

Research Article

# Mammalian herbivory in post-fire chaparral impacts herbaceous composition but not N and C cycling

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## Abstract

**Aims** Classical theory predicts that herbivores impact herb assemblages and soil nitrogen (N) cycling through selective plant consumption and the deposition of N-rich waste, with effects dependent upon ecosystem N availability. Herbivores are predicted to accelerate N cycling when N availability is high and decelerate cycling when availability is low. However, experimental tests of these theories in natural systems are limited and have yielded contradictory results. California's widespread chaparral shrublands provide a tractable system in which to test these theories. They are prone to periodic crown fire, which temporarily removes living shrub cover, deposits mineral N on soils and allows diverse herbaceous assemblages to dominate the landscape for 3–5 years. Chaparral is also increasingly vulnerable to herbaceous invasion; mammalian herbivory may limit the establishment of non-native herbs in the shrub understory.

**Methods** We implemented a 2-year herbivore-exclosure experiment (Hopland, CA) to assess the impact of mammalian herbivory during early post-fire chaparral succession, both on herbaceous plant assemblages and soil N and C cycling. We predicted that, in high-N post-fire conditions, mammalian herbivory would not demonstrate a strong preference for N-fixing herbs, would accelerate N cycling and would reduce the abundance of non-native herbs.

**Important Findings** Excluding mammalian herbivores increased herb standing biomass by 54%, but changed neither the relative abundance of N-fixing vs. non-N-fixing herbs nor any measure of N or C cycling. Herbivore impacts on nutrient cycling may not be significant over the 2-year time scale of the experiment and physical effects of herbivore activity could have counteracted the influence of plant litter and animal dung/urine inputs. Mammalian herbivores concentrated their feeding on typical non-native herbs, slightly decreasing their relative abundance; however, mammalian herbivory was not sufficient to stem the invasion of chaparral by invasive herbs or alter C and N cycling over the first 2 years after fire.

**Keywords** herbivory, fire, plant invasion, chaparral, N cycling

## 火后灌丛中哺乳动物食草性影响草本植物组成而不影响氮和碳循环

**摘要:** 经典理论预测, 草食动物通过选择性植物啃食和富含氮的排泄物的沉积影响草本植物组成和土壤氮循环, 其影响取决于生态系统氮的有效性。据预测, 草食动物在氮有效性高时可加速氮循环, 而在氮有效性低时减慢其循环。然而, 在自然系统中对这些理论的实验验证比较有限, 并且有些结果相互矛盾。

盾。美国加利福尼亚州广泛分布的灌木林地验证这些理论提供了一个可行的系统。它们易于发生周期性的林冠火，这会暂时移除活的灌木覆盖物，使土壤中的矿质氮沉积，并利于各种草本植物组合在3–5年内形成主导景观。灌丛也越来越容易受到草本植物的入侵；哺乳动物的食草性可能会限制灌丛下层非本地草本植物的建立。我们设置一项为期两年的植食动物-围封实验，评估火灾后早期灌丛演替期间哺乳动物食草性对草本植物组合和土壤氮和碳循环的影响。我们预测，在火灾后高氮条件下，哺乳类食草动物不会更倾向于取食固氮草本植物，会加速氮循环并减少非本地草本植物的丰富度。研究表明，排除哺乳类食草动物后，尽管草本植物的现存生物量增加了54%，但固氮与非固氮草本植物的相对丰富度没有改变，也没有改变氮和碳循环的指标。在两年的实验期间内，草食动物对养分循环的影响可能并不显著，草食动物活动的物理影响可能抵消了植物凋落物和动物粪/尿输入的影响。哺乳类食草动物主要以典型的非本地草本植物为食，略微降低了它们的相对丰度；然而，在火灾后的前两年，哺乳动物的食草性并不能有效地阻止侵入性草本植物对灌丛的入侵，也不能改变植物的碳和氮循环。

**关键词：**草食，火灾，植物入侵，灌丛，氮循环

## INTRODUCTION

Community ecologists have long appreciated the role of herbivory in limiting plant biomass and abundance, mediating species coexistence and influencing successional change. Herbivores can also have important effects on nutrient cycling in ecosystems (Sitters and Olde Venterink 2015). The effect of herbivores on ecosystem nutrient cycling depends on both top-down and bottom-up effects, namely the preferences and nutritional needs of grazers and the C:N ratio of plants (Hobbie and Villóeger 2015). Classic theory predicts that herbivory's effect on nitrogen (N) cycling depends on the N availability of a given ecosystem. Herbivory is thought to accelerate N cycling in high-N systems and decelerate cycling in low-N systems (Ritchie *et al.* 1998; Wardle *et al.* 2004). However, experimental tests of these theories have been limited to a few study systems, namely grassland systems with ungulate grazers, and results have been contradictory (Sitters and Olde Venterink 2015). Testing this prediction in more natural settings could provide evidence to support the development of context-specific frameworks relating herbivory to N cycling; an updated framework will increase the generalizability of this ecological theory.

