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Top-down effects on biological soil crust function

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ABSTRACT

Biological soil crusts (BSCs) are communities of microorganisms, mosses, and fungi that control ecosystem functions in drylands. Despite their importance, little is known about how trophic interactions affect BSC function. We conducted a series of mechanistic experiments to tease out the direct (i.e., consumption) and indirect (i.e., fecal and mucus deposition) pathways by which crustivores (i.e., consume BSCs) and detritivores affect BSC functions— complemented by a manipulative field experiment exploring the integrative effect of these pathways. We showed that detritivore feces, mucus, and grazing increased the BSCs' CO_2 respiration. Detritivore feces also increased BSC N content by 9% compared to BSCs not exposed to snail consumers. Crustivorous snail feces increased BSC CO_2 respiration, and their mucus decreased BSC %C and %N. In the field, detritivorous and crustivorous snails increased BSC %C by 15% and 17%, respectively, but did not affect BSC CO_2 respiration. Combined, our findings highlight that macro-invertebrate consumers exert top-down regulation on BSC function, opening the door for a new avenue of trophic research.

1. Introduction

Biological soil crusts (hereafter, BSCs) are a thin encrusted soil layer comprised of communities of photosynthetic (e.g., cyanobacteria, moss) and heterotrophic (e.g., bacteria) organisms (Weber et al., 2016). In drylands, BSCs account for up to 70% of the biological ground cover and play a critical role regulating key ecosystem processes such as carbon (C) and nitrogen (N) cycling, soil-water relationships, dust emissions, soil properties and erosion, albedo, plant community composition, and crust eating invertebrate "*crustivore*" abundance (Weber et al., 2016; Chamizo et al., 2022; Rodriguez-Caballero et al., 2022). In fact, BSCs generate ~7% of the net primary productivity of terrestrial ecosystems and account for ~20% of global nitrogen fixation (Elbert et al., 2012; Weber et al., 2015). Revealing factors regulating BSC properties and function is thus crucial for understanding dryland ecosystems.

Most studies of BSC function have focused on abiotic conditions, such as precipitation, air temperature, light availability, soil chemistry, and physical disturbances (Weber et al., 2016). For instance, temperature and water content explained 67–70% of the variation in C-fixation of late-successional BSCs (Grote et al., 2010), and physical disturbances reduced runoff from early-successional cyanobacteria-dominated BSCs (Faist et al., 2017).

Biotic interactions can also influence the structure and function of BSCs. Vascular plants affect BSCs through shading, litterfall, and alteration of soil properties (Maestre et al., 2010; Weber et al., 2016). In the Negev desert, plant litter kills cyanobacteria and other organisms in the BSC community (Boeken and Orenstein 2001). Animals have been found to affect BSC function mostly through trampling and burrowing. High levels of sheep activity can decrease BSC cover, reduce N input and soil stabilization, or cause total BSC loss (Liu et al., 2009; Weber et al., 2016). Such animal-induced disturbances; however, are not fundamentally different from physical disturbances caused by other factors, like those created by off-road vehicles (see Ferrenberg et al., 2015).

Animals may also affect BSCs via trophic interactions. BSCs are consumed by many crustivorous animals including mole crickets, isopods, beetles, termites and snails (Darby and Neher 2016). By consuming BSCs, animals may exert direct and indirect top-down control over key ecosystem properties and functions. This primary producer-consumer relationship is analogous to plant-herbivore interactions. Yet, BSC- crustivore interactions have received minimal consideration, especially compared to the wealth of literature documenting herbivore effects on the productivity of vascular plants (Gruner et al., 2008), seaweeds (Burkepile and Hay 2006), and aquatic microalgae (Buffan-Dubau and Carman 2000).

The few studies exploring crustivore effects on BSC function focused primarily on micro- and mesoscopic species of soil fauna. Ghabbour

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et al. (1980) found that protozoa selectively consumed the vegetative cells (over heterocysts) of BSCs, which increased the rate of BSC nitrogen-fixation. Birkemoe and Liengen (2000) showed that low and intermediate levels of BSC grazing by the springtail, *Hypogastrura viatica*, increased the N-fixation of cyanobacteria-dominated BSCs. To the best of our knowledge, only one study has tested the effect of macroscopic crustivores on BSC function— showing that mucus deposition by snail crustivores increased BSC CO_2 efflux (Rinehart et al., 2021). These studies suggest that crustivores have the potential to control BSC function, but the detailed regulatory pathways and the overall effects remain largely unknown. We aimed to fill this knowledge gap by testing well established top-down regulatory pathways drawn from the rich plant-herbivore literature in a crustivore-BSC system.

Herbivores often selectively graze, exerting unequal grazing pressure on different plant species. Combined with above- and belowground plant biomass loss, this can alter plant community composition, plant C-fixation (Strickland et al., 2013), and soil C storage (Sun et al., 2011). Herbivores can also influence plant productivity, and thus ecosystem function, through the deposition of metabolic waste products, egesta, and other excretions like mucus (Kitchell et al., 1979). For instance, mucus excretions from herbivorous homing limpets stimulated microalgal and bacterial growth in rocky intertidal communities (Conner 1986). Similarly, N-rich moth frass increased plant productivity and N-poor frass decreased plant productivity (Kagata and Ohgushi, 2011), suggesting that herbivore diet may mediate the ecosystem-level impacts of primary producer – consumer interactions.

