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Snail Mucus Increases the CO_2 Efflux of Biological Soil Crusts

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Abstract

Biological soil crusts (hereafter, biocrusts) are communities of microorganisms that regulate key ecosystem processes such as water distribution, soil erosion, and nutrient cycling in drylands worldwide. The nature of biocrust function can be influenced by multiple environmental factors, including climatic conditions (for example, precipitation), interactions with plants, and anthropogenic disturbances. Animal regulation of biocrust function has received less research attention, focusing primarily on livestock trampling and to a much lesser extent on biocrust consumption by mesofauna. Deposition of animal waste products, carcasses, and other body secretions such as mucus may also affect biocrust function. Yet, this novel regulatory pathway, to our knowledge, has never been empirically tested. Our goal was to begin bridging this knowledge gap by exploring how snail mucus affects biocrust CO_2 efflux—using two distinct biocrust communities and three snail species. We found that snail mucus increased the CO_2 efflux of both cyanobacteria-dominated and lichen/moss-dominated biocrusts. However, the magnitude of snail mucus effects on biocrust CO_2 efflux varied between snail species—possibly due to species-level differences in snail diet. Our study highlights a novel interaction between animals and biocrusts and suggests that even small quantities of animal-derived nutrients can have important consequences for biocrust carbon dynamics.

Key words: Animal-derived nutrients; Biological soil crusts; Carbon cycling; Ecosystem function; Snail mucus; Nutrient excretion.

Highlights

- Mucus increased the CO_2 efflux of cyanobacteria-dominated biocrusts by > 20%.
- Mucus enhanced the CO_2 efflux of moss/lichen-dominated biocrusts by > 86%.
- Dietary differences likely underlie species-specific effects of mucus on biocrusts.

Introduction

Biological soil crusts (hereafter, biocrusts) are a thin encrusted soil layer comprised of draught-tolerant communities of photosynthetic (for example, cyanobacteria, algae, mosses, lichens) and hetero-

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trophic (for example, bacteria, archaea, and microfungi) microorganisms (Belnap and Lange 2001; Garcia-Pichel and others 2001; Bates and others 2010; Doherty and others 2018). In drylands, biocrusts can account for up to 70% of the biological ground cover and often play a critical role in regulating key ecosystem processes such as carbon (C) and nitrogen (N) cycling, soil–water relationships, soil properties and erosion, albedo, plant community composition, and the abundance of crust eating invertebrates (Belnap and Lange 2001). Revealing what factors regulate biocrust properties and function is thus crucial for understanding ecosystem dynamics in drylands.

Most studies investigating biocrust structure and function have focused on abiotic conditions, such as precipitation, light availability, air temperature, soil chemistry, and physical disturbances (Belnap and Lange 2001; Bowker and others 2002; Barger and others 2016; Sancho and others 2016). For example, lichen-dominated biocrusts in central Spain have greater soil CO_2 flux at elevated temperatures (Maestre and others 2010). Similarly, Feng and others (2014) found that increasing water content resulted in elevated nighttime respiration in moss and lichen-dominated biocrusts, but not in cyanobacteria/algae-dominated biocrusts—suggesting that distinct biocrust communities can respond differentially to abiotic conditions. The CO_2 efflux of soil microbial communities can also depend on local nutrient conditions. For instance, soil CO_2 efflux increased with the co-addition of C and N in two polar desert sites differing in soil stoichiometry (Ball and others 2018). Biotic interactions can also influence the structure and function of biocrusts. Vascular plants can affect biocrusts through canopy shading, litterfall, and alteration of soil properties (Boeken and Orenstein 2001; Maestre and others 2010; Zhang and others 2016). For example, in the Negev desert, the accumulation of plant litter kills cyanobacteria and other organisms in the biocrust community (Boeken and Orenstein 2001). Animals have been found to affect biocrust function through physical disturbance (for example, trampling and burrowing) and biocrust consumption. High levels of sheep activity (that is, ≥ 16 sheep ha^{-1}) can decrease biocrust cover (Huajie and others 2009) or cause total biocrust loss (Memmott and others 1998; Warren and Eldridge 2001). Additionally, low and intermediate levels of grazing by the springtail, *Hypogastrura viatica,* was found to increase the N-fixation of cyanobacteria-dominated biocrusts in Artic wetlands (Birkemoe and Liengen 2000).

