

1 Running Head: Herbivores, decomposers, and soil carbon

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3 **Large mammalian herbivores and the paradox of soil carbon in grazing ecosystems: role of**  
4 **microbial decomposers and their enzymes**

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14 Author contributions

15 S.R. and S.B. designed research; S.R. performed research; S.R. and S.B. analysed data; and S.R.

16 and S.B. wrote the paper.

17

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19

20 **Abstract**

21 Grazing is the dominant land-use across the world, and large mammalian herbivores exert strong  
22 influence over biogeochemical cycles. Grazing ecosystems feature C-rich soils, even though  
23 herbivores consume a major fraction of plant production to reduce detrital input to soil. Yet,  
24 counterintuitively, moderate grazing can promote net soil-C storage in many ecosystems  
25 compared to grazer-exclusion. We address this enigmatic influence of grazers on soil-C and test  
26 their indirect effect on proximate drivers of decomposition—microbial extracellular enzyme  
27 activity. We used a replicated long-term grazer-exclusion experiment to measure responses in  
28 above- and belowground plant biomass, soil-C stock, microbial biomass, labile/recalcitrant C-  
29 pools, and three enzymes relevant to the C-cycle: peroxidase – which initiates decomposition of  
30 recalcitrant matter, alongside beta-glucosidase and cellobiohydrolase – which act further  
31 downstream on more labile fractions. Consistent with other ecosystems, 12 years of herbivore-  
32 exclusion did not increase soil-C in the fenced plots despite higher plant biomass and higher  
33 potential detrital C-inputs. Grazer-exclusion did not alter microbial biomass; peroxidase  
34 increased three-fold and beta-glucosidase was doubled; cellobiohydrolase was unaffected.  
35 Grazer-exclusion also led to two-fold increase in recalcitrant-C and in microbial respiration, but  
36 it did not influence labile-C. Structural equation models supported the hypothesis that grazing  
37 favours soil-C via its indirect effect on peroxidase, but they did not support that the effects can  
38 run in the opposite direction where soil-C affects enzymes. Grazer-mediated shifts in how  
39 microbes deploy enzymes emerge as a plausible mechanism that affects soil-C. These linkages  
40 may be important to maintain soil-C sequestration in drylands which support large-mammalian  
41 herbivores.

42

43 **Keywords:** Grassland; Herbivory; Decomposition; Carbon sequestration; Lignin; Structural  
44 equation modelling (SEM); Soil organic matter

45

## 46 **Highlights**

- 47 • Grazing-exclusion did not increase soil-C despite higher detrital C-input.
- 48 • Peroxidase, a lignin-modifying enzyme, regulates soil-C storage in grazing ecosystems.
- 49 • Grazing reduces peroxidase activity to help net soil-C sequestration.

50

## 51 **Introduction**

52 Grazing ecosystems with large mammalian herbivores have been a characteristic feature of the  
53 earth since the Miocene. Today they occupy c. 42% of the world's terrestrial where large  
54 mammalian herbivores play important roles in global biogeochemical cycles (Hobbs 1996; Frank  
55 and others 1998; Schmitz and others 2014). Herbivores can consume, metabolise, and respire a  
56 large fraction of the carbon fixed by plants before it enters the soil as detritus (McNaughton and  
57 others 1989; Cebrian 1999; Cebrian and Lartigue 2004; Jia and others 2018). Despite supporting  
58 herbivores who divert carbon into secondary production and away from soil organic matter,  
59 grazing ecosystems contain over a third of the global soil-C (c. 500 Pg) (Lal 2004b). Further,  
60 empirical estimates from around the world have repeatedly found that moderate grazing often  
61 leads to net soil-C sequestration, compared to no grazing at all, while overgrazing depletes soil-C  
62 stocks (Conant and Paustian 2002; Pineiro and others 2009; Witt and others 2011; Tanentzap and  
63 Coomes 2012; Chen and others 2015; Conant and others 2017; Wilson and others 2018; Derner  
64 and others 2019; Sitters and others 2020; Zhan and others 2020). Counter-intuitively, reducing  
65 the quantity of C-input from plants to soil and diverting it towards secondary production by

66 grazing can result in higher soil-C. Meta-analyses and reviews have quantified this apparent  
67 paradox where grazer-exclusion did not necessarily result in long-term soil-C gains in many  
68 ecosystems (Pineiro and others 2009; McSherry and Ritchie 2013; Conant and others 2017;  
69 Abdalla and others 2018; Liu and others 2020; Zhan and others 2020). This enigmatic effect of  
70 herbivores on soil-C occurs across a broad range of temperature, precipitation, seasonality, and  
71 productivity (Fig. S1). It can be found in tropical savannas (Sitters and others 2020), temperate  
72 grasslands (Chen and others 2015; Derner and others 2019), alpine-steppes (Liu and others  
73 2020), sub-tropical grasslands (Pineiro and others 2009), and subtropical shrub-steppes (Witt and  
74 others 2011). This net positive effect of grazers on soil-C tends to be stronger in drier and less  
75 productive environments than in more mesic conditions (McSherry and Ritchie 2013; Liu and  
76 others 2020). Importantly, removing plant biomass inputs to detritus by other means, such as fire,  
77 reduces soil-C (Pellegrini and others 2018). Identifying the underlying mechanisms which  
78 explain this paradox can have implications for managing a large soil-C pool across the world  
79 (Lal 2004a; Follett and Reed 2010; Paustian and others 2016; Conant and others 2017).

80         Several mutually non-exclusive explanations have been proposed to account for these  
81 observed effects of grazers on soil-C. The include grazing-induced changes in vegetation  
82 composition and plant biomass allocation (Bagchi and Ritchie 2010b; Eldridge and others 2018;  
83 Koerner and others 2018), change in quality of C-input (Hobbs 1996; Olofsson and Oksanen  
84 2002), and in soil microbial processes such as their metabolism and community composition  
85 (Sankaran and Augustine 2004; Bagchi and others 2017; Wilson and others 2018). Different  
86 studies have found evidence either in support of, or against these individual explanations under  
87 differing environmental settings. These lend support to a viewpoint that grazers can have  
88 context-dependent effects as distinct mechanisms become important in different environmental

89 settings (McSherry and Ritchie 2013; Abdalla and others 2018). Amidst such heterogeneity, one  
90 aspect that is common across environmental settings is the indirect effect of grazers on soil  
91 microbial decomposers. While there is increasing evidence that grazing influences soil microbial  
92 processes (Sankaran and Augustine 2004; Bagchi and others 2017; Wilson and others 2018), it is  
93 not yet clear how this could explain the counter-intuitive relationship between grazers and soil-C.  
94 Potential explanations could involve indirect effects where grazers alter the proximate drivers of  
95 decomposition, i.e., microbial extracellular-enzymes. Indeed, recent studies show that grazer-  
96 exclusion increases the activity of lignin-modifying enzymes in soil (Fig. S2). Since lignin is an  
97 important component of soil organic matter, this can potentially alter decomposition when  
98 grazers are excluded. But it remains unknown whether such a microbial response translates into  
99 long-term effects on soil-C.