In terrestrial systems, grazing is predicted to affect the rate of nutrient cycling through consumptive and non-consumptive mechanisms. Herbivore preferences and grazing intensity will change plant functional diversity, influencing litter quality supplied to decomposers and ultimately N cycling rates, which are largely controlled by litter N content (Parton *et al.* 2007). In N-limited systems, herbivores are predicted to selectively consume plants with low C:N ratios, such as legumes, facilitating dominance by

higher C:N ratio species. Thus, the low-quality litter of the surviving plants is supplied to decomposers, slowing N cycling and decreasing N availability to growing plants. These conditions may even promote the growth of nutrient-poor plants that can tolerate low-N soils, further decelerating N cycling (Ritchie *et al.* 1998; Wardle *et al.* 2004). In high-N systems, herbivores may stimulate compensatory growth of fast-growing N-rich species that can tolerate defoliation (*sensu* McNaughton 1983). This effect may accelerate N cycling by increasing rates of plant N uptake, N content of litter and dung/urine N (Sitters and Olde Venterink 2015).

Chaparral ecosystems, the fire-prone shrublands that cover over 7% of California's land area (Keeley and Davis 2007), have been a classic study system for botanists, ecologists and resource managers over the past 80 years. Comprised mainly of *Adenostoma fasciculatum*, as well as interspersed *Ceanothus* spp., *Arctostaphylos* spp. and other taxa, chaparral is susceptible to high-intensity crown fires, typically occurring in the late summer and fall dry seasons. Nutrient dynamics following chaparral fire (Christensen 1973; Hanan *et al.* 2016a) and patterns of species replacement during chaparral succession (Keeley *et al.* 1981) have been well characterized. Mature *Adenostoma* chaparral is considered N-limited and hosts almost no herbaceous understory beneath its dense shrub canopy (Christensen and Muller 1975a).

Disturbance by fire temporarily eliminates canopy cover, liberates N and stimulates the germination of fast-growing herbs, resulting in accelerated N cycling in recently burned stands (Hanan *et al.* 2016a). During fire, large net losses of N occur through volatilization from shrub biomass, litter and soils (Debano and Conrad 1978). Despite net

losses of total N, labile N is highly elevated after fire. The ash layer deposited on chaparral soils is rich in ammonium ( $\text{NH}_4^+$ ) (Christensen 1973; Deban and Conrad 1978), which is available for immediate uptake by plants. Within 2–3 months after fire, the onset of winter rains stimulates rapid nitrification, conversion of  $\text{NH}_4^+$  to nitrate ( $\text{NO}_3^-$ ) (Christensen 1973). While both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are available for plant uptake,  $\text{NO}_3^-$  is highly soluble and vulnerable to leaching if not taken up immediately by plants (Deban and Conrad 1978). Thus, post-fire plant assemblages, and herbivore interactions that mediate their composition, have an important influence on N cycling in recently burned chaparral. Post-fire chaparral is characterized by a flush of herbaceous species (Keeley *et al.* 1981; Potts and Stephens 2009), which may dominate for 3–5 years before the shrub canopy closes over, accompanied by the basal regrowth of *Adenostoma* (Potts *et al.* 2010).

In the present study, we established an herbivore-exclosure experiment immediately after chaparral fire in northern California to test the effect of mammalian herbivores on chaparral herb assemblages and soil N cycling. The immediate post-fire environment is generally characterized by elevated soil mineral N (Christensen 1973; Hanan *et al.* 2016a) and high plant tissue N (Rundel and Parsons 1980, 1984). Despite luxury consumption of N in the immediate post-fire years (Rundel and Parsons 1980), the plant tissue of typical N-fixing herbs is richer in N than co-occurring non-N-fixers in post-fire chaparral. One year after a 2015 chaparral fire in the nearby Stebbins Cold Canyon Reserve (Solano County, CA), the dried standing litter of *Acmispon* spp., the dominant N-fixing herbs from the present study, had a significantly lower C:N ratio ( $27.8 \pm 0.5$ , S.E.M.) than co-occurring typical non-N-fixing herbs *Emmenanthe penduliflora* ( $70.7 \pm 2.3$ ) and *Chlorogalum pomeridianum* ( $115.9 \pm 14.3$ , Wahl *et al.*, unpublished work). Thus, we considered the post-fire chaparral environment a natural setting temporarily elevated in soil N availability and with differential forage quality, distinguished by the functional groups of N-fixers and non-N-fixers.