Building upon plant-herbivore regulatory pathways, we explored how two sympatric snail species, which differ in their preference for BSCs (i.e., a crustivore and a detritivore), affect the performance of BSCs. In controlled laboratory conditions, we tested how feces, mucus, and grazing affect the CO_2 respiration and C and N stoichiometry of lab grown BSCs. We complemented these mechanistic experiments with a manipulative field study testing the combined effects of these pathways on natural BSCs. We hypothesized that detritivores would promote BSCs' CO_2 respiration and increase BSC C and N content since they facilitate the recycling of plant litter-derived nutrients. We also anticipated that crustivores would have negatively impacts BSC performance through direct grazing effects. Ultimately, we demonstrated that macrocrustivores can regulate BSC function via direct and indirect pathways, establishing a framework for future work evaluating BSC-crustivore interactions.

2. Methods

2.1. Study site and species

The Avdat Research station (30°47′02″ N, 34°46′09″ E; hereafter, Avdat) is in the central Negev highland and receives an average of 93 mm of rainfall year^{-1} (range = 32–134 mm year^{-1}) across 19–42 days (Israel Meteorological Survey, 2021; Station: 253010). Avdat is characterized by 1–2 mm thick cyanobacteria dominated BSC and has multiple common snail species, including *Xerocrassa simulate* and *Sphincterochila prophetarum*, which reach densities of 12 snails m^{-2} (Rinehart et al., 2021). *X. simulate* (hereafter, detritivore) consumes plant litter and BSCs (Ward and Slotow 1992), while *S. prophetarum* (hereafter, crustivore), feed exclusively on BSCs (Shachak and Brand 1981; Appendix S1). A single crustivore can consume 203–577 mg of BSC day^{-1} (Appendix S1). Both snail species break their aestivation and become active after precipitation events. During these periods of activity, snails will forage on BSCs, leaving behind feces and mucus trails. Snails are active 8–27 days year^{-1}, with these days of activity spread across 1–8 active periods (continuous days of activity; Shachak and Steinberger 1980).

2.2. Mechanistic laboratory assays

2.2.1. Laboratory grown BSCs. We grew laboratory BSCs by adding 25 ± 1.0g of soil, followed by 25 ± 1.0g of disaggregated BSCs to 120 mm Ø petri dishes. We used laboratory generated BSCs to minimize natural variation in BSC function. This method has been employed previously in BSC experimentation (Doherty et al., 2015; Rinehart et al., 2021). Soils and BSCs were collected from Avdat. Laboratory-reared BSCs were watered with DI water every-other day for 80 days then left in an incubator at 16 °C with 40% humidity and a 16:8 light cycle (light intensity: 240 Mmol $m^{2\text{-}1}$ s^{-1}) until used (80–194 days). These conditions do not represent the natural conditions experienced by BSCs but did generate homogenous, well-developed cyanobacteria-dominated BSC. This approach allowed us to begin evaluating the potential impacts of top-down effects on BSC functions under standardized conditions. Prior to every laboratory assay, we quantified the initial CO_2 respiration rate of each laboratory grown BSC to be used in that assay by watering all BSCs with a standard volume of water (3 ml of DI water). We placed the BSC plates in airtight plastic chambers [155 mm × 155 mm × 61 mm (length × width × height) Lock & Lock HPL 823; www.s-d.co.il]. We then flushed the chambers with CO^2-free air at a rate of 2L minute^{-1} for a total of 5 min. The BSC were incubated in the flushed, airtight chambers at 16 °C with no light for 48 h. After the dark incubation, we used a LI-7000 CO2/H2O infrared gas analyzer (IRGA; LI-COR, Inc.) with a designated self-manufactured injection system to quantify the initial amount of CO^2 released by each BSC (Appendix S1).

2.2.2. Fecal deposition. We collected feces from snails housed in the laboratory and fed BSCs and plant litter (see Appendix S1). Crustivores generated 77 ± 7.9 mg DM (mean ± 1SE) of feces day^{-1}, while detritivores only generated 13 ± 0.2 mg DM of feces day^{-1}. This difference in fecal production is not due to differences in snail biomass (crustivores: 1.29 ± 0.1 g WM; detritivores: 1.24 ± 0.2 g WM). We allocated fecal samples, by consumer species, to laboratory BSCs in the following three consumer treatments: crustivore, detritivore, and no snail We used 15 replicates for each experimental treatment. We added 130 ± 10 mg DM of homogenized detritivore feces to detritivore BSC replicates and 770 ± 10 mg DM of crustivore feces to crustivore BSC replicates. The amount of feces added to each BSC replicate was equal to ten days' worth of snail fecal production. We chose to add ten days' worth of snail feces since snails are commonly active for 1–15 days at a time under field conditions (Shachak and Steinberger 1980). We did not standardize the DM of feces added across species because we wanted to know the mean impact of feces snail^{-1}. No feces were added to no snail BSC replicates. All BSCs were watered every-other day with 3.5 ml of DI water and housed in an incubator at 18 °C with 40% humidity and a 16:8 dark: light cycle. We watered all BSCs with 3.5 ml of DI water, as this was the maximum amount of water that we could add to the BSCs before run-off was generated. This volume represents 0.3 mm of rainfall per watering event. Ten days into our watering regime we paused our study for 30 d due to Covid-19. After the lockdown, we resumed our watering regime for eight days before measuring the CO_2 respiration, C (%), and N (%) of each BSC (Appendix S1). The delay in our study should not affect our results since all treatments were exposed to the same pause in watering regime. To quantify BSC CO_2 respiration rate, we watered all BSCs with a standard volume of water (3 ml of DI water). We placed the BSC plates in airtight plastic chambers [155 mm × 155 mm × 61 mm (length × width × height) Lock & Lock HPL 823; www.s-d.co.il]. We then flushed the chambers with CO^2-free air at a rate of 2L minute^{-1} for a total of 5 min. The BSC were incubated in the flushed, airtight chambers at 16 °C with no light for 48 h. After the dark incubation, we used a LI-7000 CO2/H2O infrared gas analyzer (IRGA; LI-COR, Inc.) with a designated self-manufactured injection system to quantify the final amount of CO^2 released by each BSC (Appendix S1). We measured the C (%) and N (%) content using a C:N:H analyzer.

