1   Running Head: Can livestock be substitutes for native herbivores?

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3   **Functional substitutability of native herbivores by livestock for soil carbon depends on**

4   **microbial decomposers**

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17   analysed data; and S.R., DGTN, and S.B. wrote the paper.

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19   Conflict of Interest: The authors declare that they have no conflict of interest.

20   Preprint server: bioRxiv, DOI: 10.1101/2022.02.07.479355

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Abstract

Grazing by large mammalian herbivores impacts climate as it can favor the size and stability of a large carbon (C) pool in soil. As native herbivores in the world's grasslands, steppes, and savannas are progressively being displaced by livestock, it is important to ask whether livestock can emulate the functional roles of their native counterparts. While livestock and native herbivores can have remarkable similarity in their traits, they can differ greatly in their impacts on vegetation composition which can affect soil-C. It is uncertain how their similarities and differences impact soil-C via their influence on microbial decomposers. We test competing alternative hypotheses with a replicated, long-term, landscape-level, grazing-exclusion experiment to ask whether livestock in the Trans-Himalayan ecosystem of northern India can match decadal-scale (2005-2016) soil-C stocks under native herbivores. We evaluate multiple lines of evidence from 17 variables that influence soil-C (quantity and quality of C-input from plants, microbial biomass and metabolism, microbial community composition, veterinary antibiotics in soil), and asses their inter-relationships. Livestock and native herbivores differed in their effects on several soil microbial processes. Microbial carbon use efficiency (CUE) was 19% lower in soils under livestock. Compared to native herbivores, areas used by livestock contained 1.5 kg C m^{-2} less soil-C. Structural equation models showed that alongside effects arising from plants, livestock alter soil microbial communities which is detrimental for CUE, and ultimately also for soil-C. Supporting evidence pointed toward a link between veterinary antibiotics used on livestock, microbial communities, and soil-C. Overcoming the challenges of sequestrating antibiotics to minimize their potential impacts on climate, alongside microbial rewilding under livestock, may reconcile the conflicting demands from food-security and ecosystem services. Conservation of native herbivores and better management of livestock is crucial for soil-C stewardship to envision and achieve natural climate solutions.

48   Keywords: Grassland, Soil microbial enzymes, Microbiome rewilding, Restoration,

49   Ecosystem disservice, Tetracycline, Carbon use efficiency

50

51   **Introduction**

52   Grazing by large mammalian herbivores is integral to the zoogeochemistry of the global

53   carbon (C) cycle (Schmitz et al., 2014, 2018). Herbivores exert a strong influence on climate

54   via their impacts on a large soil-C pool—over 500 Pg C in the world's grasslands, steppes,

55   and savannas (Derner et al., 2019; Liu et al., 2020; Naidu et al., 2022; Roy & Bagchi, 2022;

56   Sitters et al., 2020; Witt et al., 2011). Long before the industrial era, humans started

57   influencing global climate via their impacts on the distribution and abundance of large

58   mammalian herbivores—prehistoric humans may have hunted many megafauna to extinction,

59   and modern humans are progressively replacing native herbivores with domestic livestock

60   across the world (Asner et al., 2004; Bar-On et al., 2018; Schowanek et al., 2021). Intact

61   assemblages of native herbivores are now increasingly confined to parks and reserves;

62   elsewhere, livestock have not only become the most abundant grazers, but they are also the

63   most expansive land-use. Today, livestock biomass (10^{11} kg) exceeds that of all other

64   mammals; they occur at high densities ($>10^3$ individuals km^{-2}) across more than 60 million

65   km^2 which contain over 295 Pg of soil-C (Bar-On et al., 2018; Brus et al., 2017; Gilbert et al.,

66   2018). Further displacement of native herbivores seems inevitable due to steadily rising

67   demands for livestock-products to accommodate evolving human-diets (Leclère et al., 2020;

68   Thornton, 2010). Increasingly, it is important to ask whether livestock can maintain the

69   functional roles of native herbivores they displace (Cromsigt et al., 2018; Lundgren et al.,

70   2020; Malhi et al., 2016) because grazing can favor soil-C to decarbonize the atmosphere and

71   offers an important natural climate solution (Naidu et al., 2022; Roy & Bagchi, 2022). If

72   livestock can maintain the ecological functions of their native counterparts, then such

73   substitutability can have implications for the future of biodiversity, ecosystem functions and

74   services, food-security, and other social and environmental dimensions (Delabre et al., 2021;

75   du Toit et al., 2012; Sayer et al., 2013; Veblen et al., 2016).

76   The extent to which livestock can approximate the functional influence of native

77   herbivores on soil-C is important to inform and steer grazing management as a natural

78   climate solution (Bossio et al., 2020; Cromsigt et al., 2018; Reinhart et al., 2022; P. Smith,

79   2014). On one hand, the opportunity for livestock to be functional surrogates of displaced

80   native herbivores arises from the mutual similarity in their traits (Lundgren et al., 2020;

81   Malhi et al., 2016; Schowanek et al., 2021). Analyses of key traits, such as body size, diet-

82   choice, and fermentation type, hypothesize that livestock can be approximate surrogates for

83   native herbivores, i.e., nearest neighbors in trait-space have similar ecological roles

84   (Lundgren et al., 2020; Malhi et al., 2016; Schowanek et al., 2021). On the other hand, in

85   many ecosystems, livestock differ from native herbivores in how they impact vegetation

86   composition (Bagchi et al., 2012; Bagchi & Ritchie, 2010b; Price et al., 2022; Ratajczak et

87   al., 2022; Wells et al., 2022). This alters the quantity and quality of C-input to soil from

88   plants by changing the underlying distribution of plant traits (e.g., root:shoot ratio, C:N

89   stoichiometry of leaf and litter, etc.) that is ultimately consequential for soil-C (Bagchi &

90   Ritchie, 2010b; Laliberté & Tylianakis, 2012). While there is clarity on how herbivore

91   assemblages influence vegetation communities (Augustine & McNaughton, 1998; Bagchi et

92   al., 2012; Bakker et al., 2006), we have a limited understanding of the downstream

93   consequences for soil-C that are ultimately controlled by microbial actions on soil organic

94   matter (Falkowski et al., 2008; Fontaine et al., 2004; Roy & Bagchi, 2022; Sinsabaugh et al.,

95   2013; Six et al., 2006). For instance, differences in aboveground responses may percolate

96   belowground to alter the labile and recalcitrant fractions of soil organic matter (Bardgett &

97   Wardle, 2003), and depending on the direction and magnitude, this can either promote or

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98   deplete soil-C (Fontaine et al., 2004; Kuzyakov, 2010). Further, livestock also release

99   veterinary antibiotics into soil through their dung and urine which can alter soil microbial

100   communities (Lucas et al., 2021; Wepking et al., 2019). But we do not know whether soil

101   microbes respond differently to grazing by livestock and native herbivores, and how this

102   influences soil-C under the two types of grazers. In other words, though the promise of

103   functional substitutability between livestock and native herbivores might be constrained by

104   aboveground responses by plants (Bagchi et al., 2012; Bagchi & Ritchie, 2010b), it remains

105   tangled with belowground responses over how soil microbes respond to the two types of

106   grazers. This limits our ability to envision and derive natural climate solutions from livestock

107   (Bossio et al., 2020; Cromsigt et al., 2018; Reinhart et al., 2022; P. Smith, 2014) across a

108   large fraction of the earth's terrestrial surface (Asner et al., 2004; Bar-On et al., 2018).