100         The role of soil microbes and their extracellular enzymes now becomes a prime candidate  
101 to resolve why grazers can have net positive effects on soil-C. Decomposition is initiated by  
102 enzymes such as peroxidase that act on large polymers (e.g., lignin) (Burns and Dick 2002;  
103 Sinsabaugh 2010). Further downstream, other enzymes catalyse the release of assimilable end-  
104 products from oligomers, e.g., glucosidase, cellobiohydrolase (Burns and Dick 2002; Sinsabaugh  
105 2010). Lignin is recalcitrant and is considered a key influence on soil-C sequestration, as lignin  
106 breakdown can often be a rate-limiting step in the decomposition process (Hall and others 2020).  
107 Therefore, any increase in activity of lignin modifying enzymes can decrease soil-C stocks  
108 (Schmidt and others 2011; Lehmann and Kleber 2015; Chen and others 2018a, 2018b). In  
109 comparison, downstream enzymes that act on more labile fractions should not have any long-  
110 term term effect on soil-C. From these, we hypothesise that grazer-induced shifts in lignin-  
111 modifying peroxidase can regulate soil-C storage, and any parallel response in glucosidase and

112 cellobiohydrolase should be weakly related to soil-C storage. Here we use a long-term grazer-  
113 exclusion experiment to investigate whether soil microbial responses – via their deployment of  
114 key enzymes – are consistent with the hypothesis that grazing suppresses decomposition to  
115 favour net soil-C storage.

116

## 117 **Methods**

### 118 **Study site**

119 Our study site in Spiti, northern India (32° N, 78° E), is a high-altitude (4400 m elevation)  
120 grazing ecosystem in the Trans-Himalayas which covers the Tibetan highlands and adjacent  
121 mountains in Central Asia (Fig. S3). The region experiences cold and semi-arid climate with a  
122 short vegetation growing season during the warm months (May-August) with a peak in July.  
123 Plants are senescent by August-September, and litter gets buried under snow and ice during  
124 winter (November-March) when temperatures drop below -30 °C. Annual precipitation of c. 450  
125 mm yr<sup>-1</sup> occurs mostly as snow (200-300 mm in November-April), and as 50-200 mm of rain  
126 (July-August) due to the summer monsoon (Fig. S4). Soil pH is neutral to alkaline, water holding  
127 capacity ranges from 0.8 to 1.2 ml g<sup>-1</sup> soil, bulk density is 1.05-1.25 g cm<sup>-3</sup>, and soil texture is  
128 sandy-loam (Fig. S4). The ecosystem supports native herbivores (bharal *Pseudois nayaur*, ibex  
129 *Capra sibirica*, yak *Bos grunniens*) and livestock (goat, sheep, donkey, horse, cattle, and yak-  
130 cattle hybrids) who consume c. 60% of the annual plant production during the growing season  
131 (Bagchi and Ritchie 2010b, 2010a). In the 50-60 km<sup>2</sup> area around village Kibber in Spiti,  
132 herbivore biomass of c. 1.41×10<sup>5</sup> kg (Bagchi and Ritchie 2010b, 2010a; Singh and others 2015)  
133 falls within theoretical estimates for carrying capacity of 2500-3200 kg km<sup>-2</sup> calculable from  
134 productivity, precipitation, and soil nutrients (Fritz and Duncan 1994).

135 **Sampling**

136 We established  $n=30$  fenced exclosures starting in 2005-06, each  $10 \times 10 \text{ m}^2$ , with a paired-  
137 adjacent control plot. We collected soil samples during peak season in July 2017, with a 5 cm  
138 diameter corer till 20 cm depth and analysed enzyme activity with laboratory assays within 4-5  
139 days from the time of collection. We measured standing live above- and belowground biomass at  
140 the end of the growing season in August. We collected shoot biomass from  $0.5 \times 0.5 \text{ m}^2$  quadrats  
141 in each paired plot, and root biomass using 20 cm deep and 5 cm radius cores. Biomass samples  
142 were sun-dried, and subsequently oven-dried to constant weight at  $40 \text{ }^\circ\text{C}$  to obtain their dry-  
143 weight ( $\text{g m}^{-2}$ ).

144

145 **Soil and microbial analysis**

146 We analysed soil-C with an elemental analyser (TruSpec, Leco, USA) and express as carbon  
147 density up to 20 cm depth ( $\text{kg C m}^{-2}$ ). We measured soil microbial biomass using chloroform  
148 fumigation-extraction (Anderson and Domsch 1978; Bagchi and others 2017). Briefly, we  
149 incubated 4 g of soil at 60% water holding capacity with ethanol-free chloroform for 24 hr,  
150 extracted them in 0.05 M  $\text{K}_2\text{SO}_4$ , and analysed for C-content in TOC/TN analyser (Shimadzu,  
151 LCPH/CPN, Japan). We considered extraction-efficiency to be 0.54 (Beck and others 1997), and  
152 microbial biomass ( $\text{mg C g}^{-1}$  soil) as the difference between fumigated samples and unfumigated  
153 controls.

154 We measured the Beta-glucosidase (BG), Cellobiohydrolase (CBH), and Peroxidase  
155 (PER) activity following standard protocols (Saiya-Cork and others 2002; German and others  
156 2011). Briefly, we homogenised 1 g soil in 100 ml of Tris buffer at pH 7.8 to prepare a  
157 suspension. We assayed BG and CBH with a fluorogenic substrate (4-MUB  $\beta$ -D-

158 glucopyranoside and 4-MUB  $\beta$ -D-cellobioside, respectively), and PER with chromogenic  
159 substrate (L-dihydroxyphenylalanine). For BG and CBH, we added 200  $\mu$ L of 200 mM substrate  
160 to 50  $\mu$ L of soil slurry and incubated for 2 hr. For PER, we added 50  $\mu$ L of 25 mM substrate to  
161 200  $\mu$ L of slurry with 10  $\mu$ L of 0.3% H<sub>2</sub>O<sub>2</sub>, incubated for 15 hr, and then stopped the reaction  
162 with 10  $\mu$ L of 2 M NaOH. We incubation samples at 20 °C, as this approximates average  
163 temperature during the growing season. Fluorogenic measurements (BG and CBH) were done in  
164 96-well fluorimeter (Tecan Infinite M200 Pro, Switzerland) at excitation wavelength 365 nm and  
165 emission wavelength 450 nm. For PER, we measured absorbance at 460 nm. For internal quality  
166 checks, we repeated each sample for all assays four times with corresponding standards, and then  
167 averaged (median) the readings before statistical analysis of enzyme activity (nmol hr<sup>-1</sup> g<sup>-1</sup> soil).

