The Effect of Mucus and Feces from the Invasive Slug *Arion rufus* on the Growth of Annual Ryegrass *Lolium multiflorum*

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Blinks-NSF REU-BEACON 2015

Summer 2015

Please note: this paper is the result of a student project conducted over a single summer and some of our samples are still in process. We are currently working on a peer-reviewed publication. Please contact the authors to ask for an updated version and do not cite this article.

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Keywords: *Arion rufus*, *Lolium multiforum*, grassland, plant growth, nutrient cycling, herbivory

Abstract

On San Juan Island, WA, the invasive slug *Arion rufus* lives in two habitats, the Douglas-fir/red cedar forests and the grasslands. The native slug of comparable size, *Ariolimax columbianus*, the banana slug, only lives in the forests, so *Arion* represents an inherently new type of herbivore/decomposer in the local grassland ecosystems. At some sites, *Arion* is present at extremely high densities, making it likely that this new species may be altering the overall rate of nutrient cycling in its new habitat. We examined the effect of live *Arion* slugs and *Arion* feces on the growth of the annual ryegrass, *Lolium multiforum*, over a month in July 2015. We found no significant difference among our treatments (live slug, slug feces only, no slug impact) with any measure of biomass production. This finding seems contrary to what we expected based on prior research by other researchers which typically occurred in forested areas. Our results prompt the larger questions of how *Arion* impacts grassland ecosystems, whether the impact of *Arion* slugs on nutrient cycling in forests is different than its impact in grasslands, and, if this difference does occur, why it does so.

Introduction

The presence of invasive species is by now expected in almost all parts of the globe as increasingly rapid forms of human transportation transfer species from native habitats to new sites of potential colonization. Many studies have examined how such rapid and prevalent colonization influences and changes native speciesôrichness, abundance, and behavior as well as how the ecosystem in its entirety may be altered. Sometimes an invasive species may compete directly with a native species that fulfills a similar environmental niche, such as the case of the invasive crayfish *Orconectes neglectus* and native crayfish *Orconectes eupunctus*. These two crayfish have similar tolerances for drought, and have very few significant differences in how they both impact ecosystem structure and function, leading the researchers to conclude that the two species appear largely ecologically redundant (Magoulick, 2014). In other instances, an invasive species may alter the nature of an entire ecosystem, as is occuring with the introduction of zebra mussels in the Great Lakes. In Oneida Lake, zebra mussels alter carbon cycling pathways and increase the abundance of benthic organisms, while decreasing the abundance of pelagic organisms and causing an overall decrease in ecosystem productivity (Miehls et al., 2009). Prevalent invasive species have the potential to drastically alter nutrient cycling and cause reorganization at an ecosystem level.

The European slug, *Arion rufus*, is a prevalent invasive species found throughout the Pacific Northwest region of the United States. About 18 cm long when full grown, its color range includes black, brown, yellow-orange, and brown-red (GOERT, 2013). The banana slug, *Ariolimax columbianus,* is the only native slug in Washington State comparable in size to the European slug. While both live in forests, only the invasive species is found in disturbed and urban sites and in grasslands (Thompson & Iyengar, REU 2014; Cates & Orians, 1975). Thus, while not a completely new taxa to the region, this invasive species is many times larger than any native slugs in grasslands and so may represent an inherently different player in the ecosystem compared to the native slugs.

It is unknown exactly when the European slug first appeared on San Juan Island, this study site, but they were not on the island 100 years ago and have been present for at least the past 20 years. The European slug likely entered western America via the Puget

Sound, as it was collected in Seattle by 1933, and was spotted in Oregon, Vancouver, and California, as well as Washington, within the next 20 years (Hanna, 1966). Slugs from the *Arion* genus are generally thought to have arrived in North America and spread through nursery plants, potting soil and pallets delivered to fish canneries (Wittwer, 2004). There are actually three species from Europe (*Arion rufus, Arion ater,* and *Arion vulgaris*) that are extremely similar in habitat and appearance, can produced hybrid offspring, and can only be differentiated by careful dissection of their genitalia or through genetic testing. However, a study on San Juan Island in 2009 confirmed that the local *Arion* population is *A. rufus* (personal communication with D. Robinson). Because of hybridization, morphological and behavioral similarities, some authors recommend referring to any individuals of these species in the United States as members of the *Arion ater* group. We refer to our study species as *Arion rufus* because of the genetic tests done on the island, but consider previous studies on any one of these three species as potentially relevant and informative for the present study.

Within San Juan County, 3,275 acres of land are devoted to hay production (USDA, 2012). Populations of *Arion* within hayfields and fallow fields can attain extremely high densities. We have found 6 slugs under a single 0.25 m^2 shelter board (Cotton, personal observation), seen more than 10 slugs per 0.25 m^2 in patches of recently mown hayfield (Iyengar, personal observation), and easily collected more than 80 slugs in 30 minutes in intact grasslands on thistle or on a mown grass path (Iyengar, personal observation). This is not an unexpected result as *Arion* in their native European ecosystems frequently inhabit grasslands. In Europe, Wilby and Brown (2001) found that mollusks are the dominant factor regulating plant community composition in old fields through preferential feeding on forbs and seedlings. In a separate study in Switzerland, it was found that *Arion lusitanicus* similarly altered grassland community composition and diversity, reducing overall above-ground biomass and decreasing the abundance of forbs (Buschmann et al., 2005). In California, Strauss et al. (2009) discovered that nocturnal generalists, including slugs, often graze upon just-sprouted seedlings, strongly affecting seedling survival rate.