109   Here we ask how livestock and native herbivores influence soil-C via their effects on

110   soil microbial processes in addition to their aboveground effects (Falkowski et al., 2008; Roy

111   & Bagchi, 2022; Sinsabaugh et al., 2016). We assess their functional substitutability in Spiti

112   region of northern India, which is part of the greater Trans-Himalayan ecosystem spread

113   across the Tibetan plateau and surrounding mountains (Fig. S1). We investigate long-term

114   (decadal-scale) soil-C stocks under livestock and native herbivores, and their influence on

115   key microbial decomposer functions related to C-cycling in soil. We take advantage of

116   several key features in the Trans-Himalaya: (a) the livestock are a multi-species assemblage

117   that show considerable trait-overlap with the native herbivores (Fig. 1), (b) livestock and

118   native herbivores occupy replicate juxtaposed watersheds with comparable environmental

119   settings (e.g., similarity in edaphic factors, climatic conditions, Fig. S1-S4), (c) their

120   abundances are comparable such that grazing pressure on vegetation is also similar (Fig. S5).

121   Absent major confounding influences, the livestock and native herbivore assemblages in

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122 Trans-Himalaya can help evaluate unresolved questions over how they influence soil-C

123 (Cromsigt et al., 2018; Lundgren et al., 2020; Malhi et al., 2016; Schowanek et al., 2021).

124

125 **Materials and methods**

126 STUDY AREA

127 Climate in Spiti region of Trans-Himalaya is cold and semi-arid with temperature ranging

128 from 25 °C in summer to below –30 °C in winters (Fig. S1, S2). Precipitation occurs as

129 winter-snow (100-200 cm yr^{-1}) and summer-rain (150-300 mm yr^{-1}), and vegetation growth-

130 season is short (May-August, Fig. S2), peak biomass in July-August. Vegetation in this

131 treeless ecosystem consists of grasses (*Poa*, *Elymus*, *Festuca*), sedges (*Carex*, *Kobresia*),

132 forbs (*Lindelofia*, *Astragalus*) and shrubs (*Artemisia*, *Caragana*). Traditional pastoral

133 livestock production is the major land-use across much of the Trans-Himalaya. Watersheds

134 near village Kibber in Spiti are used by a multi-species livestock assemblage, and these are

135 juxtaposed with watersheds that retain native herbivores (Fig. 1, Fig. S1). Natural terrain and

136 barriers (canyons, escarpments, rivers, high ridges) restrict frequent animal movement and

137 maintain replicate watersheds under two alternative herbivore assemblages (Fig. S1)—some

138 used primarily by native herbivores and others by livestock (Bagchi & Ritchie, 2010b). Like

139 other parts of the world (Veblen et al., 2016; Western et al., 2020), local extinctions of native

140 herbivores and their replacement by livestock over the past decades remains a major

141 conservation challenge in the Trans-Himalaya (Mishra et al., 2002; Namgail et al., 2013).

142 The extant native herbivores are ibex (*Capra sibirica*), bharal (*Pseudois nayaur*), and yak

143 (*Bos grunniens grunniens*) that are related to wild yak (Bos grunniens mutus; also known as

144 B. grunniens and B. mutus, respectively; Leslie & Schaller, 2009). Currently, kiang (*Equus*

145 *kiang*) are known from the fringes of the study area (Fig. S1), and there are sporadic reports

146 of argali (*Ovis ammon*). But Tibetan antelope (*Pantholops hodgsonii*) and Tibetan gazelle

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147 (*Procapra picticaudata*) are no longer found in the study area (Mishra et al., 2002; Namgail

148 et al., 2013). The livestock consist of yak-cattle hybrids, cattle goat, sheep, horse, and donkey

149 (Fig. 1).

150

151 NATIVE HERBIVORES AND LIVESTOCK OF SPITI

152 Cumulative biomass of native herbivores (c. 1.1×10^5 kg) has remained comparable to that of

153 livestock over the past few decades (c. 1.2×10^5 kg) in the watersheds covering c. 40 km^2 near

154 village Kibber (Bagchi & Ritchie, 2010b; Singh et al., 2015) (Fig. S1). The livestock and

155 native herbivores (after including kiang and argali across the broader region) show

156 sufficiently high overlap in key traits (Lundgren et al., 2020), such as their body mass, dietary

157 guild and graminoid consumption, fermentation type, sexual-dimorphism, and limb

158 morphology (Fig. 1). Since the native herbivores appear to have a livestock counterpart (Fig.

159 1), one expects the two assemblages to be functionally similar. Yet, consistent with other

160 parts of the world, the livestock and native herbivores differ in their impacts on vegetation

161 composition (Bagchi et al., 2012; Bagchi & Ritchie, 2010b; Price et al., 2022; Ratajczak et

162 al., 2022; Wells et al., 2022). Specifically, native herbivores lead to forb-and-grass dominated

163 vegetation whereas sedges dominate under livestock (Fig. 1). Such differences in vegetation

164 composition can be attributed to diet selectivity of the two herbivore types (Augustine &

165 McNaughton, 1998; Bagchi et al., 2012; Bagchi & Ritchie, 2010b; Ratajczak et al., 2022).

166 However, peak-season live biomass, both above and below-ground, are comparable (Fig. 1).

167 In addition, standing litter biomass, and microbial biomass in soil are also comparable (Fig.

168 1). The two types of watersheds are also similar in several key abiotic variables (Fig. S3-S4).

169 Soil pH is near-neutral to alkaline in both native and livestock watersheds; electrical

170 conductivity and bulk density are also comparable (Fig. S3). Soils in both types of

171 watersheds have sandy-loam texture (Fig. S4).

172   These similarities and differences can determine whether livestock can match long-

173   term soil-C stocks under native herbivores, or not. Specifically, this not only depends on how

174   the two types of herbivores influence C-input from plants to soil, and but also how they

175   influence microbial processes during decomposition of soil organic matter (Bardgett &

176   Wardle, 2003; Falkowski et al., 2008; Roy & Bagchi, 2022; Sinsabaugh et al., 2016).

177

178   EXPERIMENTAL DESIGN

179   Humans arrived in Spiti in the pre-historic age (Bellezza, 2017), and livestock replaced the

180   native herbivores from these watersheds few decades before our study began (Mishra et al.,

181   2002; Namgail et al., 2013). Thus, *sensu stricto*, a contemporary landscape-level comparison

182   of these watersheds cannot distinguish the effects of change in animal assemblage from any

183   pre-existing differences between them. Therefore, instead of relying solely on prevailing

184   differences in above- and belowground factors that influence soil-C, we determine whether

185   any observed difference in soil-C between the watersheds can be attributed to how plants and

186   microbes respond to grazing by the alternative herbivore assemblages. We use long-term

187   experimental herbivore-exclusion in the replicate watersheds to quantify grazer-effects on

188   different variables to investigate (a) whether grazing by livestock and native herbivores has

189   different influence on soil-C, and (b) whether any difference in soil-C can be attributed to

190   how plants and microbes respond to grazing by the two assemblages. This approach overlays

191   a manipulative experiment over the natural experiment of two types of watersheds (Bagchi &

192   Ritchie, 2010b). For this, starting in 2005, we set up experimental grazer-exclusion with

193   replicated paired-and-adjacent control-and-fenced plots (10×10 m^2, each). We used four

194   watersheds used primarily by native herbivores, and another four by livestock (Fig. S1). We

195   set up 3-4 paired control-and-fenced plots in each watershed (n=15 plots under native

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196   herbivores, and another n=15 plots under livestock), with a total of 30 paired control-and-

197   fenced plots.

198

199   SOIL CARBON AND PLANT BIOMASS

200   Over the next 12 years, we measured soil-C eight times at roughly inter-annual intervals in

201   each paired plot across the different watersheds. We collected soils with a 5 cm diameter and

202   20 cm deep cores, and measured carbon content (TruSpec, Leco, USA) to estimate soil-C

203   stocks (kg C m^{-2} up to 20 cm depth) under livestock and native herbivores; soil-depth rarely

204   exceeds 20 cm in this ecosystem and in other similar mountainous landscapes (Bagchi &

205   Ritchie, 2010b). We measured the difference in standing above- and belowground biomass at

206   peak of growing season between paired fenced and grazed plots as an indicator of grazing

207   pressure (Fig. S6). We collected live shoot biomass from 0.5×0.5 m^2 quadrats in each paired

208   plot. Subsequently we first sun-dried and then oven-dried the biomass samples to constant

209   weight at 40 °C to obtain the dry-weights (g m^{-2}). We separated live roots and other

210   belowground structures such as rhizomes from the soil cores, and similarly obtained dry-

211   weights (g m^{-2} up to 20 cm depth) (Fig. S6).