168 We used two-step acid hydrolysis of soil organic matter (SOM) to measure  
169 labile/recalcitrant pools (Rovira and Vallejo 2002; Khalili and others 2016). Briefly, we added  
170 20 ml of 5N H<sub>2</sub>SO<sub>4</sub> to 500 mg of soil, and incubated for 30 min at 105 °C. After incubation, we  
171 centrifuged the mixture at 3000 RPM for 2 min (1000 G-force approx., Remi R-4C, Bangalore)  
172 and stored the supernatant. To the remaining soil residue, we added 2 ml of 26N H<sub>2</sub>SO<sub>4</sub> and  
173 incubated overnight with continuous shaking. After incubation, we diluted the acid to 2N by  
174 adding water, and incubated again at 105 °C for 3 hours. This was also centrifuged at 3000 RPM  
175 for 2 min to obtain the supernatant. Sum of carbon content in supernatants from the first and  
176 second step was considered as the labile-C pool. The difference between total organic carbon and  
177 labile-C was the recalcitrant C-pool, and we express both fractions as mg g<sup>-1</sup> soil.

178 For microbial basal respiration, we used the alkali trap method for respired CO<sub>2</sub>. Briefly,  
179 we pre-incubated 4 g of dry soil at 60% water holding capacity for 24 hours. Next, we kept a  
180 beaker containing 1 N KOH solution along with samples in a sealed container. After incubation

181 for 24 hr at 28 °C, we added 1ml of 15% BaCl<sub>2</sub> to KOH and titrated 2 ml of the solution with 0.1  
182 N HCl using phenolphthalein as indicator, and calculated CO<sub>2</sub> respired from soil (mg C g<sup>-1</sup> soil  
183 day<sup>-1</sup>).

184

## 185 **Data analysis**

186 We evaluated responses to grazer-exclusion with general linear mixed-effects models (GLM)  
187 where grazing (fenced or grazed) was a fixed-effect and plot identity was a random-effect. In this  
188 way, the GLM accounts for the paired structure of the replicated exclosures since model  
189 intercepts can vary between sampling locations (Pinheiro and Bates 2000). We summarized  
190 grazer-effect for each variable as the ln response-ratio of paired fenced and grazed plots, i.e.,  $\Delta_i =$   
191  $\ln\left(\frac{\text{Fenced}_i}{\text{Grazed}_i}\right)$  for the  $i^{\text{th}}$  pair, and calculated their mean and 95% CI. We evaluated bivariate  
192 relationships between grazer-exclusion effects in different variables (pair-wise Spearman's  
193 correlation). We used structural equation modelling, SEM (Grace 2006), to evaluate likely causal  
194 relationships from the overall variance-covariance structure in the data across grazer-exclusion  
195 effects ( $\Delta$ ) for all variables (Wang and others 2020). SEMs help evaluate hypothesized pathways  
196 over how one variable can influence another. When SEM paths are supported by data, it does not  
197 automatically imply causation (Grace 2006). Rather, it means that the hypothesized paths can  
198 offer a plausible explanation for the process. When SEM paths are not supported, it suggests that  
199 the data may not arise from the hypothesized process. To retain the paired structure in the data  
200 from fenced-and-grazed plots, we incorporated SEM paths as mixed-effect models that allowed  
201 the intercept to vary among replicates through a random-effect for plot identity (Shipley 2009;  
202 Lefcheck 2016). Here we tested whether the data support the hypothesis that grazer-exclusion  
203 effect on soil-C is mediated by changes in microbial biomass and in extracellular enzymes. SEM

204 paths were motivated by *a-priori* instances known in literature (Table S2). We started with a full  
205 model with all paths (Fig. S6) and evaluated the variation explained by the different variables  
206 (marginal  $R^2$ ). For parsimony, we simplified this full model and removed paths which explained  
207 the least variation (Borer and others 2012). In the simplified model, paths for cellobiohydrolase  
208 and basal respiration were dropped. We assessed the agreement between data and hypothesized  
209 paths with Fisher's C statistic (Shipley 2009; Lefcheck 2016). In parallel, we also evaluated the  
210 alternative hypothesis where grazer-effects on soil-C leads to changes in microbial functions;  
211 directionality is reversed in this competing model (Fig. S8). We report standardised path  
212 coefficients and their statistical significance ( $\alpha=0.05$ ). For each variable we also report individual  
213 GLM models (Table S3), alongside marginal and conditional  $R^2$  (Table S4) that represent the  
214 influence from fixed-effects and site-specific variability, respectively. We performed these  
215 analyses in R 4.0.3 (R Development Core Team 2020) with nlme (Pinheiro and others 2020),  
216 piecewiseSEM (Lefcheck 2016), and ggplot2 (Wickham 2016) libraries.

217

## 218 **Results**

### 219 **Responses to grazer-exclusion**

220 As expected, grazer-exclusion increased potential C-input to soil from plant biomass. Live  
221 aboveground (shoot) biomass was 46.8% higher in fenced plots ( $\bar{x} = 40.1 \pm 2.7$  SE,  $\text{g m}^{-2}$ ) than in  
222 grazed plots ( $27.3 \pm 2.3$   $\text{g m}^{-2}$ , Fig. 1). Grazing-exclusion had a similar positive effect on  
223 belowground (root) biomass as it was 18.3% higher in fenced plots ( $930.4 \pm 78.1$   $\text{g m}^{-2}$ ) than in  
224 grazed plots ( $786.6 \pm 85.6$   $\text{g m}^{-2}$ , Fig. 1). Even after 12 years of grazer-exclusion, soil-C stock in  
225 fenced plots ( $3.81 \pm 0.29$   $\text{kg C m}^{-2}$ ) had not exceeded that in grazed plots ( $3.66 \pm 0.24$   $\text{kg C m}^{-2}$ ,

226 Fig. 1). Microbial biomass (Fig. 1) was comparable between the fenced ( $0.086 \pm 0.02 \text{ mg C g}^{-1}$   
227 soil) and grazed plots ( $0.084 \pm 0.02 \text{ mg C g}^{-1}$  soil).

228 Peroxidase activity (Fig. 2) was 359.9% higher in the fenced plots ( $0.47 \pm 0.11 \text{ nmol hr}^{-1}$   
229  $\text{g}^{-1}$  soil) than in the grazed plots ( $0.10 \pm 0.02 \text{ nmol hr}^{-1} \text{ g}^{-1}$  soil). Beta-glucosidase activity (Fig. 2)  
230 was 139.8% higher in the fenced plots ( $71.71 \pm 13.74 \text{ nmol hr}^{-1} \text{ g}^{-1}$  soil) than in the grazed plots  
231 ( $35.82 \pm 5.49 \text{ nmol hr}^{-1} \text{ g}^{-1}$  soil). Grazing-exclusion did not influence cellobiohydrolase activity  
232 (Fig. 2). Mass-specific enzyme activity, i.e., activity per unit microbial biomass, was higher for  
233 peroxidase (176.4%,  $F_{1,29}=7.12$ ,  $P=0.012$ ) and for beta-glucosidase (74.7%,  $F_{1,29}=6.76$ ,  $P=0.015$ )  
234 in the fenced plots, but was unaffected for cellobiohydrolase ( $F_{1,29}=0.02$ ,  $P=0.883$ ).