2.2.3. Mucus deposition. We harvested mucus from snails housed in the laboratory and fed BSCs and plant litter (see Appendix S1). The

harvested mucus from each snail species was diluted in 14 ml of DI water before being allocated, by species, to laboratory BSCs in the following three consumer treatments: crustivore, detritivore, and no snail control. The sample size was 21 BSCs for the crustivore and detritivore treatments and 20 for the no snail treatment. The no snail treatment had 20 BSCs because one BSC broke during the experimental set up. We watered crustivore and detritivore BSCs with 1.5 ml of homogenized crustivore or detritivore mucus, respectively, and 2 ml of DI water every-other day. No snail BSCs were watered with 3.5 ml of DI water every-other day. All BSCs were housed in an incubator set at 18 °C and 40% humidity with a 16:8 dark: light cycle. After 14 d, we quantified the CO_2 respiration rate, C (%), and N (%) of each BSC using the same protocols listed above (see section 2.2.2; Appendix S1).

2.2.4. Grazing and mucus deposition. We manipulated snail consumer treatment (crustivore, detritivore, or no snail) within circular plastic containers (Ø = 25 cm; height = 15 cm). In crustivore and detritivore treatments, we included three snail crustivores and detritivores, respectively, all collected from Avdat and starved for 6 d prior to the assay. No snail treatments received no snails. All containers received a single pre-weighed laboratory reared BSC. Containers were then placed in a climate-controlled room at 18 °C with a 16:8 dark: light cycle. We watered all treatments with 3 ml of DI water daily to promote snail activity. All feces generated by snails were removed daily before watering occurred. After 4 d, we measured the biomass loss of BSCs, CO_2 respiration rate, C (%), and N (%) of each BSC (Appendix S1). The amount of grazing inflicted by snails during this assay is equal to 15–50% of annual potential grazing based on past observations of snail activity (i.e., 8–27 days of activity $year^{-1}$, Shachak and Steinberger 1980).

2.3. Field experiment

At Avdat, we deployed ten blocks of three 0.25 m^2 circular enclosures on natural BSCs. In each block, we added five crustivore snails to a randomly chosen enclosure, five detritivore snails to another, and the third enclosure was a no snail control (n = 10 enclosures $treatment^{-1}$). The enclosures were constructed from flexible PVC tubing (40 mm Ø) anchored in place with metal pegs and painted with antifouling paint. Antifouling paint is used to restrict gastropod movement in marine communities (Bracken et al., 2011) and effectively restricts snail movement in our system. We used antifouling paint covered enclosures to minimize effects of shading and edge effects, which are typically observed in enclosure studies. All enclosures started with no vascular plants but were equipped with 1) a fake plant constructed of plastic-coated wire and plastic leaves, 2) 1.5 ± 0.1g of *H. scoparia* litter, and 3) a rock of standard size (~6 cm long, ~4 cm wide). The fake plant and rock were included to provide refuge and aestivation sites for the snails. We monitored all enclosures weekly and replaced any snails that escaped the enclosure (no 'wild' snails entered the enclosures). Enclosures were watered bi-weekly with ~5 mm of water. This yielded 45 mm of rainfall, in addition to the 83 mm of rainfall that naturally occurred during the study, resulting in enclosures receiving a total of 128 mm of rainfall over the experiment (Avdat maximum annual rainfall = 134 mm; Israel Meteorological Survey, 2021; Station: 253010). After 113 d, we collected five, 120 mm Ø BSCs samples from each enclosure. BSCs samples were housed in an incubator at 16 °C with 40% humidity and a 16:8 light: dark cycle until CO_2 respiration rate, C (%), and N (%) were quantified using the protocols defined above (see section 2.2.2; Appendix S1).

2.4. Statistical analysis

Statistical analyses were performed using Jamovi software version 1.2 (The jamovi project, 2020; R Core Team, 2019). Kruskal-Wallis tests were used for all response variables (CO_2 respiration rate, C (%), N (%), and C:N) in mechanistic laboratory assays and our field experiment. We followed Kruskal-Wallis tests with Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons where necessary. Additionally, we compared the snail effects across all our studies by calculating the Hedges *d* in OpenMEE (Wallace et al., 2017). Effect sizes were calculated using the crustivore and detritivore treatments as the experimental groups and the no snail treatment as the control group.

