C$) (51). d) Stoichiometric constraints between C and N ensure that both size ($C_t \longleftrightarrow N_t$) and the fluctuations ($\Delta C \longleftrightarrow \Delta N$) remain coupled (26, 44). For this coupling, we used correlated paths (bidirectional paths) between variables to account for a scenario where one can affect the other. e) Finally, grazers modify the above paths (b–c) through interactive effects (15, 25, 53, 56). Collectively, these paths evaluate the hypothesis that N affects stability of soil-C. We also tested the alternative hypothesis where C affects the stability of soil-N by reversing the directionality of the paths (*SI Appendix*, Fig. S9). First, we built the SEM of a full model with the above hypothesized paths to test how soil-C stability is influenced by grazers and by the size of soil-C and soil-N pools. Since the hypothesized paths contain interactive effects of grazing, we then compared two SEMs for data from the grazed and fenced plots separately. This helped us discern whether there are any differences in the hypothesized paths in the presence and absence of herbivores. We judged the agreement between data and the hypothesized paths via Fisher's C-statistic. We report standardized path coefficients and their statistical significance; we also report marginal and conditional r^2 for the response variables (65, 66).

Data, Materials, and Software Availability. The data are available through the Zenodo repository (https://doi.org/10.5281/zenodo.7071575) (67), and the computer code is provided in *SI Appendix*.

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