212

213   SOIL MICROBIAL FUNCTIONS

214   In 2016, after up to 12 years of herbivore-exclusion, we estimated grazer-exclusion effects on

215   soil microbial processes in the paired-adjacent fenced and control plots to assess whether they

216   explain any observed differences in soil-C. These soil microbial variables represent different

217   processes that collectively influence nutrient cycling in soil (Table S1). Residence-time of

218   soil-C in such subhumid ecosystems is 10-30 years, whereas it can be in excess of 100 years

219   in swamps and wetlands (Carvalhais et al., 2014; Raich & Schlesinger, 1992). Thus, much of

220   the soil-C from the time of initial replacement of herbivore assemblages would have

221   undergone considerable turnover. So, we expect our long-term grazing-exclusion experiment

222   to reflect how the two types of herbivore assemblages influence soil-C at decadal timescales

223   that is relevant to their functional influence on climate (Naidu et al., 2022). In this way, the

224   grazing-exclusion experiment offers an opportunity to assess if differences in plant and

225   microbial responses to grazing by different types of herbivores can explain how they

226   influence soil-C.

227   In 2016, we estimated the response of key soil microbial functions to grazing: basal

228   respiration (BR), potential respiration or substrate-induced respiration (SIR), broad soil

229   microbial community profile as bacterial and fungal fractions (B and F), microbial biomass C

230   and N (i.e., MBC and MBN), labile carbon (LC), labile nitrogen (LN), and recalcitrant

231   carbon (RC) fractions of soil organic matter, microbial metabolic quotient (qCO_2), and

232   microbial carbon use efficiency (CUE). Since these microbial processes have seasonal

233   dynamics (Bagchi et al., 2017; Roy & Bagchi, 2022), we measured them at five time-points

234   during the growing season between May and September (Fig. S7). We followed standard

235   laboratory protocols to measure these microbial variables (Bagchi et al., 2017; Robertson et

236   al., 1999; Roy & Bagchi, 2022). Briefly, for BR we used an alkali-trap to incubate 4 g soil at

237   60% water holding capacity and measured respired CO_2 (mg C g^{-1} soil day^{-1}). Similarly, we

238   measured SIR (mg C g^{-1} soil day^{-1}) after adding 0.5% (w/v) of glucose to soil. We estimated

239   broad microbial community profile as fungal and bacterial contributions to potential

240   respiration under selective inhibition using Streptomycin (anti-bacterial) and Cycloheximide

241   (anti-fungal) with respect to controls. We used chloroform fumigation-extraction to estimate

242   MBC (mg C g^{-1} soil), and MBN (mg N g^{-1} soil). We measured labile and recalcitrant

243   fractions (mg g^{-1} soil) of soil organic matter (SOM) using two-step hydrolysis with H_2SO_4.

244   We estimated qCO_2 as the ratio of BR and MBC (respiration per unit biomass, hr^{-1}). We

245   estimated CUE from established relationships between key microbial extracellular enzymes

246   (carbon-acquiring β-glucosidase, alongside nitrogen-acquiring Leucine aminopeptidase and

247   *N*-acetyl-β-D-glucosaminidase), MBC and MBN, and labile fractions of C and N in SOM

248   (Sinsabaugh et al., 2013, 2016). We measured activity of the three enzymes using their

249   specific fluorogenic substrates (4-MUB-β-D-glucopyranoside for β-glucosidase, L-Leucine-

250   7-amido-4-methylcoumarin hydrochloride for Leucine aminopeptidase, and 4-MUB-*N*-

251   acetyl-β-D-glucosaminide for *N*-acetyl-β-D-glucosamindase) (German et al., 2011) in soils

252   (Fig. S8). These parameters influence microbial metabolism to determine their investment in

253   growth (*G*) and respiration (*R*), so that $CUE = \frac{G}{G+R}$ (Sinsabaugh et al., 2013, 2016).

254   Theoretically CUE can range between 0 and 1, i.e., nil to perfect efficiency, with higher

255   values indicating greater potential for C-sequestration. But, CUE in nature generally does not

256   exceed 0.6 (Sinsabaugh et al., 2013, 2016).

257

258   SOIL MICROBIAL DNA

259   Based on patterns seen in fungal and bacterial contribution to soil respiration, we evaluated

260   community composition in more detail using their DNA markers from soils collected in 2019

261   (Fierer et al., 2005; Maestre et al., 2015). We used soils from a subset of 20 paired-adjacent

262   plots and extracted microbial DNA (MOBIO Power Soil DNA Extraction Kit). Since fungal

263   and bacterial fractions can have seasonal dynamics (Bagchi et al., 2017), we covered four

264   time points during the growing season (May, July, August, and September, for a total of n =

265   160 samples). Next, we quantified the Internal Transcribed Spacer regions (ITS-1 and ITS-2)

266   of rDNA for fungal abundance, and region of 16s rDNA for bacterial abundance with

267   quantitative polymerase chain reaction, qPCR (Fierer et al., 2005; Maestre et al., 2015). We

268   used these qPCR data to confirm whether DNA-based estimates align with respiration-based

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

270   From these DNA extracts for a subset of ten paired plots, we estimated microbial

271   taxonomic diversity in soil from Operational Taxonomic Units (OTUs). To capture seasonal

272   community dynamics, we estimated OTUs at three time points during the growing season

273   (May, July, and September, for a total of n = 60 samples of community wide OTUs). We

274   amplified and sequenced the 16S rRNA hyper variable V3-V4 region in Illumina MiSeq

275   platform, followed by taxonomic assignment using the SILVA_v138 database. Additional

276   details are in Appendix.

277

278   VETERINARY ANTIBIOITICS IN SOIL

279   Herbivore-mediated changes in plant communities, with subsequent alteration in the

280   underlying distribution of plants traits, can influence the quantity and quality of C-input to

281   soil (Bagchi & Ritchie, 2010a). This can lead to differences in microbial communities

282   between livestock and native herbivores due to alteration in labile and recalcitrant fractions of

283   soil organic matter (Bagchi & Ritchie, 2010b; Laliberté & Tylianakis, 2012). A more direct

284   effect on microbial communities can arise from veterinary use of antibiotics on livestock that

285   eventually enter soil via dung and urine (Albero et al., 2018; Jechalke et al., 2014; Kemper,

286   2008; O'Connor & Aga, 2007; Wepking et al., 2019). Livestock in Spiti are frequently

287   treated with antibiotics such as tetracycline (therapeutic and sub-therapeutic dosage). Among

288   the native herbivores, veterinary care for yaks is comparatively rare, while it is practically

289   absent for ibex and bharal. So, based on the patterns seen in microbial OTUs, we quantified

290   tetracycline (μg kg^{-1} soil) in soil from all 30 paired plots in August 2022. We used enzyme-

291   linked immunosorbent assays (ELISA) to quantify water-extractable tetracycline levels in

292   soil with a detection limit of 3 ppb (Immunolab, GmbH, Germany), relative to standards and

293   controls (Aga et al., 2003; O'Connor & Aga, 2007).