2.4.1. Mechanistic laboratory assays. In our laboratory assays, CO_2 respiration rate is represented as the change in CO_2 respiration rate over the study, C (%) content is the total C (%) of BSCs, N (%) content is the total N (%) of BSCs, C: N is the ratio of C (%): N (%) in each BSC, and BSC biomass loss is the decline in BSC biomass over the study (i.e., initial – final; grazing and mucus deposition assay only). The change in BSC CO_2 respiration was calculated for each BSC by subtracting the Initial CO_2 respiration rate of the BSC from the Final CO_2 respiration rate of the BSC. We chose this approach because it allowed us to specifically calculate the change in CO_2 respiration rate of each BSC over the course of the study. We used the change in CO2 respiration for all statistical analyses of our laboratory assays. We calculated BSC C (%) and N (%) by taking the quotient of C (g) or N (g) in the soil sample divided by the total mass (g) of the soil sample. We used Mann-Whitney U tests to compare fecal % C and mucus production between crustivores and detritivores.

2.4.2. Field experiment. In our field experiment, CO_2 respiration rate is representative of the final CO_2 respiration rate, C (%) content is the total C (%) of BSCs, N (%) content is the total N (%) of BSCs, C: N is the ratio of C (%): N (%) in each BSC. We used the final CO_2 respiration rate in our field experiment, rather than the change in CO_2 respiration rate, because we were unable to measure the initial CO_2 respiration rate in the field. For all our BSC responses we averaged the five subsamples collected from each enclosure.

3. Results

3.1. Mechanistic laboratory assays

3.1.1. Fecal deposition. Crustivore feces contained 5.5% ± 0.1% (mean ± 1SE) total C, while detritivore feces contained 13.0% ± 0.6% total C (Mann-Whitney U, U = 0.00, df = 6, p = 0.029). Detritivore feces also contained 0.8% ± 0.03% total N. Total N was undetectable in crustivore feces, suggesting that these feces had a total N (%) < 0.02%. In all our laboratory studies with BSCs, CO_2 respiration rate in no snail treatments declined over the study, we attribute this to nutrient limitation from watering with DI (Rinehart et al., 2021). BSC CO_2 respiration rate was influenced by consumer treatment [$\chi2 = 18.46$, df = 2, $p \leq 0.001$; Fig. 1a], with crustivore and detritivore feces increasing BSC CO_2 respiration rate by 40% and 44%, respectively, compared to BSCs in No Snail treatments.

Consumer fecal treatment did not influence the total C (%) of BSCs [$\chi2 = 0.98$, df = 2, p = 0.614; Fig. 1b], but did affect BSC N (%) [$\chi2 = 6.09$, df = 2, p = 0.048; Fig. 1c] and C:N [$\chi2 = 7.65$, df = 2, p = 0.022; Fig. 1d]. Detritivore feces increased BSC N (%) by 9% compared to no snail BSCs, which led to a 7% reduction in BSC C:N. Crustivores had an intermediate effect on N (%) and C:N, increasing N (%) and decreasing C:N compared to the N (%) and C:N of No Snail BSCs. Remember that we added different quantities of feces to Crustivore (770 ± 10 mg DM) and Detritivore (130 ± 10 mg DM) treatments to capture effects $snail^{-1}$. The effect size (i.e., Hedges *d*) of snail consumer feces on BSC function and stoichiometry depended on the response being considered and snail physiology (Fig. 2a–d).

3.1.2. Mucus deposition. Crustivores produced 14.8 ± 4.0 mg DM (mean ± 1SE) of mucus $snail^{-1}$ day^{-1}, while detritivores produced 3.9 ± 0.4 mg DM of mucus $snail^{-1}$ day^{-1} (Mann-Whitney U, U = 0.00, df = 12, $p \leq 0.001$). Consumer treatment affected the rate of BSC CO_2 respiration rate [$\chi2 = 10.51$, df = 2, p = 0.005; Fig. 3a]. Specifically, we found that detritivore mucus increased BSC CO_2 respiration rate by 54% compared to no snail treatments [DSCF: W = 4.39, p = 0.005]. Crustivore mucus had an intermediate effect, tending to increase CO_2