The deposition of animal-derived nutrients is another possible regulatory pathway of biocrust function that, to the best of our knowledge, has yet to receive conceptual or empirical consideration. Animal-derived nutrients, such as metabolic waste products (for example, urine, guanine, ammonia), egesta and carcasses, are known to control the performance and function of foundational primary producers, including terrestrial vascular plants, seagrasses, macroalgae, and microalgae (Kitchell and others 1979; Bazely and Jefferies 1985; Day and Detling 1990; McNaughton and others 1997; Frank and others 2002; Vanni 2002; Allgeier and others 2017; Barthelemy and others 2019). Other animal excretions, such as snail mucus, have received less attention despite having documented effects on primary production and microbial activity. For instance, Conner (1986) found that mucus from herbivorous gastropods (*Lottia gigantea* and *Collisella scabra*), but not omnivorous or predatory gastropods, fertilizes the environment—increasing the prevalence of microalgae and bacteria compared to mucus-free controls. Similarly, Theenhaus and Scheu (1996) found that the addition of slug mucus to beech leaf litter increased substrate respiration and microbial biomass, leading to accelerated C, N, and P cycling. Given the wealth of evidence that animal-derived nutrients can affect primary producer and microbial activity, it is surprising that there have been no attempts to study this pathway in biocrust systems. Thus, our goal was to begin bridging this knowledge gap by testing how snail mucus affects desert biocrust CO_2 efflux.

In the Negev desert, snails are extremely abundant—reaching densities of 15–60 snails m^{-2} (Bar 1975; Genot-Lahav 1986; Degen and others 1992). For example, the density of *Xerocrassa simulata* in the lower region of a central Negev desert watershed is 49.2 ± 34.0 snails m^{-2} (Shachak and others 2002). Snails deposit mucus trails as they move along the biocrust surface, paving the desert floor with shiny mucus cover. In fact, *Xerocrassa simulata* deposits 0.5 ± 0.1 mg of mucus per mg biomass per day under laboratory conditions and can move 2.4 ± 1.9 m to 3.4 ± 1.9 m after a single rain event, depending on the local snail density (Shachak and others 2002).

Snail mucus may have several important consequences for the function of biocrusts. First, snail mucus may enhance biocrust performances through fertilization effects, as snail mucus is comprised of water (91–98%), glycoproteins (2–9%), and small quantities of sugar moieties (Campion and Staffordshire 1961; Greistorfer and others 2017). Notably, both snail diet and activity may

influence the production and nutritional composition of snail mucus (Smith and Morin 2002; Munn and Treloar 2017). Second, mucus may impede biocrust function if it contains antimicrobial properties. For example, mucus from the brown garden snail, *Helix aspersa*, inhibits the growth of several bacterial species, including strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Pitt and others 2015). Third, mucus may increase the local water availability for biocrusts via water vapor absorption (Lincoln and others 2004). Increasing the water available to biocrusts could have profound effects on their overall performance, as water is known to regulate biocrust activity and function (Huxman and others 2004).

Here, we used a series of manipulative laboratory experiments to understand how snail mucus affects biocrust CO_2 efflux. We tested the generality of this unknown interaction, by using two distinct biocrust communities (that is, cyanobacteria-dominated and lichen/moss-dominated) and considering intra- and interspecific variation in snail mucus deposition using three snail species that differ in diet. Shedding light on this unexplored pathway by which consumers may regulate biocrust function will inform models of local and global nutrient cycling—because biocrusts contribute significantly to terrestrial NPP and biological carbon fixation (Galloway and others 2004; Canfield and others 2010; Elbert and others 2012).

Methods

Study System

We focused our investigation on two LTER sites in the Negev desert that differ in climatic conditions and biocrust composition. The Avdat Research station (30°47′02″ N, 34°46′09″ E; hereafter, Avdat) is in the central Negev highland and receives ~ 93 mm of rainfall per year spread across 19–42 days (Israel Meteorological Survey, 2021; Station: 253052). Avdat is characterized by 1–2-mm-thick cyanobacteria-dominated biocrusts (also containing bacteria, algae, and fungi) that have a distinct flat, solid surface. This solid surface is due to cyanobacteria secreting polysaccharides that adhere soil particles (Mazor and others 1996). Sayeret Shaked Park (31°16′16″ N, 34°39′03″ E; hereafter, Shaked Park) is in the Northern Negev and receives 190 mm of rainfall per year spread across 25–52 days (Israel Meteorological Survey 2021; Station: 251,691). Shaked Park is characterized by moss/lichen-dominated biocrust that also contain bacteria, cyanobacteria, algae, and fungi. The moss/lichen-dominated biocrusts at Shaked Park reach thicknesses of 8–10 mm on south-facing slopes and 10–15 mm on north-facing slopes (Zaady and others 1996; Zaady and others 1998).