294

12

295   DATA ANALYSIS

296   We evaluated competing hypotheses (Betts et al., 2021; Tredennick et al., 2021) to determine

297   whether the observed variation in the data merely represent spatio-temporal heterogeneity

298   between replicates, or it is necessary to invoke grazing (fenced or grazed) and herbivore

299   assemblage-type (livestock or native) as an explanatory variable. We used a linear mixed-

300   effects model to evaluate long term trends in soil-C and grazer-exclusion effects on each

301   plant and microbial variable. For soil-C we used a null-model $\left(M_{null}^{soilC}\right)$ with time (years,

302   2006-16) and plot identity as random-effects to account for background variation due to

303   sampling locations (plots) and times (year). This model was evaluated against the alternative

304   competing model $\left(M_{alt}^{soilC}\right)$ which included assemblage-type and grazing as fixed-effects,

305   alongside the random-effects. We compared these competing models based on parsimony,

306   goodness-of-fit, and likelihood-ratio test, i.e., AIC, RMSE, and LR (Tredennick et al., 2021).

307   We estimated grazer-effect on each microbial variable as the log-ratio of values in the

308   fenced and paired-adjacent control plots i.e., $\Delta = ln\left(\frac{\text{Grazed}_i}{\text{Fenced}_i}\right)$ for the ith pair, and calculated

309   the mean and 95% CI of the log-ratio (Roy & Bagchi, 2022). As earlier, we first evaluated a

310   null-model to assess whether the observed variation in grazer-effects can simply be attributed

311   to spatio-temporal heterogeneity. For the null-model for the jth microbial variable $\left(M_{null}^{j}\right)$, we

312   used plot identity and month (May-September) as random-effects. In the competing

313   alternative model for each variable $\left(M_{alt}^{j}\right)$, we included herbivore assemblage-type as a

314   fixed-effect alongside the random-effects. As before, we used AIC, RMSE and LR for model

315   comparison to judge whether herbivore assemblage-type is a necessary explanatory variable

316   for the data (Tredennick et al., 2021). We assessed whether the data met statistical

317   assumptions from observed and theoretical quantiles of residuals; the variables required no

318   further transformation (Δ is already in log-scale). We used 'nlme' library in R (Pinheiro et al.,

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319   2020) to perform these analyses. As the data on antibiotics had many zeros (i.e., tetracycline

320   concentration in many samples was below the detectable limit of 3 ppb), we used a

321   generalized mixed-effects model with binomial distribution and logit-link.

322   We assessed the inter-relationships between grazer-effects (Δ) on different microbial

323   variables with structural equations models (SEM). SEM help evaluate hypothesized pathways

324   over how variation in one variable can influence another (Grace, 2006). We identified SEM

325   paths from *a-priori* examples in the literature (Table S2). To avoid multicollinearity in the

326   SEM arising from redundancy among the variables, we removed those variables which were

327   derived from one another (Fig. S9). So, ΔSIR, ΔBR, ΔRC and ΔqCO$_2$ were not included in

328   this step, and we built SEM using ΔMBC, ΔMBN, ΔLN, ΔLC, ΔF, ΔB, and ΔCUE. We

329   incorporated SEM paths as mixed-effects models for each variable as piece-wise structural

330   equations (Lefcheck, 2016). We used directed paths (e.g., ΔF$\rightarrow$$\Delta$CUE) as tests of

331   hypothesized causal relationships, and bidirectional paths (e.g., ΔMBC$\leftrightarrow$$\Delta$MBN) for

332   variables that are correlated or stoichiometrically coupled (Naidu et al., 2022). We did not

333   use genetic data from ITS and 16s rDNA in the SEM because these were collected from a

334   subset of plots, they were also not from the same year as the other microbial variables, and

335   because estimates from e-DNA broadly matched respiration-based estimates of community

336   composition. Similarly, we did not include tetracycline data in the SEM because when

337   tetracycline abundance was below the detection limit of ELISA (3 ppb), we considered it to

338   be zero—this makes Δ undefined (Δ becomes ∞). First, we evaluated inter-relationships

339   between grazer-effects (Δ) on different microbial variables with herbivore assemblage-type

340   as an explanatory variable. We assessed goodness of fit with Fisher's C statistic (Roy &

341   Bagchi, 2022; Shipley, 2009) and report standardised path coefficients alongside their

342   statistical significance. When SEM paths are supported by data, then they offer a plausible

343   explanation for an underlying process. When SEM paths are not supported, the hypothesized

344   process may not give rise to the observed data.

345   Next, since the temporal scale and span of the microbial variables (monthly

346   dynamics) are different from that of plant biomass and soil-C (annual or decadal dynamics),

347   the first SEM by itself cannot directly evaluate how grazer-effects ultimately influence soil-C

348   via microbes. While these different types of data differ in their temporal dimensions, they

349   have a common spatial dimension (n=30 replicate pairs). So, we evaluated their connections

350   under the expectation that effects seen on microbial processes could accumulate and

351   potentially explain variation in soil-C. For this, we first calculated the average (mean effect

352   on j^{th} variable, $\bar{\Delta}$) across all the months for each microbial variable, and across years for soil-

353   C and grazing intensity, for all replicates. In this second SEM, we evaluated paths using these

354   average values to assess whether herbivore assemblage type explains variation in soil-C via

355   grazer-effects on microbes, alongside the effects originating from plants. As before, plot

356   identity was a random-effect in the SEM paths as this allows the intercept to vary between

357   samples that may have unmeasured background difference in $\bar{\Delta}$ due to other factors such as

358   variation grazing pressure, or in edaphic conditions (Naidu et al., 2022; Roy & Bagchi,

359   2022). We summarized and visualized the variation in microbial community composition

360   with Principal Coordinates Analysis (PCoA) over Sorensen dissimilarity. We determined the

361   effect of herbivore assemblage type and grazing on microbial community composition using

362   999 randomized iterations of the dissimilarity matrix. All analyses were performed in R 4.1.0.

363

364   **Results**

365   LONG-TERM TREND IN SOIL-CARBON

366   Model comparison showed that spatio-temporal heterogeneity was not sufficient to explain

367   long-term variation in soil-C, and it could instead be attributed to herbivore assemblage-type.

368   There was stronger support for M_{alt}^{soilC} than for M_{null}^{soilC} (ΔAIC=-74.39, ΔRMSE=-0.662,

369   LR=80.386, P<0.001). However, soils under native herbivores stored 1.55$\pm$0.3 SE more

370   carbon (kg C m^{-2}) than under livestock ($F_{1,184}$=40.11, P<0.001). Effectively, areas under

371   livestock could realize up to 71% of the soil-C found under native herbivores (Fig. 2).

372   Grazer-exclusion, by itself ($F_{1,178}$=3.66, P=0.06) and as interaction ($F_{1,178}$=1.25, P=0.26), had

373   weak influence on soil-C (Fig. 2) even though the fenced plots received higher C-inputs from

374   plant biomass that was not consumed by grazers (Fig. 3).

375

376   GRAZER-EFFECTS ON PLANT AND SOIL VARIABLES

377   Analysis of grazer-effects (grazed/fenced comparison as log-ratio Δ) indicated that the

378   observed difference in soil-C (Fig. 2) was related to how soil microbes respond to difference

379   in herbivore assemblage-type (whether livestock or native herbivores, Fig. 3). Livestock and

380   native herbivores had comparable effects on B, 16s rDNA abundance, MBC, MBN, LC, LN,

381   RC, qCO$_2$, and shoot biomass (Fig. 3, Table S3). For these variables, the competing

382   alternative models were not supported, and their variability in grazer-effects could be

383   attributed to spatio-temporal heterogeneity rather than to herbivore assemblage-type (Fig. 3,

384   Table S3). Experimentally removing livestock and native herbivores had comparable effects

385   on soil-C as well (Fig. 3). But livestock and native herbivores differed in their influence on

386   BR, SIR, F, ITS rDNA abundance, below-ground biomass, and on CUE. For these six

387   variables, there was stronger support for the competing models than the null models (Table

388   S3), as assemblage-type was an important explanatory variable over and beyond background

389   spatio-temporal heterogeneity (Fig. 3). Grazing by livestock increased both basal and

390   potential microbial respiration (BR and SIR), compared to grazing by native herbivores (Fig.