235 Grazing-exclusion did not influence labile-C (Fig. 3). Recalcitrant-C (Fig. 3) was 26.9%  
236 higher in fenced plots ( $10.10 \pm 1.26 \text{ mg g}^{-1}$  soil) than in grazed plots ( $7.96 \pm 1.05 \text{ mg g}^{-1}$  soil).  
237 Microbial basal respiration (Fig. 3) was 113.4% higher in fenced plots ( $0.49 \pm 0.03 \text{ mg C g}^{-1}$  soil  
238  $\text{day}^{-1}$ ) than in grazed plots ( $0.22 \pm 0.03 \text{ mg C g}^{-1}$  soil  $\text{day}^{-1}$ ).

239

#### 240 **Inter-relationships between variables**

241 Pair-wise correlations between grazer-exclusion effects on the different variables showed that  
242 they were inter-related (Fig. 4). Expectedly, changes in soil-C and in peroxidase were negatively  
243 related. The full SEM model with all paths accounted for the variance-covariance structure  
244 among the different variables (Fisher's  $C = 11.60$ ,  $P=0.70$ ,  $df = 16$ , Fig. S7). Removing the least  
245 influential variables for a simplified model also showed there was no random match between the  
246 data and hypothesized paths (Fisher's  $C = 6.72$ ,  $P=0.34$ ,  $df = 6$ , Fig. 5). Path coefficients  
247 supported the hypothesis that grazer-exclusion effect on soil-C is mediated by changes in  
248 microbial biomass and in peroxidase (Fig. 5, Fig. S7). Grazer-exclusion led to changes in plant

249 biomass ( $\Delta$ Root) that were related to changes in microbial biomass, which was in-turn related to  
250 changes in peroxidase. Changes in peroxidase activity affected recalcitrant-C pools, but not the  
251 labile-C pool. Expectedly, soil-C were affected by changes in both pools (Fig. 5, S7). Marginal  
252 and conditional  $R^2$  indicated that while the fixed-effects were influential, there was considerable  
253 site-specific heterogeneity among replicates in many variables (Table S4).

254 The data did not support the competing alternative hypothesis where soil-C influences  
255 microbial processes (Fig. S8). This alternative model (Fisher's  $C = 3.66$ ,  $P=0.88$ ,  $df = 8$ )  
256 revealed relationships between soil-C and its constituent pools, and between the different  
257 microbial variables (Fig. S8). There was no support for an influence of soil-C on peroxidase (Fig.  
258 S8), but there was good support in the opposite direction (Fig. 5).

259

## 260 **Discussion**

261 Since herbivores divert a considerable fraction of the carbon fixed by plants toward secondary  
262 production and metabolism to reduce detrital C-input to soil, it is puzzling why grazing  
263 ecosystems feature C-rich soils. Consistent with other studies (Pineiro and others 2009, 2010;  
264 Witt and others 2011; Chen and others 2015; Abdalla and others 2018; Derner and others 2019;  
265 Liu and others 2020; Sitters and others 2020; Zhan and others 2020), we also find that fenced  
266 plots did not contain more soil-C than grazed plots even after long-term (12-yr) grazer-exclusion.  
267 Hence, the paradox (Fig. 1). Univariate and bivariate analyses, and SEM, offer insights into  
268 grazer-effects on microbial extracellular enzymes as the proximate drivers of soil-C. As grazer-  
269 exclusion effects on total and mass-specific enzyme-activity were in the same direction (Fig. 2,  
270 Table S1), this indicates microbes deploy enzymes in response to grazing. The influence of  
271 grazers on soil-C were explained by their effects on peroxidase activity which in turn affected

272 changes in soil-C via the labile and recalcitrant pools (Fig. 5). Unlike peroxidase, there were no  
273 links involving grazer-effects on beta glucosidase and cellobiohydrolase which act further  
274 downstream in the biochemical decomposition of soil organic matter. SEM also revealed that  
275 change in belowground root biomass exercises stronger indirect influence over soil-C storage  
276 than changes in shoot biomass (Fig. 4). This is consistent with previous studies in the Trans-  
277 Himalayan ecosystem (Bagchi and Ritchie 2010b, 2010a), and with patterns seen in other  
278 ecosystems (Derner and others 2006; Gao and others 2008a; Yang and others 2010). High  
279 root:shoot ratio is a general pattern across dry regions of the world (Gao and others 2008b;  
280 Bagchi and Ritchie 2010b, 2010a; Qiu and others 2013).

281         Our results offer a connection between the roles of consumers and decomposers (Schmitz  
282 and others 2014) where herbivores affect soil-C via their influence on soil microbial processes  
283 (Fig. 5). Grazing-induced shifts in peroxidase activity and the resultant impacts on recalcitrant  
284 soil organic matter appear to favour soil-C storage. Change in labile/recalcitrant fractions can  
285 stem from difference in both the quantity (Fig 5) as well as quality of litter-input. Herbivore-  
286 exclusion is known to increase C:N ratio of litter by 50% in this ecosystem (Bagchi and Ritchie  
287 2010a). Therefore, the grazer-effects on litter quality and on its eventual fate after entering soil  
288 organic matter (Fig. 3) are consistent with each other and in the same direction. Since litter can  
289 eventually transform into soil organic matter within days or weeks (Djukic and others 2018), the  
290 effects arising from labile/recalcitrant pools are indicative of the dual response in both the quality  
291 and quality of C-inputs from plants (Fig. 3-5).