At Avdat and Shaked Park, snails are relatively abundant—reaching densities of 12 and 89 snails per m^2, respectively. Avdat has multiple common snail species, including *Sphincterochila zonata* (SZ), *Xerocrassa simulata* (XS), and *Sphincterochila prophetarum* (SP) that overlap in their distribution and use of biocrust surfaces (Bar 1975; Degen and others 1992; Genot-Lahav 1986). Meanwhile, Shaked Park is mainly dominated by XS, but also has a small population of SZ. The diets of all three snail species include biocrusts (Yom-Tov and Galun 1971; Shachak and Steinberger 1980; Shachak and Brand 1981). However, XS preferably consumes plant litter (Yom-Tov and Galun 1971).

The activity of snail species in the Negev is regulated by moisture, with all species only becoming active when the ground is damp (Yom-Tov 1971; Hermony and others 1992). Consequently, snail movement along biocrusts in search of food, mates, and egg laying habitat is limited to short bouts following substantial precipitation events (Shachak and Steinberger 1980). For example, *S. zonata* are active for only 8–27 days annually (Shachak and Steinberger 1980). These short bouts of snail activity likely have important consequences for biocrust function, as snails deposit considerable nutrient-rich mucus while moving along the biocrust surface—covering approximately 15% of the biocrust surface in mucus trails (Figure 1).

General Experimental Approach

We used three complementary laboratory experiments to reveal how desert snails affect biocrust activity. To achieve a comprehensive answer, we used two different biocrust types, and three species of snails. We also used two common biocrust cultivation methods to control for variation in biocrust performances that may reflect specific rearing conditions (Doherty and others 2015). In *experiment 1*, we explored how a mix of mucus from three abundant snail species affects laboratory grown cyanobacteria-dominated biocrusts from Avdat. In *experiment 2* we tested how *X. simulata* (XS) mucus from the Avdat and Shaked Park populations affect field-collected moss/lichen-dominated biocrust from Shaked Park. In *experiment 3* we assessed how the mucus of each of the three common snail species at Avdat affect field-collected cyanobacteria-dominated biocrusts from Avdat. In all three

Figure 1. Example of *Sphincterochila zonata* (SZ) depositing mucus trails on the biocrust surface following a light overnight rain event at Avdat Research Station during March of 2019.

experiments we measured the CO_2 efflux as a measure of biocrust activity.

Experiment 1

We collected biocrust (top 2 mm) and sediment (2–10 cm depth) from Avdat. After sieving the biocrust and sediment with a 2-mm metal sieve to remove rocks and plant litter, we added 63.7 ± 0.3 g (mean ± SE) of sediment topped with 50.1 ± 0.2 g (mean ± SE) of biocrust to 50, 145 mm (diameter) × 20 mm (depth) plastic petri dishes. We grew these crusts in growing chambers at 16 °C with 70% humidity and a 16:8 light/dark cycle for 111 days. For this first experiment, we chose to cultivate biocrusts using techniques designed for biocrust restoration (see Doherty and others 2015). This method may be less realistic but reduces variation within and between our biocrusts. Then, we randomly allocated 25 of our laboratory-grown crusts to mucus and no mucus (that is, mucus-free) treatments. In the mucus treatment, we watered biocrusts with 1 ml of our diluted snail mucus mixture (equal to 1 days' worth of snail mucus) and an additional 2.5 ml of DI every other day for 13 days. In the no mucus control, we repeated the same watering protocol but with 3.5 ml of DI water. We chose this arbitrary protocol for logistical reasons. Yet, both the overall water addition and the distribution are well within the range of natural precipitation events in our study site, as the median number of rain events from 2008 to 2020 at Avdat in the wettest months (January and February) is seven (Israel Meteorological Survey 2021; Station: 253052).

To produce the mucus, we collected wild snails (Species: XS, SP, and SZ; n = 20 individuals/species) from Avdat and randomly placed groups of 10 individuals by species (n = 2 containers per species) in plastic containers (249 mm length × 190.5 mm width × 94.0 mm height). The containers were placed in a room, maintained at 14–17 °C with a 11:13 light/dark cycle. We extracted mucus from housed snails twice weekly by placing single species groups of 10 snails on a 280 mm × 216 mm transparent plastic sheet (3 M Write-on Overhead Projector Transparency Film) covered by a 140 mm × 115 mm × 50 mm height plastic lid. We wetted the snails daily with DI to ensure their activity. After 48 h, we scrapped the mucus off the transparent plastic sheets and homogenized all collected snail mucus (across all species) in 60 ml of DI, generating a mixed mucus solution containing the equivalent of 60 days' worth of snail mucus production (that is, 60 snail^{-1} days^{-1}, 1 ml equals 1 snail^{-1} day^{-1}). Mixing all species' mucus is representative of mucus deposition in nature, where all species co-exist on moist biocrusts (Yom-Tov 1971). Our mucus harvesting technique should capture mainly the water-soluble components of snail mucus. Diluted mucus was then frozen at −80 °C until use. All snail species studied consume biocrusts. Thus, we chose to use pre-extracted mucus (rather than have snails directly deposit mucus trails on biocrusts) to isolate the effects of mucus deposition from biocrust consumption.