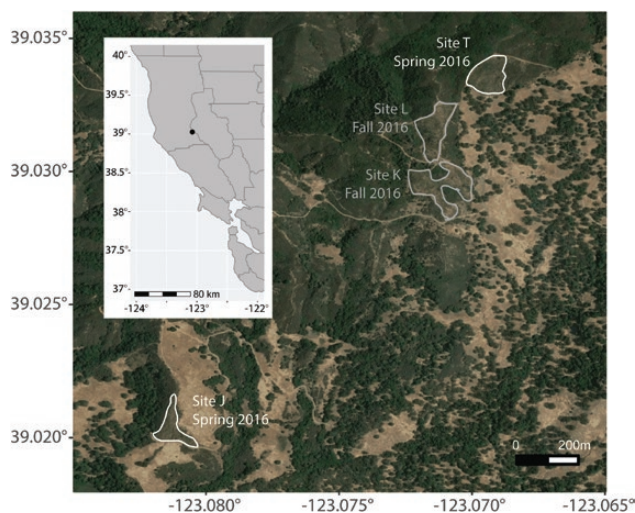
We hypothesized that mammalian herbivores would accelerate N cycling in the high-N post-fire environment. We also tested hypotheses regarding herbivore-mediated apparent competition between herbaceous functional groups. Contrary to predictions for N-poor environments, in which herbivores may disproportionately consume N-fixers and lead to dominance by non-N-fixers, we hypothesized that

herbivores would not demonstrate a clear preference for N-fixing herbs, given the generally high plant tissue N after fire. Additionally, we hypothesized that mammalian herbivores would limit the growth of non-native herb species compared with natives. Invasive herbs, many of which are non-native grassland species, are thought to be reduced in abundance by small mammals in chaparral (Park and Jenerette 2019). While several herbivore-exclosure experiments in post-fire chaparral have demonstrated effects of mammalian herbivory on shrub growth and survivorship (Mills 1986; Potts *et al.* 2010), fewer investigations have addressed herbivore effects on post-fire herbaceous assemblages (Tyler 1995) and N cycling.

## MATERIALS AND METHODS

### Study location and site selection

We conducted this study at the Hopland Research and Extension Center (Mendocino County, CA,  $39^\circ 0' \text{ N}$ ,  $123^\circ 4' \text{ W}$ , Fig. 1). Hopland is located in the North Coast Mountain Range of California. The vegetation is a mosaic typical of Mediterranean California, including grasslands, chaparral shrublands, oak woodlands and mixed evergreen forests. Hopland experiences a typical Mediterranean-type climate with a warm dry spring through summer (annual averages 1981–2010: 11.2 cm precipitation) and a mild rainy fall through



**Figure 1:** Location of research sites within the northern California region and perimeters of each prescribed fire. Basemap was built with Google Maps from imagery captured in 2018 and copyrighted (2019) by Mazar Technologies, USDA Farm Service Agency. Black dot in inset map identifies project location.

winter (83.7 cm precipitation). The first season of monitoring had a particularly rainy fall and winter (2016–2017: 117.0 cm precipitation), while the second was comparatively dry (2017–2018: 18.3 cm precipitation). Temperatures were fairly consistent between years of sampling, with mean lows–highs of 10.2–20.5 °C (2016), 10.4–21.0 °C (2017) and 10.7–21.1 °C (2018). Short-term data (2016–2018) are from NCDC Hopland Station (Elev. 817 m; 39°1′ N, 123°4′ W) and 30-year normals are from HREC Headquarters (Elev. 263 m; 39°0′ N, 123°4′ W).