While many books claim banana slugs are key facilitators of nutrient cycling in the Pacific Northwest, there is typically little data presented to support this claim. *Arion* has been shown to accelerate decomposition of beech leaf litter, but only under densities that are almost never seen in the field (Theenhaus & Scheu, 1996). However, a study in Seattle demonstrated that *Arion rufus* can affect the growth of Oregon grape, *Mahonia nervosa*, a common perennial woody shrub, through herbivory and by accelerating nutrient cycling through defecation and mucus production. At low densities, *Arion* increased Oregon grape biomass production compared with the control; at high densities, *Arion* decreased biomass production through herbivory (Lauren & Whitlow, 2012). All of these studies, however, focus on nutrient cycling in forest ecosystems, where both banana slugs and *Arion* are found. None examine potential effects *Arion* may have on grassland ecosystems, where banana slugs do not live. Is *Arion* increasing nutrient cycling and remineralization of nutrients in the grasslands, boosting plant growth?

While more common in the southeastern United States, annual ryegrass is a common cover crop throughout the country and is often grown for hay or pasture in the Pacific Northwest (MacKinnon et al., 2004). A cover crop that grows best in cool, moist conditions and germinates in 6-10 days, germination rates are highest (80%) during summer day/night temperatures of 30/17 °C while winter temperatures of 17/4 °C have a 71% germination rate (Carey, 1995). Nitrogen is often the limiting nutrient for this species.

We assessed the impact of the presence of *Arion* (the mucus and feces as well as the activities of the slugs themselves) on the growth of annual ryegrass from planting to 4 weeks of age. Mucus and feces are both potential sources of additional nutrients. When dried, mucus from *Arion* contains 38.8% C, 8.1% N, and 2.2% P (Theenhaus & Scheu, 2012), providing a potential fertilizer when present in sufficient quantities. However, mucus might also block plant stomata, limiting grass growth. Additionally, live *Arion* may consume the plants, negating any positive effects of fertilization through feces and mucus. However, previous studies (Thompson & Iyengar, REU 2014) indicate that *Arion* slugs do not consume mature velvet grass, *Holcus lanatus*, when given a choice of other plants, so they may similarly ignore older ryegrass. If the avoidance of grass is due to silica cells in the mature grass blades, young grass such as the newly germinated seedlings in our trials may still be at risk of high herbivory rates.

We predict that the extra nutrients from the feces will promote an elevated amount of biomass production, while the activities of the slugs themselves will reduce this benefit via herbivory.

Methods

Field Site

We conducted this experiment on San Juan Island, WA (48°30ô8ò N, 123°4ô30ò W), in a fallow field adjacent to a cow pastureland, small pond, and hayfield. Dominant vegetation included *Agropyron repens*, *Phalaris arundinacea*, *Festuca arundinacea*, *Dactylis glomerata*, *Holcus lanatus*, *Agrostis* spp, and *Cirsium* spp. For the previous 5 years, the average temperature during the month of July, when this study took place, was 14.5°C; this summer the average temperature was 16°C. The average precipitation for the past 5 years has been 14 mm in July; this year the total precipitation was 19 mm (Friday Harbor Laboratories Weather Station, 2015).

Grass Growth Experiment

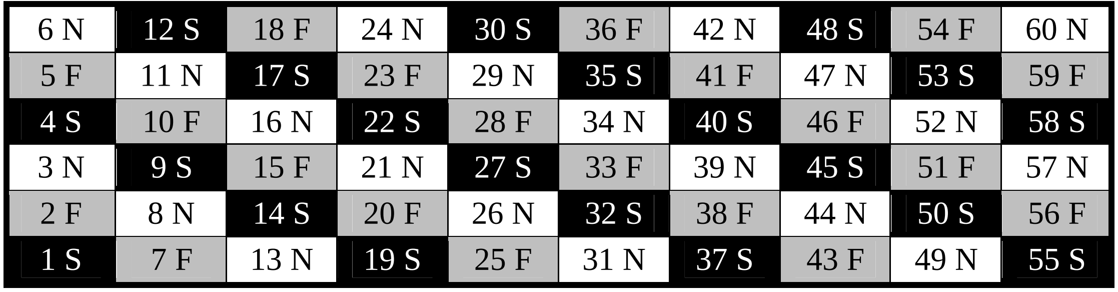
To determine the effect of *Arion* slugs on grass growth, we grew pots of grass individually in containers to prevent herbivory from any external source, allow sunlight penetration, and retain the experimental slugs in all plots. The cylindrical cages (17 cm diameter, 35 cm high) were constructed using window mesh (1 mm x 1 mm). We hot-glued three, equally-spaced bamboo stakes vertically to the sides of the cylinder for structural support and to anchor the cages to the ground. The bottom of the cage was lined with a circular plastic transparency (17 cm diameter) so we could easily collect feces excreted outside the area of grass growth in live slug treatment cages (see below). Rubber bands closed the cages on top, allowing easy access to individual cages. Each cage also contained a green plastic flower pot (10 cm diameter, 9 cm in height) with a piece of black plastic tarp (15 cm x 20 cm) attached to the north-facing side by a rubber band. A sponge (Western Family Large Cellulose Sponge 2 cm x 2 cm x 5 cm) dampened with reverse osmosis water was placed between the pot and the tarp to provide shelter for slugs and was included in all treatments regardless of slug presence to control for any effect of the cooler moisture beside the pot.