At the completion of the 13 days, we measured the effects of snail mucus on biocrust activity by measuring biocrust CO_2 efflux. We placed watered (3 ml of DI water) mucus and control laboratory-grown crusts in airtight plastic chambers [155 mm × 155 mm × 61 mm (length × width × height) Lock & Lock HPL 823; www.s-d.co.il]. We flushed the chambers with CO_2-free air at a rate of 2L minute^{-1} for a total of 5 min. Laboratory-grown crusts were incubated in the flushed, airtight chambers at 16 °C with no light for a total of 24 h. After the dark incubation, we used a LI-7000 CO_2/H_2O infrared gas analyzer (IRGA; LI-COR, Inc.) with a designated self-manufactured injection system to quantify the amount of CO_2 released by each biocrust.

Experiment 2

The goal of this experiment was to explore how XS mucus from two distinct populations affect field-collected moss/lichen-dominated biocrust from Shaked Park. We used mucus from XS because this is the main snail species found at Shaked Park. To create the laboratory biocrusts, we collected 30 intact biocrusts from Shaked Park using 120 mm diameter × 20 mm deep plastic petri dishes (fol-

lowing Weber and others 2016). We housed the field-collected biocrust in the laboratory for 25 days at 18 °C, with 60% humidity and a 8:16 light/dark cycle. During this time, we watered our field-collected biocrusts daily with 3.6–4.2 ml of DI water, using a hand-held spray bottle. Using field-collected biocrust for experiments 2 and 3 may provide more realistic, but more heterogeneous, results compared to those obtained from laboratory cultivated early successional biocrusts, complementing the approach used for experiment 1.

We randomly allocated field-collected Shaked Park biocrusts (n = 10 per treatment) to one of three treatments (1) XS-AV mucus, (2) XS-SS mucus, and (3) mucus-free control. In the XS-AV mucus treatment, we watered biocrusts with 1.5 ml of the diluted mucus (equal to 1 days' worth of snail mucus) extracted from XS snails from Avdat and 2.0 ml of DI water every other day for 16 days (until 01 April 2019). In the XS-SS treatment, we repeated the same procedures but with extracted XS mucus from Shaked Park. In the mucus-free control, we watered biocrusts with 3.5 ml of DI water every other day for 16 days. Here, we chose the same arbitrary protocol as in experiment 1 but with eight watering days, which corresponds with the median number of rain events in January and February (the wettest months) from 2008 to 2020 in this region (Israel Meteorological Survey 2021; Station: 251691).

To produce the mucus, we collected snails (Species: XS; n = 30 individuals per site) from both Avdat and Shaked Park. We randomly placed snails in 249 mm length × 190.5 mm width × 94.0 mm height plastic containers [in groups of 10 individuals by site (n = 3 containers per site)]. We placed all containers in a climate-controlled room, maintained at 15 ± 1.5 °C with a 11:13 light/dark cycle. Snails were fed biocrusts (Avdat and Shaked Park), *Hammada scoparia* litter, and *Atractylis serratuloides* litter ad libitum. We extracted snail mucus from housed snails once a week for two weeks, using the same protocol described for Experiment 1. However, at the end of each extraction session, we scrapped the mucus off the transparent plastic sheet and the small plastic container using 15 ml of DI water per box and homogenized all collected mucus (across boxes) in 50 ml falcon tubes. Falcon tubes containing diluted mucus were then frozen at − 80 °C until use. We did experience snail death during the snail extraction process (~ 10% mortality per week). When snails died, we replaced them with a new snail that was collected from the appropriate field site.

We quantified snail mucus production between XS populations (that is, Avdat vs. Shaked Park) by creating six, 1.5 ml pseudo-replicated samples of homogenized snail mucus. We then freeze-dried the mucus samples for 24 h and weighed the remaining dried material.

To account for natural variation in field-collected biocrust CO_2 efflux, we wanted to quantify the pre-experimental CO_2 efflux of each biocrust but, due to incubator malfunctions, we were unable to measure biocrust CO_2 efflux prior to experimental manipulations. We quantified the CO_2 efflux using the same protocol as for Experiment 1.