292         While the grazer effects on soil-C are recognised across tropical savannas, temperate  
293 grasslands, alpine-steppes, sub-tropical grasslands, subtropical shrub-steppes (Fig. S1), its  
294 strength varies between sites (McSherry and Ritchie 2013; Liu and others 2020). We find that

295 grazer-exclusion effects on plants, microbes, and their enzymes, accounted for a large fraction of  
296 the variation in soil-C between replicates (marginal  $R^2=0.77$ ). In comparison, high variability in  
297 peroxidase among replicates (marginal  $R^2=0.19$ ) indicates that enzymes themselves are  
298 influenced by other unmeasured factors in addition to herbivores, but they in-turn have consistent  
299 effects on soil-C across the landscape (Fig. 5, Table S4). Independently, grazer-effects on lignin-  
300 modifying enzymes, so far known from relatively mesic sites (shrub-steppes, arctic meadows,  
301 temperate prairies, conifer savannas, alpine steppes, and temperate woodlands, Fig. S2) appear  
302 weaker than at our semi-arid study-site (Fig. 2). Now, rates of decomposition of soil organic  
303 matter depend, among other factors, on moisture-availability. Therefore, even if grazing strongly  
304 influences soil microbial functions across a gradient from dry to mesic environments, the net  
305 effect on soil-C can get diluted when moisture is abundant such that soil organic matter  
306 decomposes relatively fast. But, these effects can become more influential in dry environments  
307 when decomposition rates are sluggish (McSherry and Ritchie 2013; Liu and others 2020).  
308 Photodegradation can also influence soil organic matter decomposition (Austin and Ballare  
309 2010). Dry ecosystems also tend to receive high levels of solar radiation at ground level (e.g.,  
310 daily direct radiation flux at our study site can be 5-6 kWh m<sup>-2</sup>, and can exceed 2000 kWh m<sup>-2</sup>  
311 annually). While photodegradation could be a possible explanation for net carbon losses seen in  
312 Fig. 1, it does not explain the patterns seen in Fig. 2. Many field experiments have also revealed  
313 that N-amendment to soil reduces the activity of lignin-modifying enzymes, and this favours net  
314 soil-C sequestration (Chen and others 2018b). Specifically, slower lignin decomposition under  
315 N-amendment leads to increased soil-C (Argiroff and others 2019; Tian and others 2019), such  
316 that lignin-modifying enzymes like peroxidase play an important role (Chen and others 2018b).  
317 N-amendment can help microbes meet their metabolic N-demand without investing in enzymes

318 to extract N from well-shielded recalcitrant polymers in organic matter (Argiroff and others  
319 2019; Tian and others 2019). Therefore, offsetting N-limitation can lower microbial investment  
320 in lignin-modifying peroxidase (Chen and others 2018b). Qualitatively, grazing can have the  
321 same effect as N-amendment because it returns dung and urine to soil (Hobbs 1996; Frank and  
322 others 1998), whereas the fenced plots receive litter (Chen and others 2021). Effectively, from  
323 the microbes' perspective, herbivores alter low-quality litter (high C:N) into high-quality dung  
324 (low C:N). This net N-enrichment can enable microbes to invest less in peroxidase, when  
325 compared to the fenced plots, and subsequent consequences for soil-C are consistent with known  
326 biochemical processes (Chen and others 2018b; Argiroff and others 2019; Tian and others 2019).

327 Our results reveal an indirect linkage between herbivores and soil-microbes that can  
328 effectively lengthen the residence time for soil-C to yield net soil-C sequestration. Knowledge on  
329 biomass of producers, consumers and decomposers maybe inadequate to determine the  
330 consequences for soil-C, since their effects on each other are also important. Maintaining the  
331 influence of large mammalian herbivores on grazing ecosystems through conservation and  
332 rewilding (Stalmans and others 2019; Lundgren and others 2020) may be of high importance for  
333 soil-C sequestration. Human activities have imposed high extinction pressures on wild free-  
334 ranging herbivores via hunting, poaching, and habitat-loss (Ripple and others 2017), as well as  
335 repurposed natural grazing ecosystems for livestock-production which often leads to overgrazing  
336 and soil-C loss (Bagchi and Ritchie 2010b). Conservation and rewilding efforts (Stalmans and  
337 others 2019; Lundgren and others 2020) can arrest and reverse the declining influence of  
338 herbivores on ecosystem functions (Brodie and others 2021), but they face a variety of  
339 challenges (Veldhuis and others 2019). Sustaining these efforts may be a crucial determinant of

340 whether grazing ecosystems can continue to sequester soil-C and decarbonise the atmosphere to  
341 provide a valuable climate-mitigation service.

342

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350

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546

547 **Figures and legends**

548 **Figure 1.** Response to experimental grazer-exclusion in standing aboveground biomass (**a**),  
549 standing belowground biomass (**b**), soil-C (**c**), and microbial biomass (**d**). Grazer-exclusion  
550 increases above and belowground biomass, whereas bulk soil-C content and microbial biomass  
551 remain unchanged. Grazer-effect ( $\Delta$ , ln response-ratio) on these variables is summarised as mean  
552  $\pm$  95% CI (**e**). Data are from  $n=30$  paired fenced and grazed plots in Spiti, Trans-Himalaya.

553 **Figure 2.** Response to experimental grazer-exclusion in peroxidase (PER) activity in soil (**a**),  
554 beta-glucosidase (BG) activity (**b**), and cellobiohydrolase (CBH) activity (**c**). Grazer-exclusion  
555 increases peroxidase, and beta-glucosidase, but cellobiohydrolase is unchanged. Grazer-effect  
556 ( $\Delta$ , ln response-ratio) on these variables is summarised as mean  $\pm$  95% CI in (**d**). Data are from  
557  $n=30$  paired fenced and grazed plots in Spiti, Trans-Himalaya.

558 **Figure 3.** Response to experimental grazer-exclusion in labile-C (LC) pool in soil (**a**),  
559 recalcitrant-C (RC) pool (**b**), and basal respiration (BR) of microbes in soil (**c**). Grazer-exclusion  
560 increases recalcitrant-C, and basal respiration, but did not affect labile-C. Grazer-effect ( $\Delta$ , ln  
561 response-ratio) on these variables is summarised as mean  $\pm$  95% CI (**d**). Data are from  $n=30$   
562 paired fenced and grazed plots in Spiti, Trans-Himalaya.

563 **Figure 4.** Correlation between grazer-effects on eleven interrelated soil and microbial variables  
564 (**a**). Effect of experimental herbivore-exclusion ( $\Delta$ , ln response-ratio) was calculated from paired  
565 fenced and grazed plots.  $\Delta$ Shoot: aboveground biomass,  $\Delta$ Root: belowground biomass,  $\Delta$ MB:  
566 microbial biomass,  $\Delta$ PER: peroxidase activity,  $\Delta$ BG: beta-glucosidase activity,  $\Delta$ CBH:  
567 cellobiohydrolase activity,  $\Delta$ Soil-C: bulk soil carbon content,  $\Delta$ LC: labile-C pool,  $\Delta$ RC:  
568 recalcitrant-C pool,  $\Delta$ BR: basal respiration. Pair-wise relationships between significantly

569 correlated variables in **b–h**. Data are from  $n=30$  paired fenced and grazed plots in Spiti, Trans-  
570 Himalaya.

571 **Figure 5.** Summary of structural equation modelling to account for variance-covariance between  
572 grazer-effects on different variables, to evaluate the hypothesis that grazing affects soil-C via its  
573 influence on soil microbial functions. For each variable, grazer-effect ( $\Delta$ , ln response-ratio) was  
574 calculated from paired fenced and grazed plots and paths are from mixed-effects models.