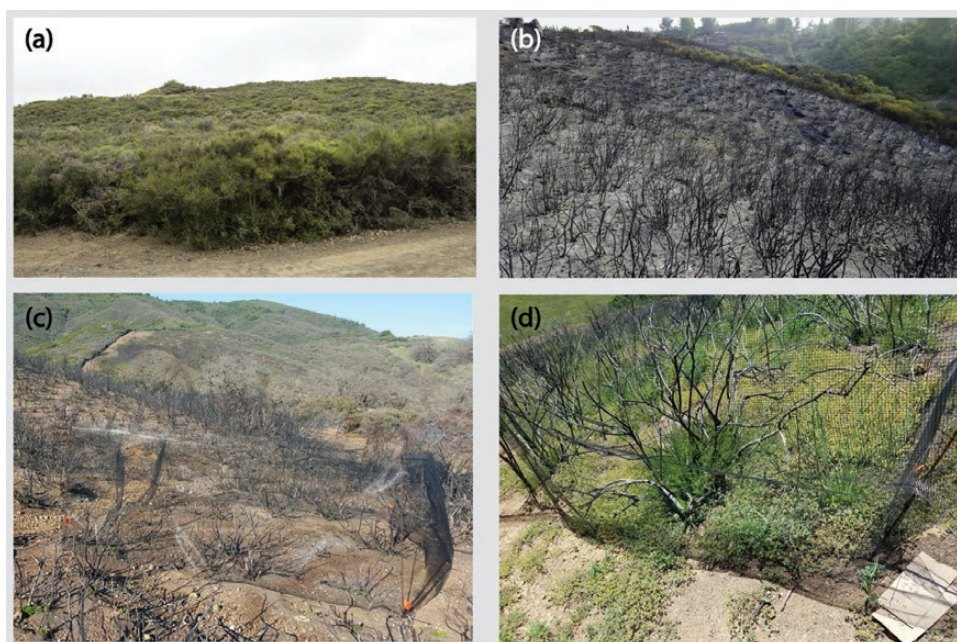
Herbivore exclosures were placed in the footprint of four prescribed fires: two conducted in spring (April 21) and two in fall (October 23) 2016. Burn areas ranged from 1 to 2 ha in extent. The California Department of Forestry and Fire Protection (CalFire) set upslope prescribed fires (headfire) using a combination of drip torches with a gasoline/diesel mixture and mounted terra torches with gelled aviation fuel. To prevent fire escape, the borders of each site were cleared by bulldozer and the uphill side of the fire was backburned. The elevation of these sites ranges from 556 to 907 m. All except for one were south-facing. Chaparral prescribed fires conducted outside of the typical summer burn window (June–November; after spring drying and before first fall rain) are typically much less intense than wildfires (Stephens *et al.* 2008) and may favor a different suite of species (Wilkin *et al.*

2017). Before fire, shrub vegetation was thick and continuous (Fig. 2a); after fire, almost all living aboveground plant biomass was consumed, leaving only woody skeletons (Fig. 2b). Soils at these sites are derived from weathered sandstone and shale and are classified as moderately acidic sandy loam with a gravel component (Soil Survey Staff 2020).

Several species of small herbivorous mammals have been observed in the early post-fire chaparral at Hopland, including brush rabbit (*Sylvilagus bachmani*), black-tailed jackrabbit (*Lepus californicus*), Botta's pocket gopher (*Thomomys bottae*), Townsend chipmunk (*Neotamias townsendii*), Heermann's kangaroo rat (*Dipodomys heermanni*), deer mice (*Peromyscus* spp.) and the dusky footed woodrat (*Neotoma fuscipes*). Black-tailed deer (*Odocoileus hemionus*) are also common (Longhurst 1978). The post-fire herbaceous assemblage is specious and functionally diverse (Supplementary Table S1).

### Experimental design

We placed six experimental blocks within each of the four prescribed fire sites, with a minimum of 20 m between blocks. Each of the blocks in a site included an open control plot, paired with an herbivore-exclusion treatment (installed December 2016–January 2017). The herbivore exclosures were rectangular plots of 3 m × 4 m staked at the corners with rebar stakes. The plots in a block were arranged side by side along the



**Figure 2:** Photos of vegetation and setup, including: (a) mature pre-burn chaparral (Site T, 21 April 2016), (b) immediate post-fire chaparral (Site K, 23 October 2016), (c) herbivore exclosure before herb growth (Site L, 28 March 2017) and (d) herbivore exclosure after 1 year of growth (Site J, 9 May 2017).

hillside contour so that no plot within a block was uphill of another. The fencing material was a heavy UV-resistant plastic mesh with 0.64 cm openings. Fences were approximately 130 cm high. The mesh was installed flush to the ground but was not buried (Fig. 2c and d). Thus, some small mammals may have been able to burrow underneath the fence. However, deer should not have been able to enter and small mammal entry was likely substantially reduced. We did not find deer droppings inside of exclosures and we monitored regularly for damage to the fences.