Experiment 3

The goal of this experiment was to reveal how interspecific variation in snail mucus affects field-collected cyanobacteria-dominated biocrusts from Avdat. We collected 40 field-collected biocrusts from Avdat and reared them in the laboratory using the exact same protocol as for Experiment 2. We randomly allocated 10 field-collected biocrusts to each of the four treatments: (1) XS mucus, (2) SP mucus, (3) SZ mucus, and (4) mucus-free control. In each mucus treatment, we watered field-collected Avdat biocrusts with 1 ml of the corresponding diluted mucus (equal to 1 days' worth of snail mucus) and an additional 2.5 ml of DI water every other day for 16 days. In the mucus-free control, we watered Avdat biocrusts with 3.5 ml of DI water every other day till the end of the experiment. We used identical watering protocol as for Experiment 2 to allow better comparisons of the XS mucus effect on biocrust CO2 efflux between biocrust types.

To harvest mucus, we collected snails (XS, SP, and SZ; n = 60 individuals per species) and reared them using the same protocol as for Experiment 2. During the mucus excretion period, all snail species were able to feed ad libitum on biocrusts and *Hammada scoparia* litter collected from Avdat. We extracted snail mucus from housed snails once weekly for two weeks using a similar extraction protocol as in Experiment 2. At the end of each extraction, we scrapped the mucus off the transparent plastic sheet and the small plastic container and diluted the collected mucus from each box in 10 ml of DI water and homogenized all mucus by snail species in 50 ml falcon tubes. Falcon tubes containing diluted mucus were then frozen at − 80 °C until use. We did experience snail death between snail extractions (~ 10% mortality per week). When snails died, they were replaced with new snails also collected from Avdat. We quantified

the production of snail mucus between the three species of snails (XS, SP, and SZ) by creating three, 1.5 ml pseudo-replicated samples of homogenized snail mucus per species. We then freeze-dried the mucus samples for 24 h and weighed the remaining dried material. We measured the CO_2 efflux of field-collected biocrusts at the beginning and end of the two-week mucus addition period using the protocol described in Experiment 1.

Data Analysis

In Experiment 1, we compared the final CO_2 efflux (ug C day^{-1} g^{-1}) of laboratory-grown biocrusts between mucus treatments using a two-sample T-test. In Experiment 2, we compared the dry mass of snail mucus between snails from Avdat and Shaked Park using a two-sample T-test. We compared the final CO_2 efflux of biocrusts between treatments using a generalized linear model (GLM) with a Tukey's HSD test. We used a GLM because they accommodate variance in heterogeneity, non-normal distributions, and uneven sample sizes (Venables and Dichmont 2004; Bolker 2008). Additionally, we used goodness-of-fit statistics to determine the best distribution for each model. Prior to data analysis, we removed two outliers that were 1.5-times the interquartile range above the third quartile [No mucus (n = 8); XS-AV (n = 10); XS-SS (n = 10)]. In Experiment 3, we compared the snail mucus dry mass between our three snail species using a one-factor ANOVA with snail species (that is, XS, SP, and SZ) as a fixed factor. We compared the change in CO_2 efflux of Avdat biocrusts between treatments using a GLM and a Tukey's HSD test. Additionally, we used goodness-of-fit statistics to determine the best distribution for each model. Prior to data analysis, we removed five outliers that were 1.5-times the interquartile range above the third quartile or below the first quartile [No mucus (n = 7); XS mucus (n = 9); SP mucus (n = 9); SZ mucus (n = 10)]. We ran all our statistical analysis in jamovi version 1.217 using the ''jmv'' and ''gamlj' modules (Gallucci 2019; R Core Team 2019; The jamovi project 2020).

We calculated the effect sizes of mucus impacts on biocrusts to compare across snail and biocrust type using OpenMee software (Wallace and others 2017). Specifically, we calculated the Hedges d by comparing the means of the mucus treatment(s) to the mean of the mucus-free control in each of our experiments. We interpreted our effect sizes using the benchmarks set by Cohen (1988), who suggested that an effect size of 0.2 is small, 0.5 is moderate, and anything greater than 0.8 is large. Although Cohen's benchmarks are general, we have no other standard at which to compare our effect sizes too—as mucus effects on biocrusts are a novel interaction in the literature. Thus, we have chosen to use Cohen's benchmarks to simply consider the relative magnitude of each interaction in our study.

Results

Experiment 1

Adding homogenized mixed mucus from three abundant snail species (that is, XS, SP, and SZ) increased the CO_2 efflux of our laboratory-grown biocrusts from Avdat ($t_{48} = -4.90$, $p < 0.001$; Figure 2). Specifically, we noted that the addition of snail mucus increased the CO_2 efflux of cyanobacteria-dominated biocrusts by almost 20% in only 13 days. The Hedges d calculation suggests that homogenized mucus has relatively large positive effects on the CO_2 efflux of cyanobacteria-dominated biocrusts (Table 1; Figure 3).