During the initial set-up, the pots were filled with premium topsoil (G&B Organics) to a height of 6 cm and watered with 210 mL of reverse osmosis water. One hundred annual ryegrass seeds (Skagit Farmers Supply) were evenly distributed on the top of the soil and covered with another 2 cm of soil, then an additional 120 mL of water was added to the pot. After the initial planting, each pot received 50 mL of water and sponges were re-soaked on alternating days for a total of three days a week, coinciding with the days when new slugs were added (see below).

We examined three treatments at our study site: a live slug treatment, a feces only treatment, and a no addition treatment to serve as the control. Twenty replicates of each treatment were interspersed in Latin square blocks, each cage 50 cm apart from any adjacent cage (Figure 1). Three days a week on alternating days, 80 slugs were collected from the field adjacent to the study site within 90 minutes after sunrise to insure the slugs had experienced a full night of feeding. Forty slugs were immediately deployed in the live slug treatment cages, and the remainder were placed in pairs in separate Tupperware containers (17 cm x 17 cm) with a mesh screen top to collect feces for the feces treatment plots. Each Tupperware also had a moist sponge inside (Western Family Large Cellulose Sponge 2 cm x 2 cm x 5 cm) and was placed under a large shelter board to avoid exposing the slugs to direct sunlight. An attempt was made to create pairs that overall represented the same biomass across all replicates and the live slug and feces treatments. However, as different locations in the field were sampled on various days, the average size of slugs varied across days.

After 24 hours, slugs were removed from the live slug cages and feces from the Tupperware containers were placed in the feces cages. After another 24 hours, a new set of 80 slugs was collected from the field to allow renewed application of feces from a natural diet. Thus, the live slug treatment grass grew for 24 hours with no slugs in the container every 48 hours. No slugs were ever used more than once in the experiment. Both the live slug treatment and feces treatment were repeated on alternating days three times a week, with one day a week at the end of the cycle where no treatment was provided. Overall, slug density averaged a bit less than 1 slug/day/pot in these treatments. On the days slugs were removed from the experimental setup, the animals were brought back to laboratory, immediately weighed, and euthanized by freezing.

Figure 1. Diagram of Treatment Plot Set Up. Cages were numbered 1-60 and marked with either an S (live slug treatment, black box), F (feces treatment, grey box), or N (no addition treatment, white box).



6 N	12 S	18 F	24 N	30 S	36 F	42 N	48 S	54 F	60 N
5 F	11 N	17 S	23 F	29 N	35 S	41 F	47 N	53 S	59 F
4 S	10 F	16 N	22 S	28 F	34 N	40 S	46 F	52 N	58 S
3 N	9 S	15 F	21 N	27 S	33 F	39 N	45 S	51 F	57 N
2 F	8 N	14 S	20 F	26 N	32 S	38 F	44 N	50 S	56 F
1 S	7 F	13 N	19 S	25 F	31 N	37 S	43 F	49 N	55 S

North →

Grass was planted and treatments set up in the field site on 29 June 2015. On this date, only slugs for the feces treatment were collected; the first set of feces for the feces treatment and the first set of slugs for the live slug treatment were placed in the cages on 30 June 2015. Until the grass germinated, we applied treatments every day. 2 July 2015 was the first day no slugs were collected. From this point onward until the last set of treatments was applied on 27 July 2015 (28 days), the treatments were applied three times a week as described previously. Germination was first observed on 3 July 2015, four days after planting. Grass was allowed to grow until 1 August 2015, thirty three days after planting, and five days after the last treatment was applied.

At the termination of the experiment, qualitative plant health, biomass production above ground (number of blades, average length of the 5 longest blades, grass blade wet and dry weight, average weight/blade) and biomass production belowground (sieved root wet and dry weight) were measured. To determine seedling germination and survivorship, three days after germination was first observed, the number of grass blades in each pot was counted and recorded to assess percent germination. On 22 July 2015, twenty-three days after planting, grass health was assessed qualitatively by estimating the amount of green/brown grass and blade width, and the amount of herbivory. The categories of scoring included: all in pot were dead, mostly brown and thin, brown tips and thin, brown tips and full blades, or green and full blades. Herbivory was scored as ñplentifulò (>50% of blades show signs of herbivory), ñsomeò (<50% of blades show signs of herbivory), or ñnone.ò At the end of the experiment, the total number of blades in each pot was counted, as well as the number of brown blades and the length of the 5 longest blades. Grass blades were cut at the soil surface and wet and dry masses were recorded. Roots were sieved from the soil using a 2 mm sieve and wet and dry masses were recorded to determine below ground biomass. To dry grass and roots, samples were placed in Flat Open Clear Plastic Poly Bags (2 Mil, 4" x 6", Plymor) and dried in a drying oven at 60°C. Grass was dried for 48 hours; roots were dried for 72 hours, then sieved a second time through 1 mm mesh to remove any remaining soil particles before the dry weight was recorded. Separate ANOVAs were conducted to examine the impact of the different treatments (independent variable) on the various parameters of growth and biomass production (the dependent variable in each ANOVA).