391   3). But grazing by livestock reduced both the fungal fraction of microbial respiration and the

392   abundance of fungal genetic markers (F and ITS). Respiration-based estimates of fungal and

16

393   bacterial fractions were consistent with DNA-based estimates (Fig. 3). Importantly, grazing

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

395

396   INTER-RELATIONSHIPS BETWEEN SOIL MICROBIAL RESPONSES

397   To assess how differences in grazer-effects on one microbial variable may influence other

398   variables, we used mixed-effects structural equation model. The model was supported by the

399   data (SEM: Fisher's C=4.409, P=0.633, df=6, AIC=66.41). This showed, that change in

400   herbivore assemblage-type influences microbial community composition to alter CUE

401   (Assemblage→ΔF→ΔCUE, Fig. 4). Specifically, change in herbivore assemblage from

402   native to livestock alters the soil microbial community, which in turn is detrimental for CUE

403   (Fig. 4). This SEM indicates that direction and magnitude of grazer-effects are consistent

404   with higher CUE under native herbivores suggesting higher potential for net soil-C storage,

405   compared to livestock (Fig. 2, Fig. 4).

406

407   INFLUENCE OF PLANT AND MICROBIAL RESPONSES ON SOIL-C

408   We considered the role of herbivore assemblage-type (identity of herbivores) and vegetation

409   biomass (shoot and root biomass), quantity and quality of C-input to soil (labile and

410   recalcitrant pools), together, as a thought experiment to connect the inter-relationship

411   between different microbial responses with soil-C (Fig. 5). This SEM showed that changing

412   herbivore assemblage-type from native to livestock is detrimental to soil-C via two paths.

413   First, herbivore assemblage-type affects soil-C through a decline in quantity of C-input to soil

414   particularly from belowground root biomass (Assemblage→$\overline{\Delta Root}$→$\overline{\Delta soilC}$, Fig. 5).

415   Second, it also affects soil-C through effects on microbial community composition and

416   microbial CUE (Assemblage→$\overline{\Delta F}$→$\overline{\Delta CUE}$→$\overline{\Delta soilC}$; Fig. 4-5). In this way, it supported our

17

417   expectation that effects arising from microbial processes at monthly scales (seen in Fig. 4)

418   could indeed explain variation in soil-C (Fisher's C=8.92, P=0.71, df=12, AIC=128.92).

419

420   MICROBIAL COMMUNITIES AND VETERINARY ANTIBIOTICS IN SOIL

421   Following the connections between herbivores, microbes, and soil-C (Fig. 5), we evaluated

422   microbial community composition in more detail using 16S rDNA sequencing and

423   classification. Overall, Actinobacteriota, Acidobacteriota, Chlorofelxi, Planctomycetota, and

424   Proteobacteria were the common and abundant taxa (Fig. S10). Microbial community

425   composition differed between native herbivores and livestock ($F_{1,56}$=8.03, P<0.001; Fig. 6a).

426   However, grazer-exclusion did not alter microbial communities regardless of herbivore

427   assemblage-type (Fig. 6a).

428   To explore whether the difference in microbial communities could be related to

429   veterinary use of antibiotics on livestock, we quantified tetracycline concentration in soil. As

430   expected, grazed plots under livestock (mean 6.44 ± 1.42 SE, µg kg^{-1} soil) contained nearly

431   three times more tetracycline residues than under native herbivores (2.59 ± 1.27 SE, µg kg^{-1},

432   Fig. 6b). Expectedly, grazer-exclusion resulted in depletion of antibiotics in the fenced plots

433   and this effect was stronger for livestock (t_{28}=-3.37, P=0.002; Fig. 6b).

434

435   **Discussion**

436   We assessed whether livestock could provide climate-mitigation services through soil-C to

437   the same extent as the native herbivores they displace (Asner et al., 2004; Bar-On et al.,

438   2018; du Toit et al., 2012; Schowanek et al., 2021), as this has implications for grazing

439   management (Bossio et al., 2020; Cromsigt et al., 2018; Reinhart et al., 2022; P. Smith,

440   2014). We find that:- (1) multi-species herds of livestock can have remarkable functional

441   similarities with the native assemblages they displace (Fig. 1), (2) but livestock do not

18

442 emerge as perfect substitutes since they store less soil-C, compared to the native herbivores

443 (Fig. 2), (3) differences in soil microbial responses can be the proximate explanation for why

444 livestock are not perfect substitutes, in addition to above-ground grazer-effects that are

445 propagated via plants (Fig. 3-5), and (4) there is supporting evidence that veterinary

446 antibiotics can restrict soil-C under livestock (Fig. 6). These results can have implications for

447 how we manage environmental impacts of livestock (Delabre et al., 2021; du Toit et al.,

448 2012; Sayer et al., 2013; P. Smith, 2014; Veblen et al., 2016), and meet the rising demand for

449 livestock-products in the coming decades (Leclère et al., 2020; Thornton, 2010).

450   To determine the scope of improved management of a large fraction of the earth's

451 terrestrial surface (Asner et al., 2004; Bar-On et al., 2018), it is important to identify the

452 potential drivers and mechanisms that explain why livestock lag behind native herbivores in

453 maintaining soil-C. Below we discuss explanations for the observed differences in soil-C

454 stocks and the candidate underlying mechanisms which can be subjects for further study.

455

456 PRE-EXISTING CONDITIONS—Since our study began after humans had already settled in

457 the Trans-Himalayan landscape and the livestock had already displaced the native herbivores,

458 *sensu stricto*, we cannot resolve whether livestock occupied the watersheds that were

459 inherently low in soil-C (i.e., $time_0$). Arguably, any pre-existing difference in soil-C can be

460 maintained over subsequent decades due to comparable grazing pressures and because

461 turnover of soil-C can occur over 10 to 30-year timescales. While pre-existing differences

462 would explain Fig. 2, but they do not explain why livestock and native herbivores differ in

463 their influence on soil microbial decomposers (Fig. 3). Pre-existing conditions also do not

464 explain why the direction and magnitude of microbial responses is related to soil-C (Fig. 4-

465 5). Alternatively, one can invoke a related argument that livestock have historically

466 overgrazed their watersheds, thus, depleting their soil-C stocks. If this was a primary reason,

467   then one expects soil-C to recover after cessation from grazing in the fenced plots. One also

468   expects appreciable recovery within the decadal timescale of our study (Jones & Schmitz,

469   2009). However, the fenced plots in livestock watersheds did not show such a response even

470   after 12 years of grazing-exclusion (Fig. 2). Instead, excluding native herbivores and

471   livestock led to comparable responses in soil-C (Fig. 3). This undermines the argument that

472   livestock watersheds are yet to recover from historical overgrazing (Fig. 2). Rather, we find

473   that under grazing by both types of herbivores, soils effectively retain a greater fraction of

474   their C-input from plants (Roy & Bagchi, 2022). Contrastingly in the absence of grazing,

475   while soils receive greater C-input from plants, but are "leaky" as they fail to store soil-C,

476   relative to the grazed plots. Such responses are known from many different ecosystems

477   around the world and are attributable to how microbes respond to grazing (Roy & Bagchi,

478   2022). Finally, antibiotic residues in soil (Fig. 6) also point away from the primacy of pre-

479   existing differences, and toward more recent factors that may have altered the microbial

480   processes affecting soil-C. Seen together, pre-existing conditions cannot be the only

481   explanation for the observed differences between livestock and native herbivores. Instead, it

482   is plausible that cascading effects of antibiotics on microbial community composition and

483   ultimately on microbial CUE play an important role, alongside the effects which originate

484   from plants (Fig. 4-6).