Experiment 2

The presence of XS snail mucus, regardless of source population, increased the CO_2 efflux of field-collected biocrusts from Shaked Park [GLM (inverse Gaussian), $\chi^2 = 14.0$, $df = 2$, $p < 0.001$; Figure 4]. In fact, we found that XS mucus from Avdat and Shaked Park enhanced the CO_2 efflux of moss/lichen-dominated biocrusts by 86% and 100%, respectively, when compared to biocrust grown with no mucus additions. Our Hedges d calculations suggest that XS mucus from Avdat and Shaked Park have relatively large positive effects on the CO_2 efflux on moss/lichen-dominated biocrusts (Table 1; Figure 3). We did not find a difference in the mucus production rate of XS snails collected

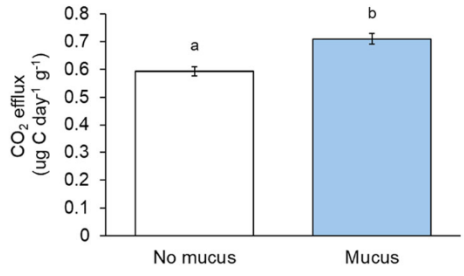

Figure 2. CO_2 efflux (ug C day^{-1} g^{-1}; mean ± SE) of laboratory-reared biocrusts from Avdat grown in the presence and absence of homogenized snail mucus. The sample size is 25 for both treatments. Treatments with different letters are statistically different at $\alpha = 0.05$.

Table 1. Hedges *d* Calculations of Mucus Effects on Biocrust CO_2 Efflux.

Study	Snail	Hedges *d* (mean ± 1 var.)
Experiment 1	All species combined	1.36 ± 0.10
Experiment 2	*X.simulata*-Avdat	1.44 ± 0.28
	X. simulata-Shaked Park	1.34 ± 0.28
Experiment 3	*S. prophetarum*	0.64 ± 0.27
	X. simulata	1.28 ± 0.31
	S. zonata	1.75 ± 0.33

Mean (± 1 var.) Hedges d for mucus effects on biocrust. For each calculation, we compared the means and standard deviations for each treatment including snail mucus with its corresponding no snail mucus treatment. Calculations were performed using OpenMee software.

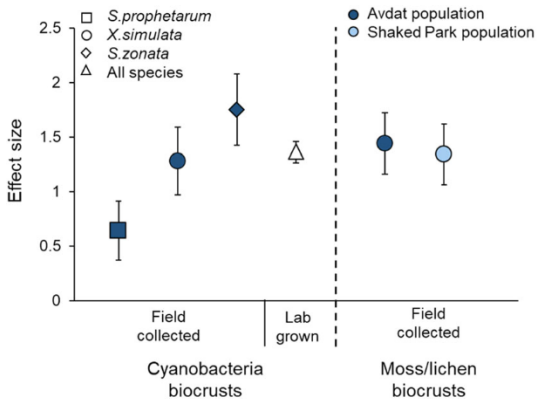

Figure 3. Hedges *d* effect size calculations (mean ± 1SD) for the impact of snail mucus additions on biocrust CO_2 efflux (ug C day^{-1} g^{-1}) in each of our three experiments. Points in all shades of blue represent data obtained using field-collected biocrusts, whereas points in white represent data obtained using laboratory-grown biocrusts. Cyanobacteria biocrusts were collected from Avdat (Experiments 1 and 3) and moss/lichen biocrusts were collected from Shaked Park (Experiment 2).

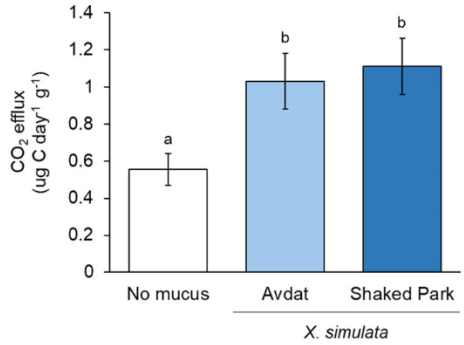

Figure 4. CO_2 efflux (ug C day^{-1} g^{-1}; mean ± 1SE) of field-collected biocrusts from Shaked Park exposed to *Xerocrassa simulata* (XS) collected from Avdat and Shaked Park. The sample size is 10 for both mucus addition treatments and 9 for the no snail mucus treatment. Treatments with different letters are statistically different at $\alpha = 0.05$.

from Avdat and Shaked Park populations (Appendix S1: Table S1).