To evaluate potential artifacts due to cage installation and presence, we included half-cage controls in three of the six experimental blocks per site. Half-cage controls consisted of rebar stakes in the four corners of the plot as well as fencing on the upper and lower edge. This was intended to control for fencing effects, including sediment capture, shading and dew collection while allowing for mammal entry. These half cages were monitored for the first season only.

Plots were intentionally placed in regions dominated by chamise (*A. fasciculatum*) chaparral. Regions with apparent N-fixing shrubs (*Ceanothus* spp. and *Pickeringia montana*) were rejected. After 1 year of the experiment, some plots contained an unexpectedly high component of recovering non-chamise shrubs, including *Quercus* spp. These blocks were dropped from the analyses (see statistical analysis). Vegetation sampling (described below) confirmed dominance by *Adenostoma*, with standing dry biomass increasing from  $107 \pm 16 \text{ g m}^{-2}$  in 2017 to  $202 \pm 19 \text{ g m}^{-2}$  in 2018 ( $F = 22.11$ ,  $P < 0.001$ ). *Adenostoma* biomass varied neither by Exclosure ( $F = 0.01$ ,  $P = 0.916$ ) nor Exclosure  $\times$  Year ( $F < 0.01$ ,  $P = 0.949$ ). No other shrub exceeded average standing biomass of  $4.4 \text{ g m}^{-2}$  in either year.

## Vegetation sampling

We monitored vegetation at peak biomass at the end of each growing season (June–July 2017 and May 2018). Because the 19500 ha River Fire (27 July–13 August 2018, River Fire was part of the Mendocino Complex Fire) burned the experimental plots, it was not possible to conduct a third year of vegetation sampling. We estimated biomass using the point intercept method with a pin-frame sampler. In each  $3 \text{ m} \times 4 \text{ m}$  plot, we subsampled three  $0.5 \text{ m} \times 0.5 \text{ m}$  quadrats, one in the center of the plot, one in the top left and one in the bottom right. The top and bottom plots were always placed 0.5 m inside of the plot, measured from the top and the side. The pin-frame

quadrat held 20 pins vertically over the shrub and herbaceous vegetation growing in the plot. The pins were placed so that five pins were in each quarter of the quadrat. We counted how many times each hanging pin hit each plant species.

We used the total number of pin hits as a proxy for plant dry biomass (Jonasson 1988). To relate this value to standing biomass, we collected and dried plants that were sampled using this method. We sampled 32 quadrats using the pin frame, principally outside of the experimental plots to minimize vegetation disturbance. We then clipped aboveground growth of plants and sorted it by plant functional group: shrubs, N-fixing herbs and non-N-fixing herbs. In the laboratory, we thoroughly rinsed these herbs in water to remove all soil and dried them at  $65 \text{ }^\circ\text{C}$  for at least 48 h before weighing their dry biomass. We estimated equations relating pin hits in each of these groups to biomass using a linear regression for each group. In order to avoid regression estimates of negative biomass values, we set the  $y$ -intercept to zero:

$$\text{biomass} = 0 + \beta_1 \times \text{hits} \quad (1)$$

where biomass is dry mass of plants ( $\text{g m}^{-2}$ ) and hits is the total number of pit hits per group in a plot (Supplementary Fig. S1). For all  $\beta$  estimates,  $r^2$  was greater than 0.9:  $\beta_{\text{shrubs}} = 4.56$  ( $R^2 = 0.96$ ),  $\beta_{\text{non-N-fixer}} = 3.97$  ( $R^2 = 0.91$ ) and  $\beta_{\text{N-fixers}} = 3.78$  ( $R^2 = 0.97$ ).

## Soil physical and chemical properties

### Soil collection

For all soil measurements in this project, we collected soils to a depth of 10 cm to capture the dynamics most likely driven by herbaceous growth and litter decay. Throughout the 2017 and 2018 spring growing seasons, we collected soil samples monthly from each plot (March–June 2017 and February–May 2018). Generally, we collected four 2.5 cm diameter, 10 cm deep soil cores at each plot, and pooled the soil to minimize plot-level variation. The exception was a collection in late January 2017, during which we pooled soil from cores across the whole block to capture seasonal conditions and block-level characteristics, without respect to a specific treatment. At each sampling, the soil cores to be pooled were first collected into a common plastic bag, and then immediately homogenized by gently massaging the bag contents to break up large aggregates and passing the soils through a 4 mm sieve. We refrigerated soils at  $4 \text{ }^\circ\text{C}$  soon after

collection and processed soils for analysis (described below) within 48 h.