Feces Experiment

Slug body mass was recorded to determine whether it differed significantly across individual pots or between the live slug treatment and feces treatment. To determine whether the weight of feces produced correlated with body mass, 41 pairs of size-matched slugs ranging across the sizes used in our grass growth experiment were placed in Tupperware containers (17 cm x 17 cm) with a damp sponge (Western Family Large Cellulose Sponge 2 cm x 2 cm x 5 cm) for 24 hours in the laboratory. Because the average slug size seemed larger closer to the pond than in the middle of the field (Cotton & Iyengar, personal observation), slugs were collected from both locations to ensure the size range in this experiment was comparable to the size range in the grass growth experiment. (Collection of slugs for the grass growth treatment always occurred on the same property as the experiment, but we collected slugs from different areas within the property on different days). At the end of the 24 hour period, slug mass and feces mass in each Tupperware were recorded. Feces were then placed in a drying oven at 70°C for 24 hours, and dry weights were recorded. The relationship between slug mass and feces (i) wet and (ii) dry weight was examined using separate regressions. Data from the two collection sites were analyzed separately.

Soil Analysis Experiment

To determine how the commercial soil in our pots for the grass growth experiment compared with the soil in the field, we collected soil samples from multiple sites on the property where the experiment was conducted (near the pond, in the middle of the field, and in the distant corner of the field). We plan to do nutrient analysis on this soil, but have not yet had time to process the samples.

Mature Grass Feeding Experiment

To assess potential impacts of slug herbivory on grass older than what we grew in the grass growth experiment, we collected leaves from *Agropyron repens* at our field site. *Agropyron repens*, while not in the *Lolium* genus, is part of the same subfamily, allowing it to be an acceptable stand-in for mature *Lolium*, which we, unfortunately, did not have available. In 20 Tupperware containers (17 cm x 17 cm), three *Agropyron* blades with no signs of herbivory were placed on damp soil with one *Arion* slug. After 24 hours, all blades were examined visually for any signs of herbivory.

Desiccation Experiment

A possible reason for the habitat difference between native banana slugs and invasive *Arion* may be differing tolerances in their ability to withstand desiccation at higher temperatures. To examine this possibility, mass loss of grassland *Arion*, Douglas-fir *Arion*, and Douglas-fir banana slugs were measured at 9.5°C and 20°C. Temperature was measured using an iButton thermochron. Slugs were placed individually in Tupperware containers (20 cm x 15 cm x 10 cm) with window mesh (1 mm x 1mm) insets on the two long sides of the container. Slug mass was recorded at the beginning of the experiment and 5.75 hours after the start time. The percent of body mass lost was used to calculate the resistance of the slugs to desiccation. The relationship between initial slug mass and percent body mass loss was examined using separate regressions. Each slug subpopulation was analyzed separately.

Results

Grass Growth Experiment

Across all three treatments, we found no significant difference in initial germination rates (averages ranged from 79.2-81.3%; df=2, F=0.69, p>0.5). There was also no significant difference in the percentage of blades that survived to the end of the experiment (averages ranged from 93.8-98.8%; df=2, F=1.14, p>0.2). The qualitative determination of plant health in the third week of the experiment did not reveal a large discrepancy in health across the treatments (Table 1), and, as expected, only the live slug treatment plants showed visible signs of herbivory (Table 2). The total average mass of slugs for each pot used in the live slug treatment and the feces treatment differed significantly between the two treatments over the duration of the experiment (averages ranged from 161.5-194.3 g; df=1, F=34.76, p<0.0001).

Table 1. All grass pots were visually sorted into one of 5 categories of grass health on July 22, 2015. No pot contained grass that was completely dead, so this category is not shown below. N=20 for each treatment.

	Health			
Treatment	Green, Full Blades	Brown Tips, Full Blades	Brown Tips, Thin Blades	Mostly Brown, Thin Blades
Live Slugs	5	14	1	1
Feces	0	12	7	1
No Inputs	0	14	5	1

Table 2. All grass pots were visually evaluated for herbivory on July 22, 2015. N=20 for each treatment.

	Herbivory		
Treatment	Plentiful	Some	None
Live Slugs	3	13	4
Feces	0	0	20
No Inputs	0	0	20

There was also no significant difference across treatments in any measure of aboveground biomass production: the average number of blades present in a pot at the end of the experiment (Figure 2, df=2, F=0.36, p>0.5), the average length of the 5 longest blades (Figure 2, df=2, F=0.50, p>0.5), the average grass wet mass (Figure 2, df=2, F=0.01, p>0.9), the average grass dry mass (Figure 2, df=2, F=0.23, p>0.5), or the average mass per blade (Figure 2, df=2, F=0.79, p>0.4).