Experiment 3

We observed strong species-specific effects of snail mucus on the CO_2 efflux of field-collected biocrusts from Avdat [GLM (Gaussian), $\chi^2 = 14.5$, $df = 3$, $P = 0.002$; Figure 5]. Specifically, the addition of mucus from XS and SZ enhanced the CO_2 efflux of cyanobacteria-dominated biocrusts by 106% and 127%, respectively (compared to Avdat biocrusts in mucus-free controls). However, mucus additions from SP did not promote the CO_2 efflux of cyanobacteria-dominated biocrusts. These species-specific effects of snail mucus on cyanobacteria-dominated biocrusts are likely not attributed to differences in snail mucus production, as we found no difference in mucus production between our three snail species (Appendix S1: Table S1). The Hedges *d* calculations suggest that XS mucus and SZ mucus have relatively large positive effects on the CO_2 efflux of cyanobacteria-dominated biocrust, whereas SP mucus has, at most, a moderate positive effect on the CO_2 efflux of cyanobacteria-dominated biocrusts (Table 1; Figure 3).

Discussion

The deposition of snail mucus on biocrust surfaces resulted in enhanced biocrust CO_2 efflux. This positive effect of snail mucus was observed in both cyanobacteria and lichen/moss-dominated biocrusts. However, snail mucus effects appear to be species-specific—as one species' mucus had no effect and two species' mucus had positive effects. Our series of experiments highlights that snail mucus may be an important resource for biocrust communities, regulating their carbon dynamics.

Biocrusts in our study generally exhibited rates CO_2 efflux comparable to previous studies. For instance, the CO_2 efflux of our field-collected

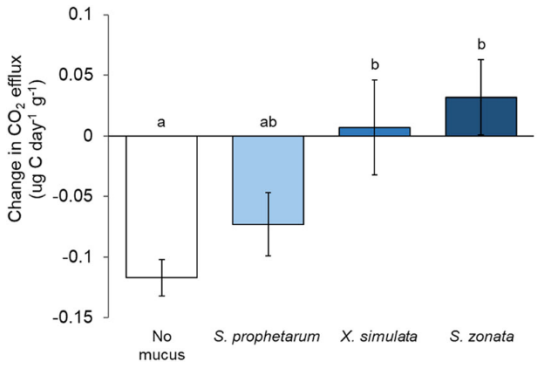

Figure 5. Change in CO_2 efflux (ug C day^{-1} g^{-1}; mean ± SE) of field-collected biocrusts from Avdat exposed to mucus from *Sphincterochila prophetarum*, *Xerocrassa simulata*, and *Sphincterochila zonata*. All snails were collected from Avdat. The sample size is 9–10 for the SP, XS, and SZ mucus treatments and 7 for the no mucus treatment. Treatments with different letters are statistically different at $\alpha = 0.05$.

cyanobacteria-dominated biocrusts was 0.57 umol CO_2 m^{-2} s^{-1}, which falls in the range of 0.55–2.00 umol CO_2 m^{-2} s^{-1} documented by Tucker and others (2019). Similarly, the CO_2 efflux of our field-collected moss/lichen-dominated biocrusts was 1.16 umol m^{-2} s^{-1}, which falls in the range of 0.05–7.62 umol m^{-2} s^{-1} observed by Yao and others (2020). The only biocrust that we worked with that did not exhibit comparable rates CO_2 efflux was our laboratory-grown cyanobacteria-dominated biocrusts, which had a mean CO_2 efflux of 0.006 umol CO_2 m^{-2} s^{-1}. This is likely due to early successional biocrust communities having lower rates of CO_2 efflux than late successional biocrusts communities (Tucker and others 2019). It should be noted that despite the large functional difference between the laboratory and field-collected biocrusts, mucus effect size on biocrust CO_2 efflux was comparable.

Snail mucus increased the CO_2 efflux of both cyanobacteria-dominated and lichen/moss-dominated biocrusts. Thus, unlike other microbial systems where mucus inhibits microbe activity (Pitt and others 2015), we found no inhibitory effects of mucus on biocrust CO_2 efflux. Mucus may stimulate biocrust CO_2 efflux by adding key nutrients or by increasing biocrust water retention. Given our decision to use standardized, extracted snail mucus (rather than mucus trails deposited directly by snails on biocrusts), our study should have primarily captured the nutritional effects of mucus for biocrusts. Nutritional effects of mucus are not necessarily surprising, as mollusk mucus is known to contain carbohydrates, proteins, lipids, amino acids, and various minerals (Campion and Staffordshire 1961; Greistorfer and others 2017). Additionally, studies using slug (*Arion rufus*) mucus found that the nitrogen and phosphorous excreted increases microbial growth and respiration—resulting in faster local mineralization rates (Theenhaus and Scheu 1996). Many studies have reported positive effects of anthropogenic nutrient supplementation on biocrust growth (for example, Bowker 2007; Roncero-Ramos and others 2019), but we provide the first evidence that snail mucus can serve as a natural fertilizer for biocrust.