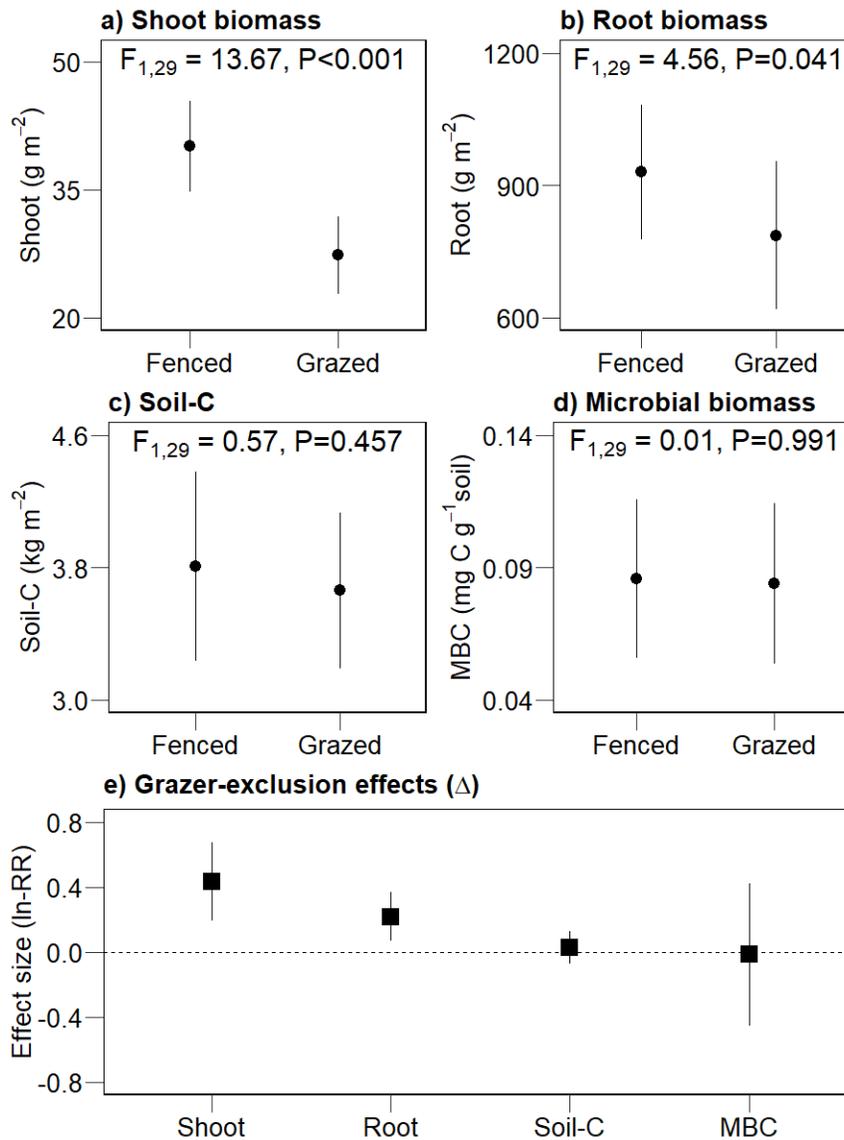
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576  $\Delta$ PER: peroxidase activity,  $\Delta$ BG: beta-glucosidase activity,  $\Delta$ CBH: cellobiohydrolase activity,  
577  $\Delta$ Soil-C: soil carbon,  $\Delta$ LC: labile-C pool,  $\Delta$ RC: recalcitrant-C pool,  $\Delta$ BR: basal respiration.

578 Thick arrows (green and red) are statistically significant paths; white arrows are paths that were  
579 statistically non-significant ( $\alpha = 0.05$ ).  $R^2$  values indicate variance explained (marginal) by fixed-  
580 effects. SEM model diagnostics were Fisher's  $C = 6.72$ ,  $P=0.34$ ,  $df = 6$ ;  $AIC = 98.72$ . Asterix  
581 represent statistical significance ( $***P \leq 0.001$ ,  $**P \leq 0.01$ , and  $*P \leq 0.05$ ).

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583

Fig. 1



585

586 **Figure 1.** Response to experimental grazer-exclusion in standing aboveground biomass (a),

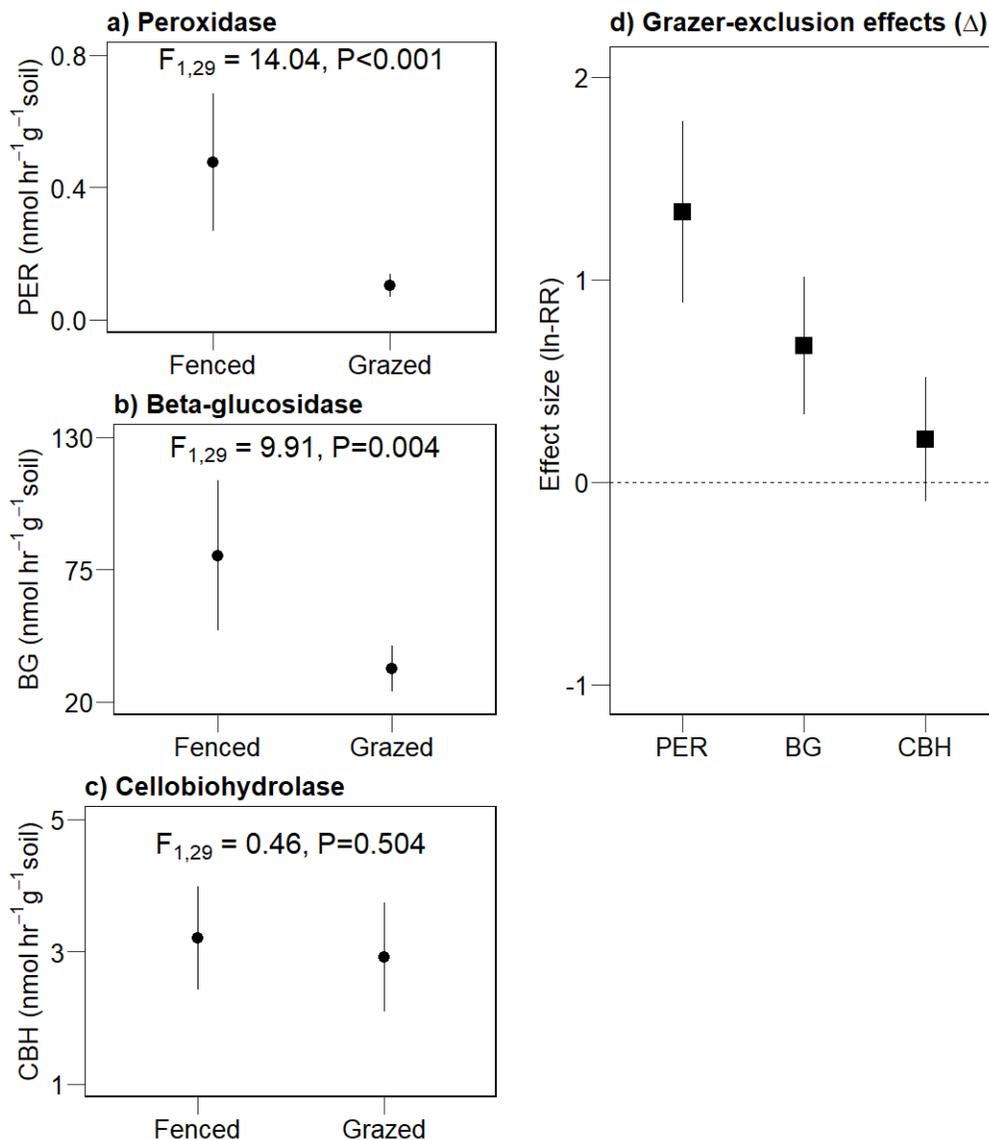
587 standing belowground biomass (b), soil-C (c), and microbial biomass (d). Grazer-exclusion