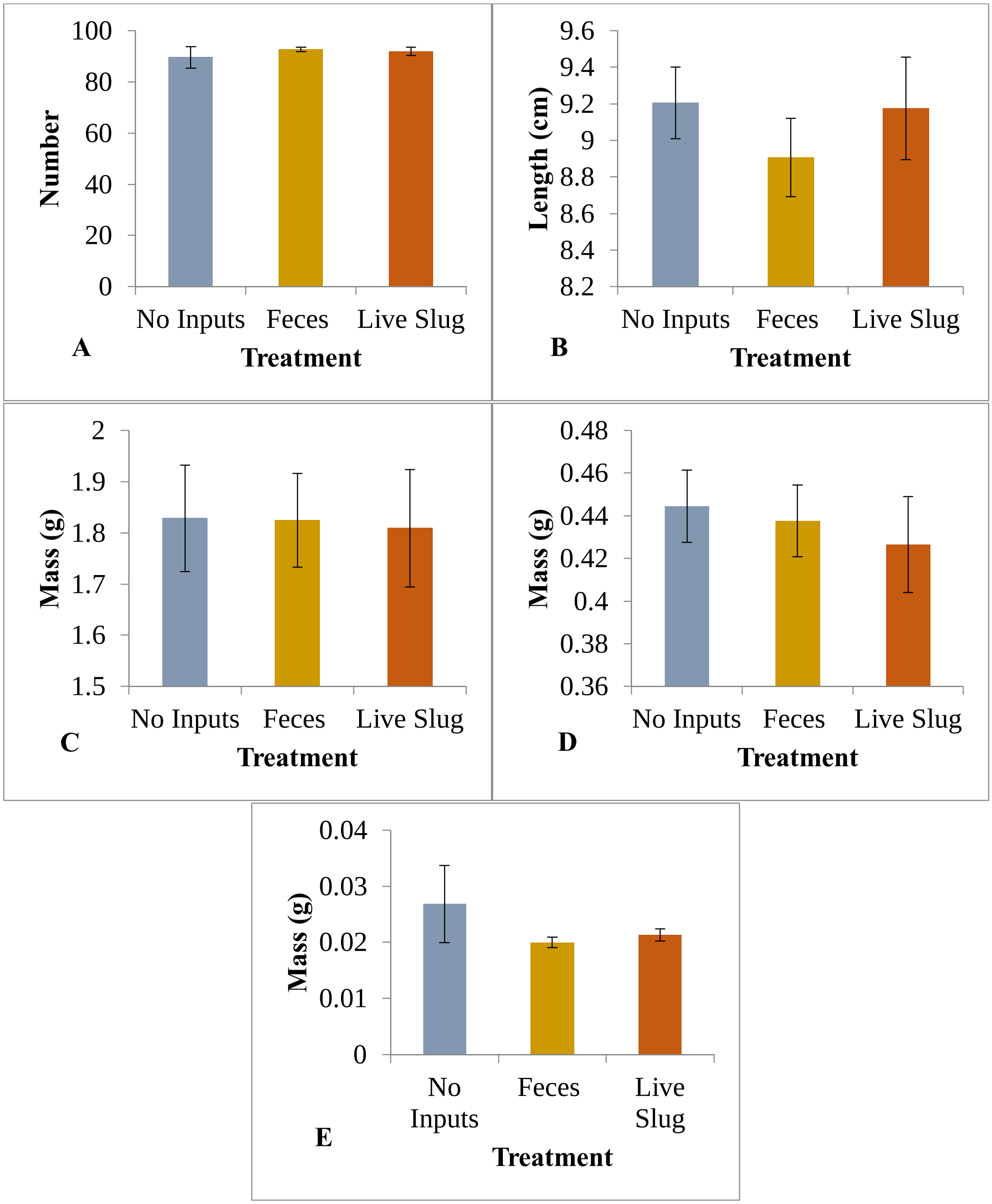


Figure 2. Measures of aboveground biomass did not differ significantly across the treatments. N=20 for all treatments. Measurements included (**A**) the average number of blades per pot, (**B**) the average length of the 5 longest blades per pot, (**C**) the average wet mass of grass per pot, (**D**) the average dry mass of grass per pot, and (**E**) the average wet mass of a single blade. Error bars are +/- 1 SE in all graphs.

Between treatments, there was also no significant difference in measurements of belowground biomass production, wet mass (Figure 3, df=2, F=0.53, p>0.5) or dry mass (Figure 3, df=2, F=0.04, p>0.9).