485

486   PLANT PATHWAYS—We attempt to find potential links between plant- and microbe-

487   centric processes, above- and below-ground, and how they influence soil-C (Fig. 5). This

488   recapitulates previous findings in terms of differences in the quantity of C-input from plants

489   (Bagchi & Ritchie, 2010b), and accommodates downstream consequences such as quality of

490   C-input due to changes in vegetation community composition that can alter labile and

491   recalcitrant pools of soil organic matter (Roy & Bagchi, 2022). In other words, while the

20

492   difference in vegetation composition is a determinant of the differences in soil-C, microbial

493   responses also play an equally important role (Fig. 4-5). Unfortunately, these microbial

494   responses do not erase the effects arising from plants and they prevent livestock from

495   becoming perfect surrogates of the native herbivores (Fig. 4-5).

496

497   SOIL MICROBIAL PATHWAYS—Livestock and native herbivores differ in several ways

498   in how they influence soil microbial processes (Fig. 3). These microbial responses lead to

499   lower CUE in soils under livestock, compared to the native herbivores (Fig. 4). CUE is a

500   fundamental microbial trait that determines the rate at which soil organic matter is

501   metabolized, therefore, how much C can be stored in soil (Fontaine et al., 2004; Sinsabaugh

502   et al., 2013; Six et al., 2006). Not surprisingly, microbial community composition plays an

503   important role because grazing by livestock is seen to reduce CUE, compared to the native

504   herbivores (Fig. 3-4), and this can be consequential for soil-C (Fig. 5). So, the question

505   becomes—why do livestock establish a different microbial community (Fig. 6)?

506

507   LIVESTOCK AND SOIL MICROBIAL COMMUNITIES–Several overlapping factors can

508   alter microbial communities under livestock, and their individual roles may not be easily

509   disentangled from each other especially if they act in the same direction. Once established,

510   the differences in microbial community composition may persist for long periods (López

511   Zieher et al., 2020) due to reorganization of competition-cooperation interactions (Lechón-

512   Alonso et al., 2021); indeed, we find them to be unresponsive to decadal-scale grazer-

513   exclusion (Fig. 6). Differences in vegetation composition, and subsequent alteration in the

514   quantity and quality of C-input from plants can result in microbial community differences.

515   Additionally, veterinary care offered to the livestock releases antibiotics into soil via their

516   dung and urine (Albero et al., 2018; Jechalke et al., 2014; Kemper, 2008; O'Connor & Aga,

517   2007). This can alter microbial communities towards lower CUE (Lucas et al., 2021;

518   Wepking et al., 2019). Annually, about 10^7 kg of antibiotics are used on livestock around the

519   world. Within a few hours of administration, these antibiotics can enter the environment

520   either unaltered, or as metabolites that continue to remain active (O'Connor & Aga, 2007).

521   Indeed, soils under livestock had higher tetracycline residues (Fig. 6b). Moreover, select

522   microbial phyla (e.g., Bacteriodota, Firmicutes, Myxococcota, Fig. S10) that are known to be

523   particularly susceptible to antibiotics in lab-incubations (Qian et al., 2016; Shawver et al.,

524   2021), indeed also appear to have different abundance under livestock, compared to native

525   herbivores. This raises two unanswered questions: (1) does susceptibility to antibiotics lead to

526   a difference in microbial community composition? And, (2) does the resultant difference in

527   microbial community composition impact CUE to become consequential for soil-C? There is

528   scattered evidence from laboratory cultures on how veterinary antibiotics alters soil microbial

529   communities (Qian et al., 2016; Shawver et al., 2021), and how various microbial taxa differ

530   in their CUE (T. P. Smith et al., 2021). However, we are yet to fully address how antibiotics

531   can influence microbial CUE in soil in ways that ultimately also affect the global C-cycle

532   (Lucas et al., 2021; Wepking et al., 2019). Seen together, our results identify microbial CUE

533   as a proximate explanation for why livestock are not perfect functional substitutes for native

534   herbivores (Fig. 3-6), to highlight the importance of soil microbial restoration in tandem with

535   sequestering their antibiotics. In other words, livestock management may offer improved soil-

536   C services (Bossio et al., 2020; Cromsigt et al., 2018; Reinhart et al., 2022; P. Smith, 2014) if

537   it is coupled with microbial restoration and rewilding (Averill et al., 2022; Mills et al., 2017;

538   Paluch et al., 2013; Wubs et al., 2016). But several questions remain unanswered on how to

539   achieve effective microbial rewilding and whether it can sustain ecosystem-level benefits,

540   and these can be subjected to further study.

541

542   MICROBIAL REWILDLING

543   One possible path to favor soil-C under livestock may lie in rewilding and restoring microbial

544   communities to achieve improved ecosystem functions and services (Averill et al., 2022;

545   Mills et al., 2017; Wubs et al., 2016). Although microbial restoration through soil-

546   inoculations is practiced in croplands (Verbruggen et al., 2013), whether target microbial

547   communities can establish and persist under livestock remains unknown because this problem

548   is compounded by veterinary antibiotics. It is also unclear how we can sequester antibiotics to

549   prevent their impact on soil-C and on global climate. Further studies on effective rewilding of

550   microbial communities and sequestering antibiotics are needed to make livestock-production

551   more environmentally responsible (Bossio et al., 2020; Cromsigt et al., 2018; du Toit et al.,

552   2012; P. Smith, 2014).

553   In conclusion, our results highlight that multi-species and functionally diverse

554   assemblages of livestock can partially approximate the ecological roles of native herbivores.

555   This supports continued conservation of native herbivores and calls for new ideas to improve

556   livestock management. Extensive therapeutic and sub-therapeutic use of antibiotics on

557   livestock may restrict soil-C by altering soil microbial CUE. Sequestering antibiotics,

558   alongside effective restoration and rewilding of soil microbial communities may enable

559   livestock to emulate the services provided by native herbivores to achieve nature-based

560   climate solutions. These may help reconcile the rising demands for livestock-products with

561   ecosystem functions and services.

562

563   **Acknowledgements**

574

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847

848 **Figures**

849 Fig. 1
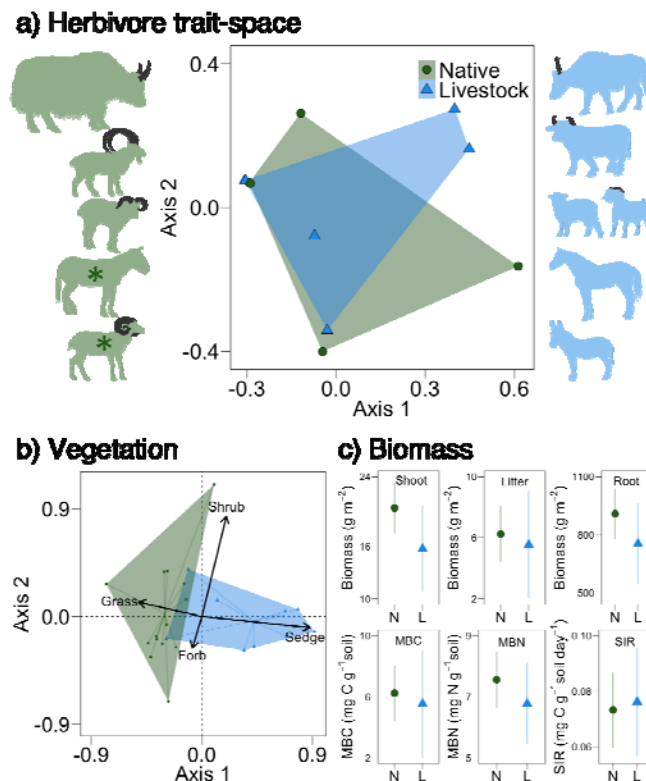
851 Figure 1. Similarities and differences between livestock and native herbivores in Spiti, India.