Despite our design's emphasis on nutritional effects, it is possible that the positive impact of mucus was mediated by shifts in sediment hydrodynamics. Specifically, the large polysaccharides (that is, carbohydrates) commonly found in snail mucus may increase the retention time of water in sediment and biocrusts—leading to increased biocrust activity (Smith and Morin 2002; Yao and others 2019). Such effects of polysaccharides on soils are well recognized. For example, exo-polysaccharides (synthesized by rhizosperic microorganisms) form complexes with mineral particles that increase sediment water retention (Theng and others 2005). Snail mucus trails may have additional effects on sediment moisture in natural conditions, where snails deposit trails directly on the biocrust surface. However, testing these effects naturally is complicated by the fact that snails also consume biocrusts, which makes these processes (consumption and mucus deposition) difficult to decouple.

The effect of snail mucus deposition on CO_2 efflux was consistent across the two distinct biocrust types. We noted that *Xerocrassa simulata* (XS) increases the CO_2 efflux of both cyanobacteria-dominated and lichen/moss-dominated biocrusts. In fact, when we calculated the Hedges *d* effect size for all snail mucus treatments (relative to their mucus-free control) we found that the effect of XS mucus on biocrust CO_2 efflux was surprisingly consistent (Table 1; Figure 3). The effect of XS mucus on cyanobacteria-dominated biocrusts ranged from 1.28 to 1.44, while the effect of XS mucus on lichen/moss-dominated biocrusts was 1.34. This suggests that disparate biocrust types may have similar functional responses to consumer fertilization effects; however, more work is necessary to test this hypothesis.

The consistent effects of XS on biocrust function also suggest that population-level differences in snails do not alter how mucus affects biocrusts. However, because we provided all snails the same four food resources (that is, Avdat biocrust, Shaked Park biocrust, Avdat plant litter, and Shaked Park

plant litter) we may have diluted natural differences in diet. This is corroborated by behavioral observations taken during the mucus extraction phase of the study, where XS showed no preference for their 'natal' resources (Appendix S2). We suggest that future work takes a more realistic approach to better capture how dietary differences influence mucus composition and, consequently, mucus–biocrust interactions.

Although we observed no population-level differences in mucus effects on biocrusts, we did observe strong species-specific effects. We found that mucus from XS and SZ increased biocrust CO_2 efflux more than mucus from SP. These species-specific effects may be attributed to quantitative and qualitative differences in snail mucus. Interestingly, we found that all snail species produced a similar amount of mucus dry mass (0.3–0.5 mg DM $snail^{-1}$ day^{-1}) despite large differences in body mass, suggesting that the species-specific effects in this experiment are likely attributed to qualitative differences in mucus.

Mucus quality may be species-specific for several reasons. First, the snail species included in our study have distinct dietary preferences, with SZ and SP primarily consuming biocrusts and XS primarily consuming plant litter. Additionally, although SZ and SP are both biocrustivores (that is, consumers that eat biocrusts), they may selectively consume different members of the biocrust community (for example, cyanobacteria, algae, bacteria, lichens, and so on). Selective grazing is commonly observed in snail species. For instance, the mud snail, *Ilyanassa obsolete*, exhibits a high degree of selectivity in the particles it consumes—preferentially grazing a specific fraction of the benthic diatom community (Connor and Edgar 1982). Second, in the field the three snail species differ in behavior and microhabitat used. Consequently, these snails may excrete mucus of different nutritional composition even under shared rearing conditions.

Biocrusts play a critical role in global carbon cycling—accounting for the net uptake of about 2.43 Pg C $y^{-1}$and about 38.5 Tg N y^{-1} (Elbert and others 2012). However, our understanding of the factors affecting biocrust function, especially over small spatial scales, is still limited (Zaady and others 2001). Our study suggests that by-products (that is, mucus) from snails can have profound effects on biocrust carbon dynamics. In fact, the positive effects of snail mucus were observed across distinct biocrust communities (that is, cyanobacteria-dominated and lichen/moss-dominated biocrusts) but did vary in magnitude depending on snail species. Our findings, when combined with the relative high densities of snails in the region and strong temporal overlap between snail and biocrust activity, suggest that snail mucus may play a key role regulating biocrust C-dynamics in the Negev desert. More generally, our work highlights the potential impacts of animals on biocrust function and emphasizes the need to develop a predictive framework for consumer–biocrust interactions that may improve our understanding of nutrient dynamics in drylands.

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DATA AVAILABILITY

Data can be accessed through Dryad (https://doi.org/10.25338/B8NK9N).

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