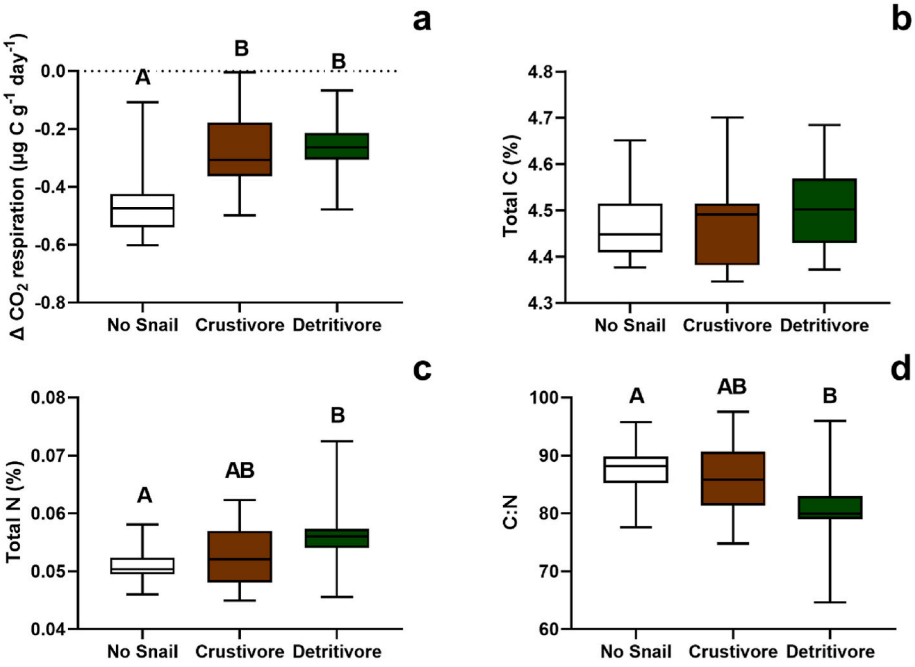

Fig. 1. Biological soil crust **(a)** change in CO_2 respiration rate (Δ μg C g^{-1} day^{-1}), **(b)** total C (%), **(c)** total N (%), and **(d)** C: N after exposure to no snail, crustivore, and detritivore feces. Lines inside the boxes are median values, box limits are the first and third quartiles, and whiskers represent the 1st- 99th percentile. Treatments with different letters are statistically different at α = 0.05. The No Snail treatment served as our no snail control. The crustivore and detritivore treatments included feces harvested from crustivorous and detritivorous snails, respectively. Different fecal dry mass was added to crustivore and detritivore treatments, based on the mean daily fecal production of each species, to capture the distinct effect of each snail species.

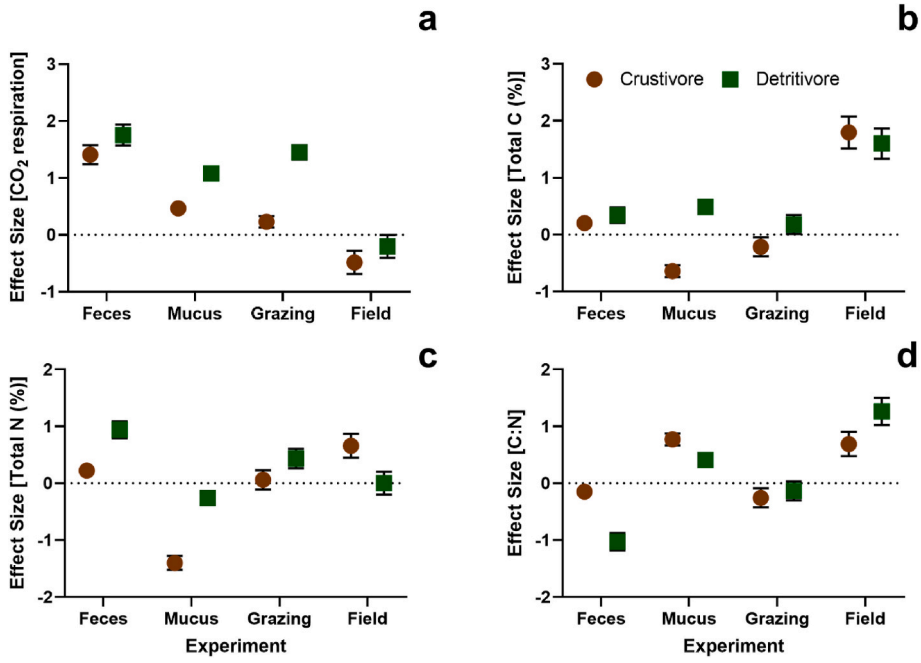

Fig. 2. Hedges *d* effect sizes ($d \pm 1$ var.) for crustivore and detritivore effects on biological soil crust a) CO_2 respiration rate, b) total C (%), c) total N (%), and d) C: N in all studies. Studies of feces, mucus, and grazing were conducted on laboratory-reared biological soil crusts, while the field study was done using natural biological soil crusts in the field. The crustivore and detritivore treatments included snail effects from crustivorous and detritivorous snails, respectively.

respiration rate compared to the no snail control treatment, but this effect was not significant [DSCF: W = 1.81, p = 0.408]. Consumer mucus treatment tended to affect BSC C (%) [$\chi 2 = 5.28$, df = 2, p = 0.071; Fig. 3b], and had strong effects on N (%) [$\chi 2 = 17.29$, df = 2, $p < 0.001$; Fig. 3c] and C: N [$\chi 2 = 6.60$, df = 2, p = 0.037; Fig. 3d]. Specifically, mucus from crustivores decreased BSC C (%) by 5% and N (%) by 15% compared to BSCs in no snail and detritivore treatments, resulting in crustivore-exposed BSCs having a higher overall C: N [DSCF: W = 3.73, p = 0.023]. The effect size of snail consumer mucus on BSC function and stoichiometry depended on the response being considered and snail diet (Fig. 2a–d).