852 Livestock (L: blue; yak-cattle hybrids, cattle, goat, sheep, horse, and donkey) and native

853 herbivores (N: green, yak ibex, bharal, kiang, and argali) show considerable overlap in

854 multidimensional trait space (a). Asterix indicates that kiang and argali are found nearby our

855 study area in Spiti. Trait-space consists of six key traits: body mass, dietary guild,

856 fermentation type, sexual-dimorphism, graminoid consumption, and limb morphology

857 (Lundgren et al., 2020) with Principal Coordinates Analysis on Gower's distance matrix

858 (PCoA). Vegetation composition summarised with NMDS ordination on Bray-Curtis
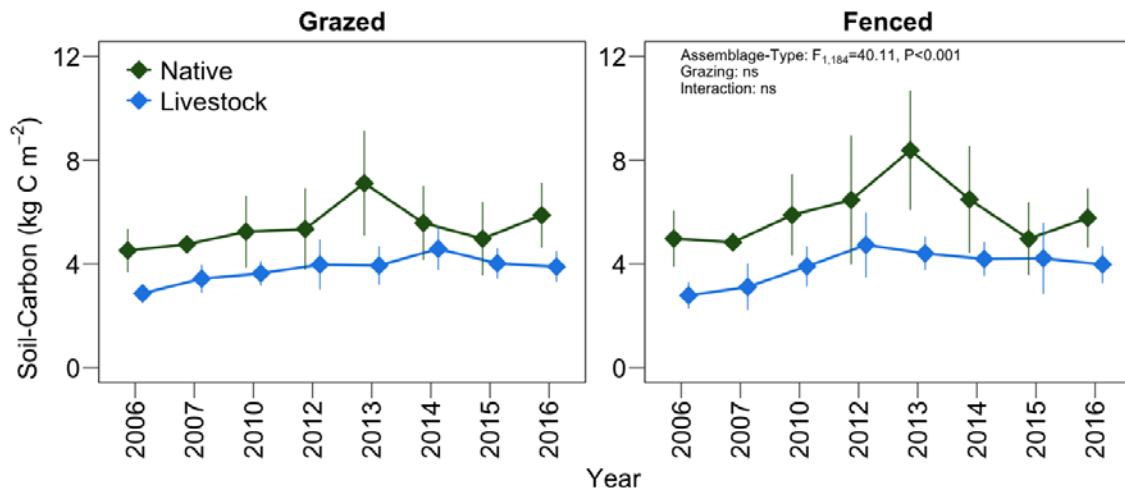
859 dissimilarity (b) shows that watersheds under native watersheds are dominated by grass,

860 whereas sedges dominate under livestock. Peak plant biomass (shoot, litter and root),

861 microbial biomass, and potential respiration (c) in native and livestock watersheds. Data are

862 from previous studies (Bagchi et al., 2017; Bagchi & Ritchie, 2010a).

863   Fig. 2

864

865

866   Figure 2. Long-term monitoring of soil-C (mean ± 95% CI) under grazed and fenced plots in

867   watersheds with native herbivores and livestock in Spiti, India. The difference in soil-C under

868   native herbivores and livestock was consistent for more than a decade and was not altered by

869   grazer-exclusion. Data are from n=30 paired control (grazed) and treatment (fenced) plots.

870

871   Fig. 3
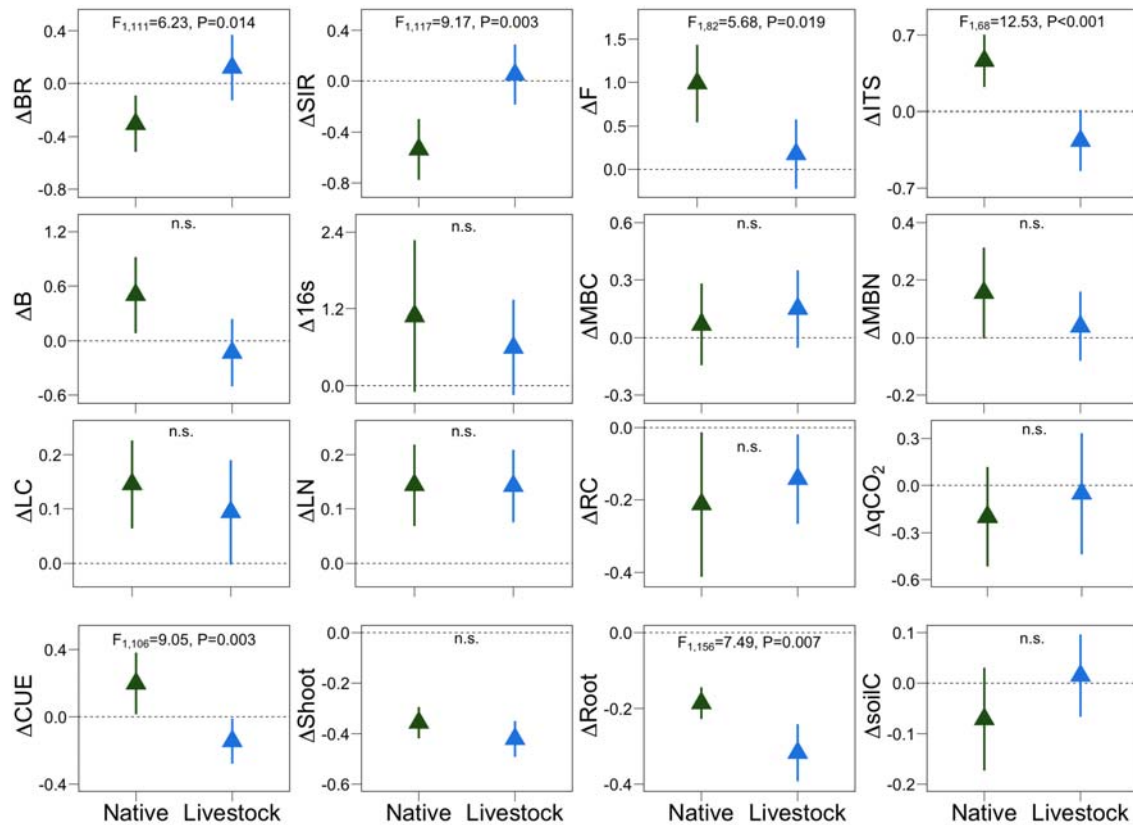
872

873   Figure 3. Grazer-effect (——— , as mean ± 95% CI) of livestock and native

874   herbivores on 15 plant and soil microbial variables, alongside soil-C in Spiti, India. These

875   variables are basal microbial respiration (BR), potential microbial respiration (SIR), Fungal

876   fraction (F), ITS DNA-amplicon abundance (ITS), Bacterial fraction (B), 16s DNA-amplicon

877   abundance (16s), Microbial Biomass Carbon (MBC), Microbial Biomass Nitrogen (MBN),

878   Labile-C (LC) and Labile-N (LN) fractions of soil organic matter, Microbial metabolic

879   quotient (qCO_2), and microbial carbon use efficiency (CUE) which were measured at

880   monthly intervals during the growth season. Vegetation biomass (ΔShoot: from difference in

881   peak aboveground biomass and ΔRoot: from difference in peak belowground biomass) and

882   grazer-effect on soil-C (ΔsoilC) are from long-term decadal measurements. Direction and

883   magnitude of differences in BR, SIR, F, ITS abundance, CUE, and Root varied with

884   herbivore assemblage-type. But the remaining variables were not influenced by herbivore

885   assemblage-type. Data are from n=30 permanent plots in Spiti region of northern India

886   (except e-DNA data, which are from a subset of 20 plots). See companion Fig. S9.