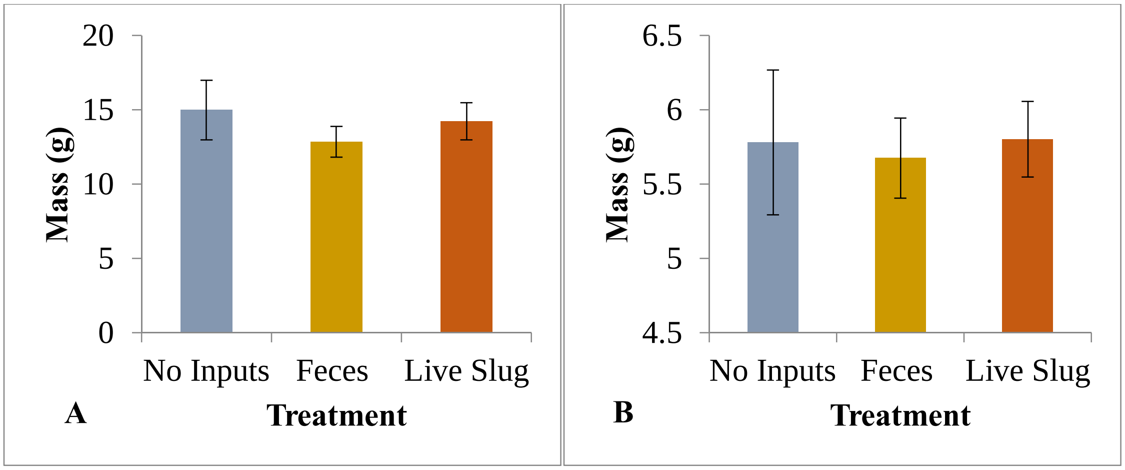


Figure 3. Average wet (**A**) and dry (**B**) mass of belowground biomass did not differ significantly across treatments. N=20 for all treatments. Error bars are +/- 1 SE in both graphs.

Feces Experiment

Initial slug size did not significantly affect the feces wet weight of grassland *Arion* found near the pond (Figure 5, df=1, R^2=0.0393, F=0.74, p>0.4), but it did affect the feces wet weight of grassland *Arion* found in the field (Figure 5, df=1, R^2=0.2504, F=6.35, p=0.0209). When the feces were dried, initial slug mass affected the weight of the pond *Arion* feces, but not significantly (Figure 5, df=1, R^2=0.1307, F=2.71, p=0.1173). The dry weight of the field *Arion* feces was significantly affected (Figure 5, df=1, R^2=0.3499, F=10.23, p=0.0047). Feces wet weight did significantly affect feces dry weight in both the pond *Arion* (Figure 5, df=1, R^2=0.4638, F=15.57, p=0.0009) and field *Arion* (Figure 5, df=1, R^2=0.6517, F=35.55, p<0.0001).

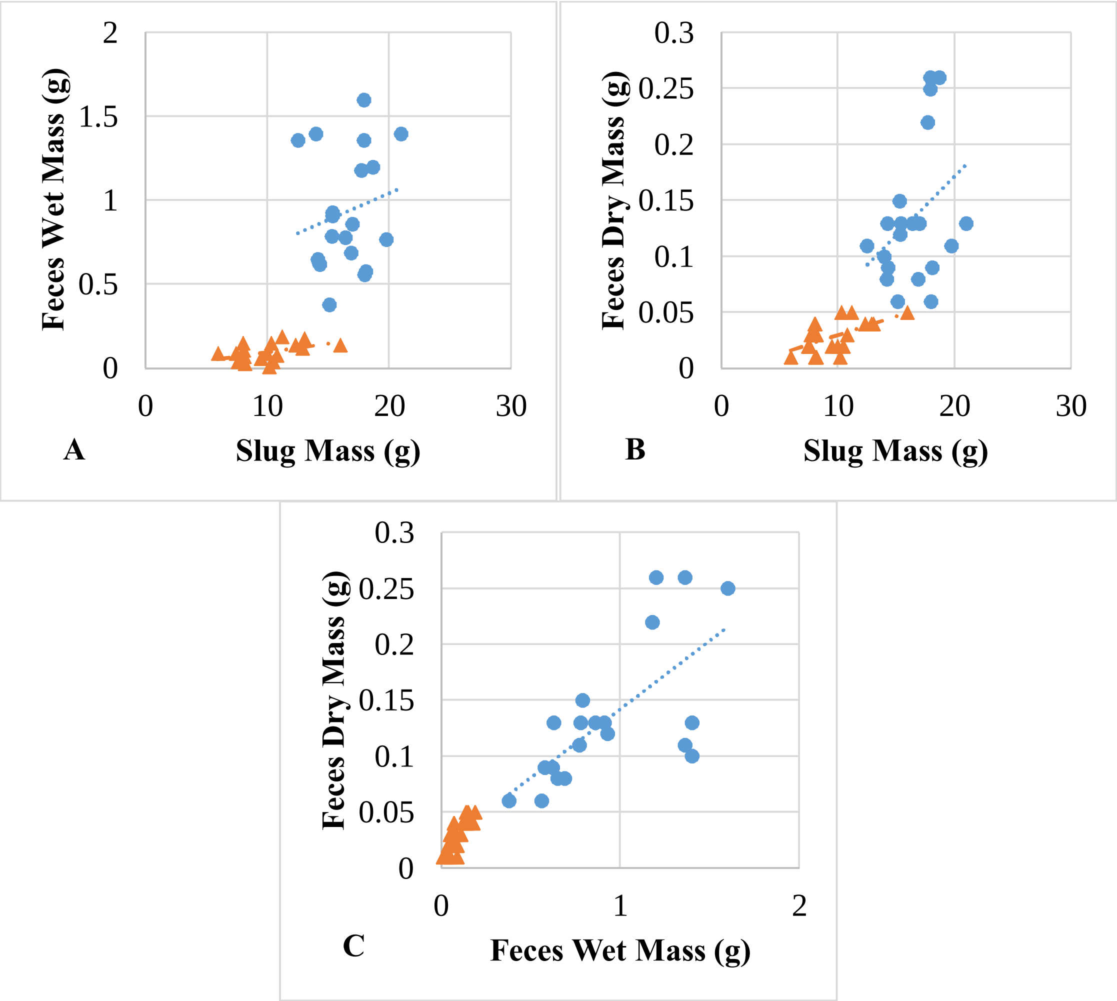


Figure 5. Linear regression of grassland *Arion* slugs at two collection sites, near the pond (circles, n=20) and further out in the field (triangles, n=21). (**A)** Regression of slug mass to feces wet mass. (**B**) Regression of slug mass to feces dry mass. (**C**) Regression of feces wet mass to feces dry mass.