3.1.3. *Grazing and mucus deposition.* Consumer grazing treatment affected the amount of mass loss experienced by BSCs in our grazing study [$\chi 2 = 42.7$, df = 2, $p < 0.001$; Appendix S1: Fig. S1]. This effect was mainly attributed to crustivores, as they increased BSC biomass loss by 627% and 1546% compared to detritivores and no snail treatments, respectively. BSC CO_2 respiration rate was also affected by consumer grazing treatment [$\chi 2 = 16.4$, df = 2, $p < 0.001$], with detritivores increasing BSC CO_2 respiration rate by 197% and 241%, respectively, compared to crustivore and no snail treatments (Fig. 4a). Consumer grazing treatment had no effect on BSC C (%) [$\chi 2 = 3.36$, df = 2, p = 0.187; Fig. 4b], N (%) [$\chi 2 = 1.80$, df = 2, p = 0.406; Fig. 4c], and C: N

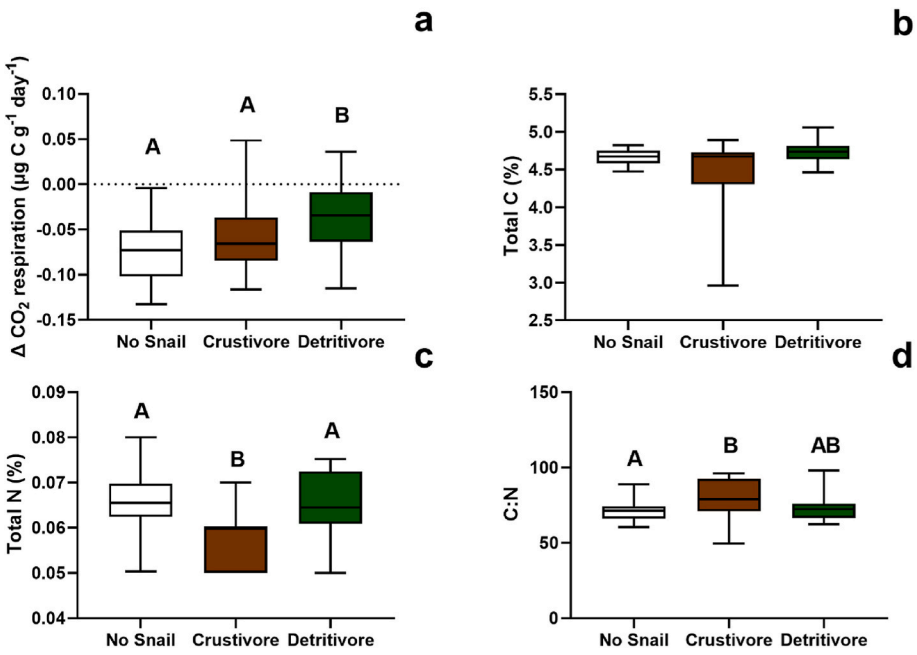

Fig. 3. Biological soil crust **(a)** change in CO_2 respiration rate (Δ μg C g^{-1} day^{-1}), **(b)** total C (%), **(c)** total N (%), and **(d)** C: N after exposure to no snail, crustivore, and detritivore mucus. Lines inside the boxes are median values, box limits are the first and third quartiles, and whiskers represent the 1st- 99th percentile. Treatments with different letters are statistically different at α = 0.05. The No Snail treatment served as our no snail control. The crustivore and detritivore treatments included mucus harvested from crustivorous and detritivorous snails, respectively.

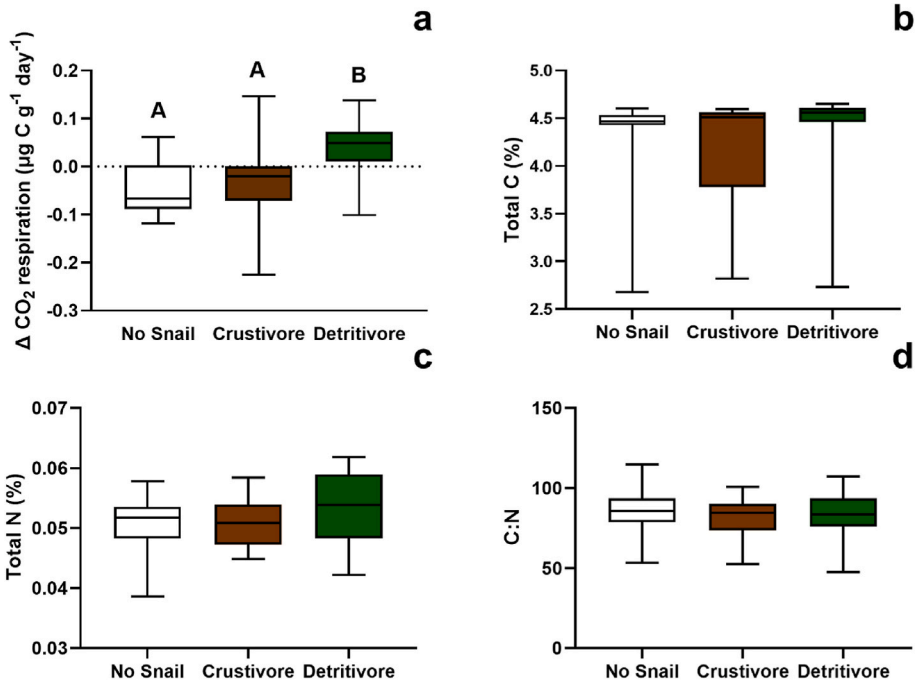

Fig. 4. Biological soil crust **(a)** change in CO_2 respiration rate (Δ μg C g^{-1} day^{-1}), **(b)** total C (%), **(c)** total N (%), and **(d)** C: N after exposure to no snail, crustivore, and detritivore grazing (and mucus). Lines inside the boxes are median values, box limits are the first and third quartiles, and whiskers represent the 1st- 99th percentile. Treatments with different letters are statistically different at α = 0.05. The No Snail treatment served as our no snail control. The crustivore and detritivore treatments included grazing (and mucus) from crustivorous and detritivorous snails, respectively.