887   Fig. 4
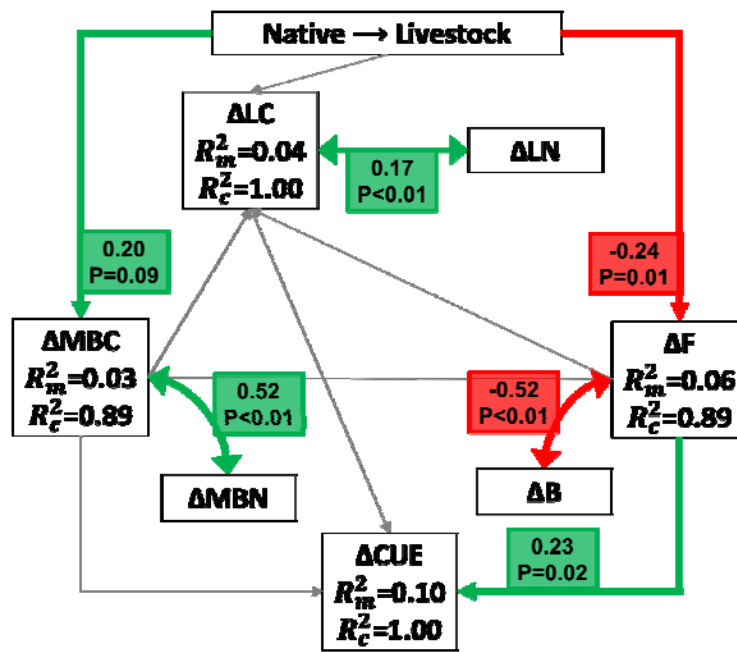
888

889

890   Figure 4. Mixed-effects structural equation model to evaluate inter-relationships between

891   grazer-effects (———) on different microbial variables with herbivore assemblage-

892   type as an explanatory variable. The variables are ΔMBC: microbial biomass-C, ΔMBN:

893   microbial biomass-N, ΔLC: labile-C, ΔLN: labile-N, ΔF: fungal biomass, ΔB: bacterial

894   biomass, ΔCUE: carbon use efficiency. SEM paths show that as herbivore-assemblage goes

895   from native to livestock, a decrease in ΔCUE via ΔF. Unidirectional arrows represent

896   hypothesized causal paths, bidirectional arrows indicate correlated paths, and rounded arrows
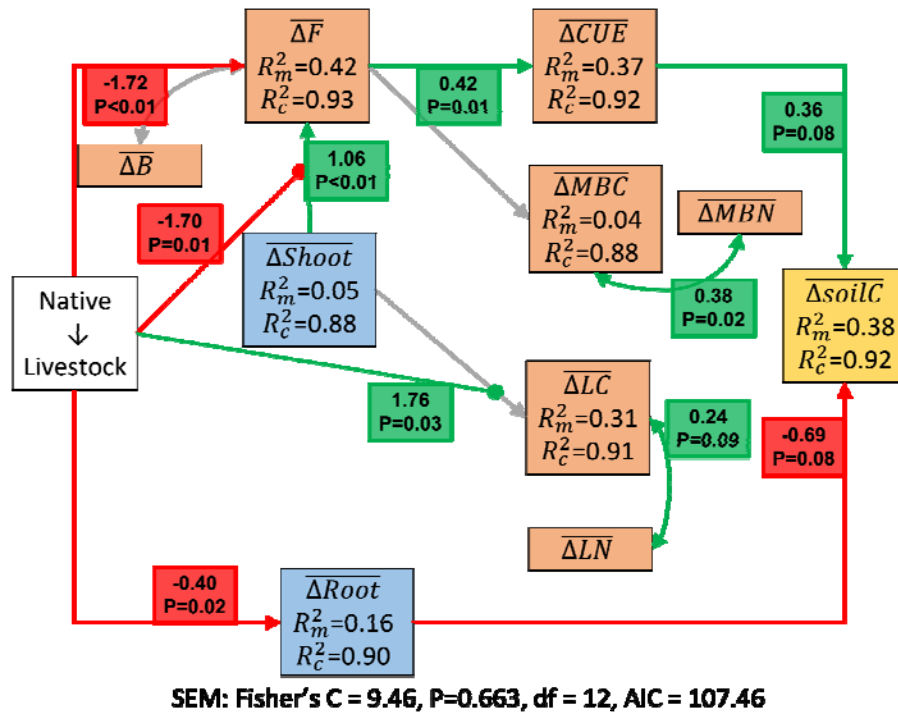
897   indicate interactions. Standardized path coefficients and their statistical significance are

898   shown alongside marginal and conditional R^2 for the different variables.

899

900

901   Fig. 5.

SEM: Fisher's C = 9.46, P=0.663, df = 12, AIC = 107.46

902

903   Figure 5. Thought experiment with mixed-effects structural equations to explore the

904   influence of plant and microbial responses to grazing by native and livestock on soil-C.

905   Unlike Fig. 4, here   is the mean grazer-effect averaged across time (months for microbial

906   variables, and years for soil-C and vegetation biomass). This helps evaluate the relative

907   influence of herbivore assemblage-type, and vegetation (and   ), and microbial

908   variables, on variation in soil-C. SEM paths suggest that replacing native herbivores with

909   livestock affects soil-C in two ways: via effects originating from plants, and through a change

910   in soil microbial communities that reduces CUE. Unidirectional arrows represent

911   hypothesized causal paths, bidirectional arrows indicate correlated paths, and rounded arrows

912   indicate interactions. Standardized path coefficients and their statistical significance are

913   shown alongside marginal and conditional R^2 for the different variables. For clarity and to

914   reduce clutter, only a few unimportant non-significant paths are shown in grey (see

915   companion Fig. S11 for all modelled paths).

916   Fig 6.
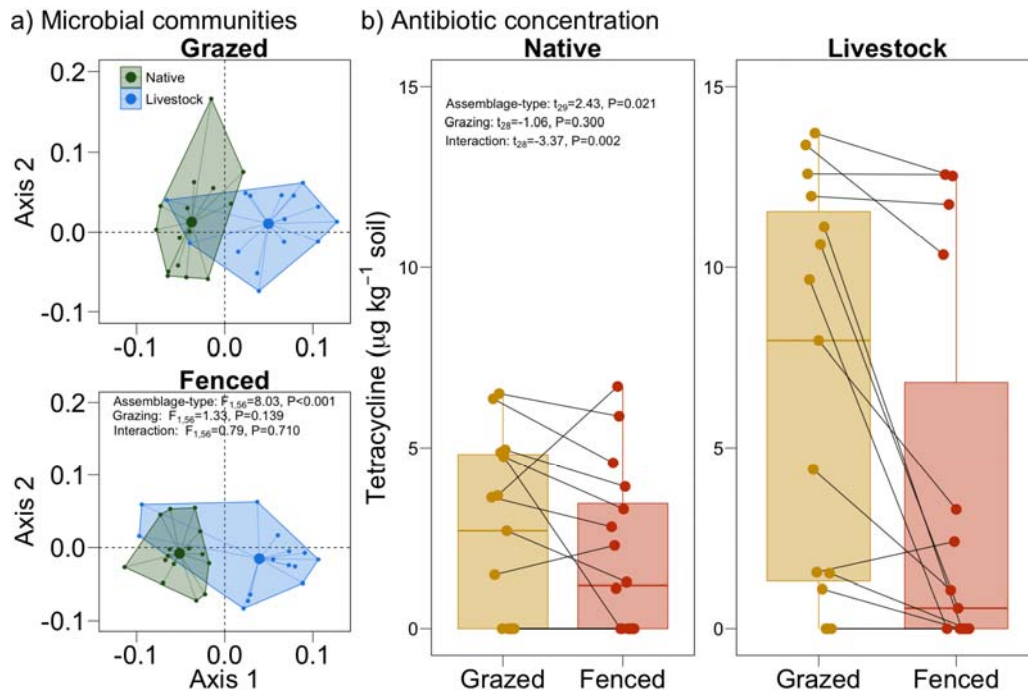
917

918   Figure 6. Differences in microbial community composition and antibiotic concentration

919   across native and livestock watersheds. Difference in microbial community composition in

920   two types of watersheds summarized with Principal Coordinates Analysis on Sorensen

921   dissimilarity matrix of DNA-based microbial OTU data (a). Microbial communities varied

922   between native and livestock watersheds, and there was no difference between grazed and

923   fenced plots. Difference in antibiotic residues (tetracycline) in soil (b). Soils under livestock

924   contain more antibiotics than soils under native herbivores, and they decline after grazer-

925   exclusion.

926