Mature Grass Herbivory Experiment

In all twenty replicates, no sign of herbivory was observed on any grass blades. This supports previous research that *Arion* does not eat mature grass (Thompson & Iyengar, REU 2014).

Desiccation Experiment

At 20°C, the grassland *Arion* loses moisture at a rate that significantly depends on the slugôs initial mass (Figure 4, df=1, R^2=0.5914, F=18.82, p=0.0008). The initial mass of the native *Ariolimax* also significantly affects how this species loses moisture at 20°C (Figure 4, df=1, R^2=0.3066, F=4.42, p=0.0618). At a lower temperature of 9.5°C, this same pattern holds true for the grassland *Arion* (Figure 4, df=1, R^2=0.3143, F=5.96, p=0.0297). However, the initial mass of the forest *Arion* slugs does not seem to significantly affect the rate at which they lose moisture at 9.5°C (Figure 4, df=1, R^2=0.0583, F=0.62, p>0.4).

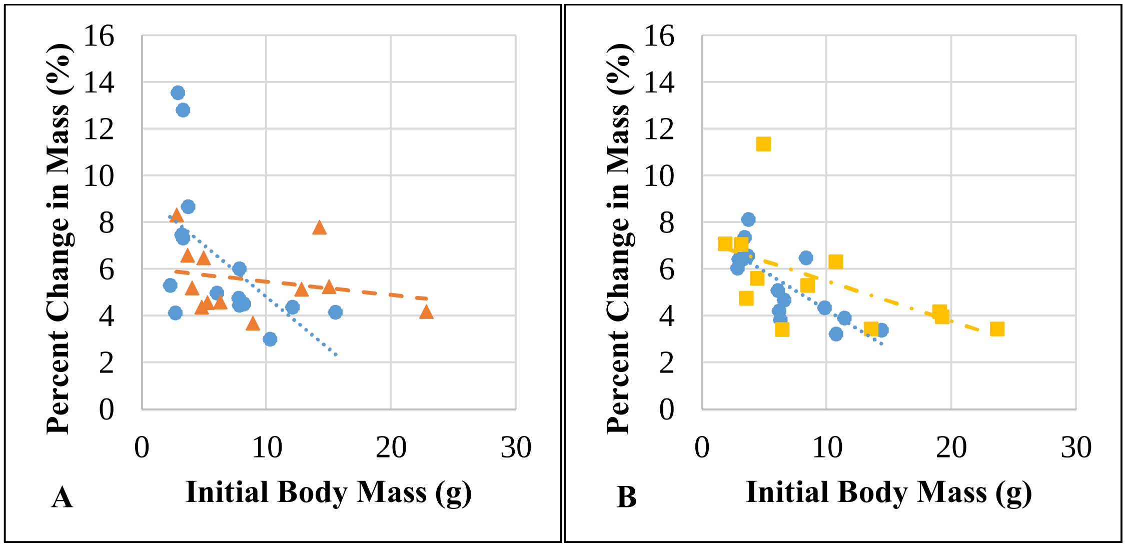


Figure 4. Linear regressions of relationship between initial slug mass and percent change in mass. (**A**) Comparing the grassland *Arion* (circles, n=15) and forest *Arion* (triangles, n=12) populations at 9.5°C. (**B**) Comparing the grassland *Arion* (circles, n=15) and *Ariolimax* (squares, n=12) at 20°C.

Discussion

We predicted that *Arion* slugs would negatively impact grass biomass production through herbivory and positively affect it through nutrient addition to the soil as their digestive processing of surrounding plant material accelerated nutrient cycling such that the slugsô feces would act as a fertilizer for the growing plants. Contrary to our hypothesis, however, in all measures of ryegrass biomass production, the data showed a remarkably uniform lack of effect across all treatments. Seedling germination and survival rates did not differ across treatments, so slug herbivory did not kill a significant number of seedlings. The measures of aboveground biomass production show that the grass was not significantly impacted by herbivory, despite the fact that we observed signs of herbivory in the live slug treatment, especially within the first week of germination. Because the average height of the 5 longest blades and the average weight per blade did not significantly differ across treatments, it appears that grass blades subject to herbivory early in the experiment made a full recovery by the end of the four week period. The grass that showed compensatory growth did not incorporate induced defenses in terms of additional silica because the average weight per blade did not differ among treatments. The lack of variation for both aboveground and belowground biomass production across treatments shows that the added nutrients from the feces and mucus of *Arion* had no discernable effect on plant growth.