[$\chi2 = 0.312$, df = 2, p = 0.855; Fig. 4d]. Snail grazing and mucus deposition had no effect on BSC stoichiometry; however, grazing and mucus deposition by detritivores facilitated BSC CO_2 respiration rate (Fig. 2a–d).

3.2. Field experiment

The CO_2 respiration rate of BSCs was unaffected by consumer treatment [$\chi2 = 1.13$, df = 2, p = 0.568; Fig. 5a]. Consumer treatment tended to influence BSC N (%) [$\chi2 = 3.12$, df = 2, p = 0.210; Fig. 5c], as BSCs exposed to crustivores had a slightly elevated N (%). Consumer treatment did affect BSC C (%) [$\chi2 = 10.97$, df = 2, p = 0.004; Fig. 5a], with BSCs in crustivore and detritivore treatments having 18% and 22% greater C (%) than BSCs in the no snail treatment. These shifts in BSC C (%) in snail treatments ultimately led snail treatments to have higher C: N than no snail treatments [$\chi2 = 7.84$, df = 2, p = 0.020; Fig. 5d]. The effect size of snails on BSC function and stoichiometry depended on the response being considered (Fig. 2a–d).

4. Discussion

Our study is the first systematic evaluation of the largely neglected trophic interactions between BSCs and macro-crustivores. Using two snail species, differing in dietary preference for BSCs, we demonstrated that consumers play a key role in regulating the function of cyanobacteria-dominated BSCs. In a series of short-term laboratory

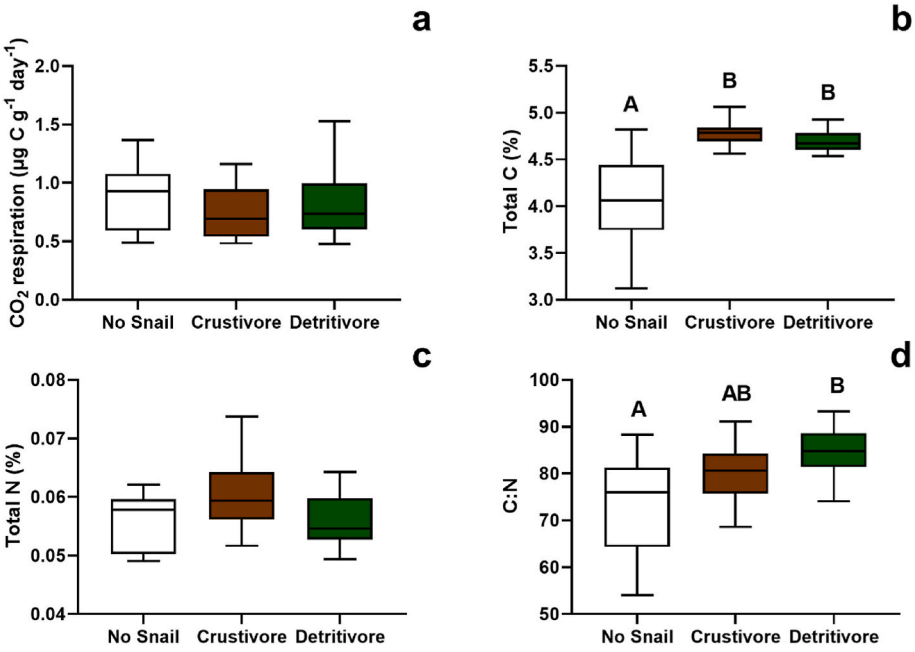

Fig. 5. Biological soil crust **(a)** CO_2 respiration rate (Δ μg C g^{-1} day^{-1}), **(b)** total C (%), **(c)** total N (%), and **(d)** C: N after exposure to no snails, crustivores, and detritivores in the field. Lines inside the boxes are median values, box limits are the first and third quartiles, and whiskers represent the 1st- 99th percentile. Treatments with different letters are statistically different at α = 0.05. The No Snail treatment served as our no snail control. The crustivore and detritivore treatments included crustivorous and detritivorous snails, respectively.

experiments, we showed that the feces, mucus, and grazing of detritivore snails increased the CO_2 respiration rate of BSCs. Detritivore feces also increased BSC N (%), lowering BSCs overall C: N. Crustivorous snails had more variable short-term effect on BSCs. Crustivore feces increased BSC CO_2 respiration and their mucus decreased BSC C (%) and N (%) (increasing BSC C:N). Crustivore feces and grazing had no short-term effects on BSC stoichiometry. In our field experiment, we found that both detritivore and crustivore snails increased BSC C content, providing important evidence that top-down control over BSC function happens in nature.