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589 remain unchanged. Grazer-effect ( $\Delta$ , ln response-ratio) on these variables is summarised as mean590  $\pm$  95% CI (e). Data are from  $n=30$  paired fenced and grazed plots in Spiti, Trans-Himalaya.

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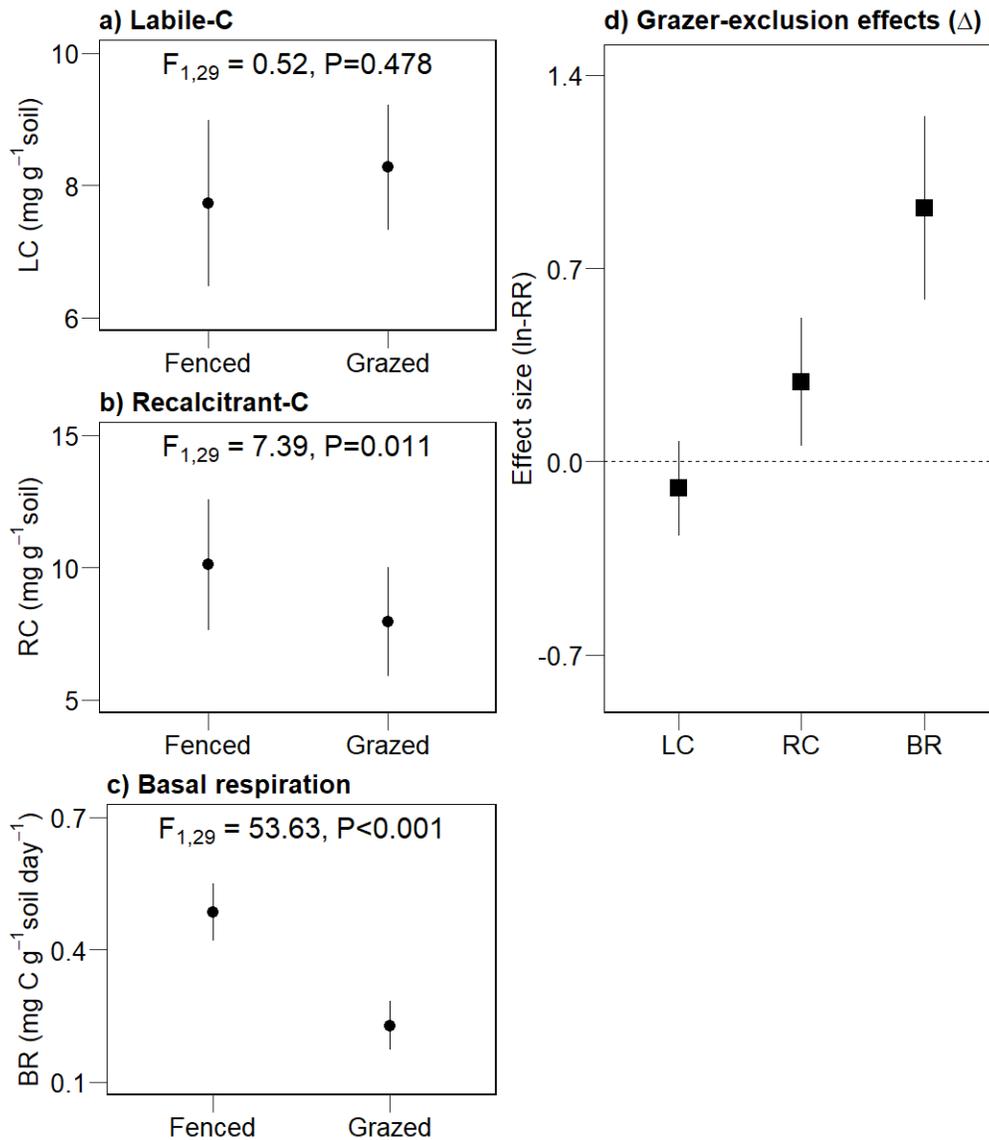
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595 **Figure 2.** Response to experimental grazer-exclusion in peroxidase (PER) activity in soil (a),  
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600