These findings are extremely unexpected. They do not seem to logically follow from previous research that demonstrates *Arion* and other slugs accelerate nutrient cycling and decomposition (Richter, 1979; Theenhaus & Scheu, 1996), thus leading to potentially accelerated plant growth. Additionally, Lauren and Wilson (2012) directly observed biomass changes caused by *Arion* in *Mahonia nervosa*, a perennial, woody plant. That a perennial, woody plant would show biomass changes while an annual, fast-growing grass would not show biomass changes surprised us. While Lauren and Wilsonõs

(2012) study extended over a much longer time period than our own, we expected that if treatment differences would occur in our experiment, it would be noticeable over the four weeks of rapid growth following germination, as ryegrass is a rapidly growing annual species.

Although *Lolium* growth was not impacted by the presence of *Arion* or the addition of their feces, the nutrients present in slug mucus and feces did not simply disappear. Although we think it is unlikely, the additional nutrients from *Arion* may have been absorbed by the grass plants, but the gain was so small that there was no significant increase in biomass. It is also possible that the topsoil where we planted the grass was already nutrient-saturated. We did not want to use soil directly from the study site because of the potential presence of other plant seeds in the soil, which could introduce accidental species into our experimental cages, and because at different locations and depths, soils have inherently different characteristics due to overlying pre-existing plants and their root masses and affiliated symbionts. However, commercial topsoils, even the organic soil we chose, have added nutrients incorporated into them, and these may have been present in sufficient quantities that the addition of *Arion* nutrients did not assist biomass production. We have collected field and commercial soil samples to conduct nutrient analyses for comparison, but these have not yet been completed. It is also possible that nutrients are simply leaching from our pots before the grass has a chance to absorb them, but this seems unlikely as feces accumulated on the surface of the soil during the experiment and likely should have acted as time release nutrient beads.

In order to control the amount of feces live slug treatments and feces treatments received, we did our best to size-match the slugs we used in our experiments each day.

The feces experiment showed that this method is somewhat justified. For *Arion* slugs collected in our field site in the middle of the field, slug body mass was significantly correlated with the wet and dry weight of feces. However, for slugs collected near the pond, body mass was not a good predictor of feces wet weight, and was not significantly correlated with feces dry weight, although there does appear to be a trend present in the data. While body mass is not a perfect indicator of the amount of feces produced, smaller slugs, as expected, generally produce less feces than larger slugs, so size-matching the slugs used in our treatments to somewhat standardize the amount of feces produced is justifiable. When examining the weights of the slugs used in the experiment, the slugs in the feces treatment weighed significantly more than the slugs in the live slug treatment. While we were initially concerned about this, we realized that this difference is likely due to differential desiccation rates of the animals. We weighed the slugs after using them in the field, which means that the feces treatment slugs had stayed completely in the shade for the past 24 hours while the live treatment slugs in the cages likely experienced more incident radiation and thus higher desiccation rates, despite the moist shelter provided for them.

Arion did not consume any mature *Agropyron repens* over a 24 hour period. Previous research (Thompson & Iyengar, REU 2014) found that *Arion* does not consume mature velvet grass when given a choice of foods; we found that *Arion* will not consume mature *Agropyron* grass, even when given no other feeding choices over a 24 hour period. While *Agropyron* is not the same species as ryegrass, the two are in the same subfamily and share many physical characteristics. *Arion*ȍs refusal to eat mature grass in both instances described above causes us to infer that *Arion* would not consume mature ryegrass, potentially explaining why signs of immediate herbivory were only seen in the grass growth treatment in the week following germination and why the grass in the live slug treatment grew as tall as in the other treatments.

In the desiccation experiment, all populations except the forest *Arion* desiccated at a rate that was proportional to initial body mass. This small-scale experiment does not have enough replicates across all variables to draw firm conclusions about desiccation rates in *Arion* and *Ariolimax* across temperature gradients, but it hints that there may be differences between forest and grassland *Arion*, as well as between grassland *Arion* and forest *Ariolimax*. These differences may partially explain why the slug subpopulations are able to exist in very different habitats, but further experiments are needed before any firm conclusions can be drawn.

From these experiments, we see the presence of live *Arion* slugs or their feces alone does not impact *Lolium* production. Different rates of desiccation are a possible explanation as to why *Arion* can tolerate life in the grasslands, while the native *Ariolimax* cannot. Because *Arion* does not directly impact the biomass production of *Lolium* nor, potentially, the biomass production of other grass species, its effects on Pacific Northwest grassland ecosystems may be more limited than we first hypothesized. However, research in Europe suggests that slugs, including species in the *Arion* genus, can significantly impact grassland community composition because the slugs preferentially feed on forbs, increasing the percentage of groundcover dominated by grass (Wilby & Brown, 2001; Buschmann et al., 2005). While the impact of *Arion* on community composition was not tested in this experiment, it is entirely possible that similar mechanisms may be at work in the Pacific Northwest, especially given slugsô consistent preference to avoid consuming mature grass in our experiments. Further studies could explore this possibility more in depth.

Improving our understanding of *Arion*ôs impact in grasslands and other ecosystems of the Pacific Northwest will increase our knowledge of the effects invasive species have on their new environments and on the role mollusks and other invertebrates play on nutrient cycling in ecosystems.

Acknowledgements:

We would like to thank the following individuals and organizations for their help with this research: the Blinks-NSF REU-BEACON program (grant number DBI 1262239), Friday Harbor Laboratories, Melissa Mayol, Rachel Merz, Eliza Habegger, Colin Hermans and E. Carrington.

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