Based on the established pathways by which herbivores regulate primary producer performance (e.g., Gruner et al., 2008), consumption of BSCs should hinder BSC function and the deposition of nutrients (as feces and mucus) should fertilize BSCs. However, grazing by detritivore snails appeared to have no effect on BSCs, since BSC responses in our grazing and mucus assay parallel our findings for the effects of detritivore mucus alone on BSCs. The lack of a detritivore grazing effect is not surprising, since detritivore snails consume relatively little BSC and did not remove significant amounts of BSC mass during the grazing study (Appendix S1).

Crustivores consumed ~2% of the available BSC mass in four days. This grazing effect did not translate to effects on BSC function or stoichiometry. Crustivorous snail grazing may have minimal effects on BSC function if their large size (relative to BSC organisms) hinders selective grazing, leading to crustivores consuming mainly mineral soil while grazing BSCs. This would explain why crustivore feces had low nutritional quality compared to detritivores. Our findings suggest that macro-consumer grazing inflicts minor damage to BSC communities and BSCs may respond to grazing via compensatory growth— like plant community responses to moderate grazing (Li et al., 2021).

Snail feces had the strongest, most consistent effects on BSCs. The deposition of crustivore and detritivore feces promoted CO_2 respiration, suggesting that fecal deposition has a priming effect on BSC communities. The effect of snail feces on BSC stoichiometry depended on snail diet. Detritivore feces increased BSC N (%) (decreasing C: N), while crustivore feces had no effect on BSC stoichiometry. These snail-specific differences were expected since detritivores consume N-rich diets and their feces contain ≥300% more N than crustivore feces $gram^{-1}$.

The effect of mucus on BSC function and stoichiometry differed between snail species. Detritivore mucus enhanced BSC CO_2 respiration while crustivore mucus had no effect on BSC CO_2 respiration, consistent with previous work (Rinehart et al., 2021). Detritivore mucus had no effect on BSC stoichiometry, but crustivore mucus decreased BSC N content— increasing BSC C:N. Mollusk mucus is known to contain carbohydrates, proteins, lipids, amino acids, and various minerals that can increase microbial growth and respiration (Theenhaus and Scheu 1996). Thus, it is surprising that crustivore mucus did not increase BSC CO_2 respiration rate. However, mucus may facilitate the growth of inhibitory bacterial strains. For instance, bacterial strains that produce ketones— found in microbial-derived volatile organic compounds— may inhibit cyanobacteria photosynthesis by disrupting electron transport through photosystem II (Voronova et al., 2019). Thus, the specific relationship between snail mucus and microbial communities may determine mucus effects on BSC function.

Based on our laboratory assays, we expected that snails would affect natural BSCs via fertilization or, in the case of crustivores, via inhibition of performance. However, we found no change in BSC CO_2 respiration rates in both detritivore and crustivore treatments, and no effect of snail activity on N content of natural BSCs. Instead, we found that snail activity enhanced BSC total C (%)— increasing BSC C: N. This positive effect of detritivores and crustivores on BSC C (%) (and consequently C: N) suggests that snail-BSC interactions are complex and cannot be predicted solely by short term laboratory studies of individual pathways. Consumer regulation of BSC performance likely involves interactions between the different regulatory pathways and with other indirect trophic pathways, such as litter decomposition (Theenhaus and Scheu 1996). Further work should use a broader set of realistic environmental conditions when exploring individual trophic pathways and combine effects of different regulatory pathways over longer timescales to accurately predict BSC performance in the field. Studies evaluating the combined effects of grazing, mucus deposition, and fecal deposition under laboratory settings may also provide key insights into the mechanisms underlying consumer effects on BSCs. Regardless, our study provides the first field-based evidence that macro-consumers regulate BSC performance, uncovering a novel form of top-down regulation on ecosystem functions.

Our pioneering study highlights that top-down trophic interactions may play a key role in regulating BSC function, using two snail species and a cyanobacteria dominated BSC. To understand the broader impacts of our findings, future work should expand such exploration to other

macro-crustivores (e.g., isopods, termites, and mole crickets) and late successional BSCs, dominated by lichens and moss. We expect the outcome of these interactions to vary substantially based on the consumer and BSC characteristics. However, just like congener interactions between consumers and plants, with enough information we can begin to explain this context dependency.

BSCs play a critical role in regulating dryland ecosystem functions and global nutrient cycling (Elbert et al., 2012; Weber et al., 2015; Chamizo et al., 2022). However, our understanding of BSC function is limited. This is especially problematic, as the global land surface covered by dryland ecosystems is expected to expand by 11–23% due to climate change (Huang et al., 2015). Our study is the first to highlight that macro-consumers can regulate BSC function and stoichiometry via top-down pathways, and that these complex interactions can affect C-cycling in the field. We provide ample evidence that consumer byproducts, such as feces and mucus, can regulate BSC performance; but no evidence that consumption by macro-consumers can affect BSC performance. However, our lack of evidence for effects of consumption in short-term laboratory assays should not undermine future attempts to explore how crustivores affect BSC function. The study of plant-herbivore interactions and their consequences for ecosystem dynamics has justifiably received considerable research attention. We hope that our findings encourage ecologists to explore analogous BSC- crustivore interactions, as these interactions have the potential to unlock insights into dryland ecosystem dynamics.

Statement of authorship

Both authors designed the study and wrote the manuscript. S.R. collected and analyzed the data.

Data statement

Data will be deposited in Dryad at time of revisions.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available in Dryad (https://doi.org/10.25338/B8CP96).

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Appendix A. Supplementary data

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