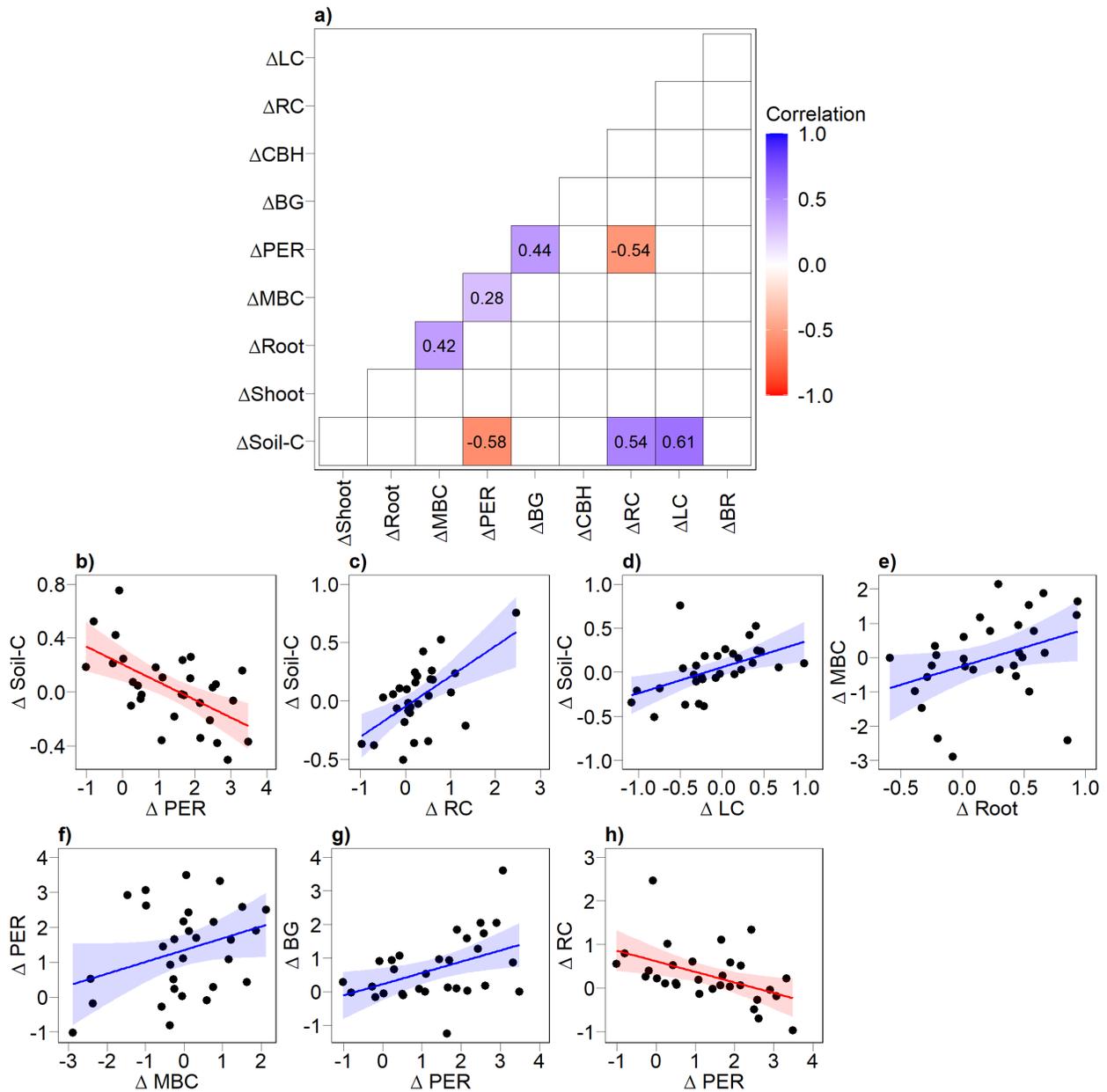


602

603 **Figure 3.** Response to experimental grazer-exclusion in labile-C (LC) pool in soil (a),  
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608

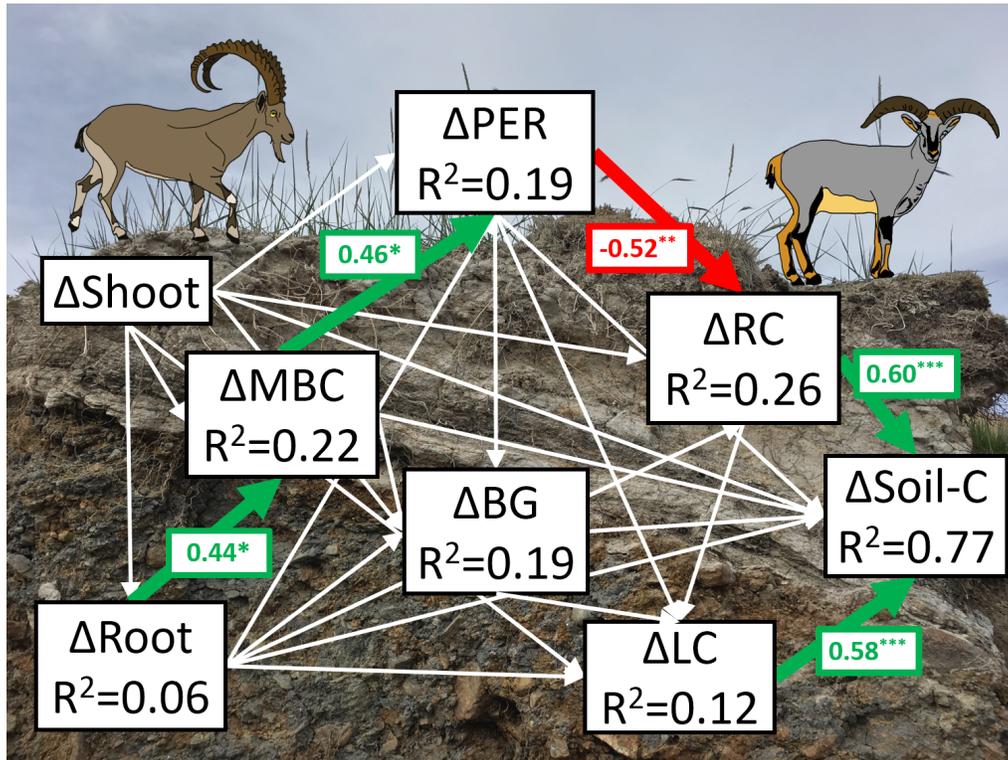
Fig. 4



610

611 **Figure 4.** Correlation between grazer-effects on eleven interrelated soil and microbial variables612 (a). Effect of experimental herbivore-exclusion ( $\Delta$ , ln response-ratio) was calculated from paired613 fenced and grazed plots.  $\Delta$ Shoot: aboveground biomass,  $\Delta$ Root: belowground biomass,  $\Delta$ MB:614 microbial biomass,  $\Delta$ PER: peroxidase activity,  $\Delta$ BG: beta-glucosidase activity,  $\Delta$ CBH:615 cellobiohydrolase activity,  $\Delta$ Soil-C: bulk soil carbon content,  $\Delta$ LC: labile-C pool,  $\Delta$ RC:

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619



621

622 **Figure 5.** Summary of structural equation modelling to account for variance-covariance between  
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627  $\Delta$ PER: peroxidase activity,  $\Delta$ BG: beta-glucosidase activity,  $\Delta$ CBH: cellobiohydrolase activity,

628  $\Delta$ Soil-C: soil carbon,  $\Delta$ LC: labile-C pool,  $\Delta$ RC: recalcitrant-C pool,  $\Delta$ BR: basal respiration.

629 Thick arrows (green and red) are statistically significant paths; white arrows are paths that were

630 statistically non-significant ( $\alpha = 0.05$ ).  $R^2$  values indicate variance explained (marginal) by fixed-

631 effects. Model diagnostics were Fisher's  $C = 6.72$ ,  $P=0.34$ ,  $df = 6$ ;  $AIC = 98.72$ . Asterix

632 represent statistical significance (\*\* $P \leq 0.001$ , \*\* $P \leq 0.01$ , and \* $P \leq 0.05$ ).

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