We also report results from soil collected shortly after the fall prescribed fires (28 November 2016) as part of a separate, earlier sampling effort to characterize the effect of prescribed fire on soil mineral N concentrations and mineralization rates at these sites. The results are included here simply to support the assumption that post-fire soils are elevated in mineral N. These soils were collected at 15 cm depth (described below) from each of the burned sites and paired immediately adjacent non-burned chaparral stands (not pictured in Fig. 1). Any visible organic debris, including limited woody litter, was gently brushed away before sampling so that the A horizon could be collected. While the results from these samples cannot be directly compared with shallower soil samples (10 cm) collected throughout the enclosure experiment (2017–2018), they demonstrate key differences between burned and non-burned sites. There were 77 burned points across four sites (Fig. 1, J = 20, T = 14, K = 21, L = 22) and 32 immediately adjacent non-burned points (J = 9, T = 9, K = 7, L = 7).

#### **Bulk density**

We collected separate soil cores for bulk density analysis during late January 2017 block-level sampling. At each block, we collected one soil core (5 cm diameter × 10 cm deep) for bulk density analysis. We carefully scraped off soil from the bottom of the core to ensure that the exact volume of the core was collected. In the laboratory, we sieved soil cores to 4 mm and used a water displacement method to measure the volume of the removed rocks. The total volume of soil was  $\text{volume}_{\text{core}} - \text{volume}_{\text{rocks}}$ . The remaining soil was dried at 105 °C for 72 h and weighed. Bulk density was the dry mass of soil divided by the total volume.

#### **Total soil C and N**

Total soil carbon (TC) and nitrogen (TN) were determined from air-dried soils. First, we tested soils for the presence of inorganic C. We gently ground soil samples and moistened with one drop of deionized water. We added one drop of 4 M HCl. As there was no effervescence, we determined that no significant inorganic C was present. Thus, TC is an appropriate measure of organic C. We determined TC and TN on a Flash 2000 Elemental Analyzer (Thermo Scientific, Germany). We analyzed duplicate samples and repeated the analysis of soils for which duplicates differed by

more than 10%. We performed this analysis on soils collected from every plot at initial (March 2017) and final (May 2018) sampling times. Although the initial date was actually shortly after enclosure installation, this was the earliest point at which plot-level soils were collected and thus the best indication of early conditions available. Final soil samples were collected at the end of two growing seasons in May 2018.

#### **Soil mineral nitrogen**

We extracted soils in 2 M KCl for analysis of mineral N:  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . We mixed 10.0 g soil samples with 40 mL of 2 M KCl in an acid-washed 50 mL centrifuge tube. Samples were shaken for 1 h at 200 rpm. The soil slurry was then gravity filtered through KCl soaked Whatman #1 filters and acid-washed funnels into separate new clean centrifuge tubes. Samples were stored at –20 °C until analysis.

Soil gravimetric percent moisture was determined for every collected soil. We measured 10.0 g subsamples into metal tins and dried the soils at 105 °C for 24 h and then weighed the dry soil. Soil gravimetric moisture content was calculated as  $(\text{mass}_{\text{wet}} - \text{mass}_{\text{dry}}) / \text{mass}_{\text{dry}}$ .

#### **Nitrogen mineralization**

We determined the rate of soil net N mineralization and nitrification using a laboratory dark incubation. This setup was combined with a soil C mineralization (respiration) incubation described below. After measuring soil subsamples for mineral N at collection (above), soils were incubated in 495 mL glass mason jars. A subsample of approximately 30 g of wet soil was gently tamped down into a tared 30 mL medicine cup and weighed. This ensured that soil samples always had the same volume, allowing for clear calculations of headspace gas exchange, while keeping soil bulk density in the jars consistent between samples at any given time point. The medicine cup was placed carefully into the base of the jar, which was sealed and allowed to incubate in the dark for 1 week. After the 7 days, we removed each incubated soil sample from its jar, mixed it thoroughly, then measured out a 10.0 g subsample for a 2 M KCl extraction. This subsample was extracted using the same procedure described above. The resulting extract samples were analyzed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations using the microplate method (Wu *et al.* 2016) (2017 samples) or Lachat Flow Injection Analyzer (2018 samples). A subset of samples from each year was analyzed using both methods to confirm correspondence between the two analysis techniques.

### Carbon mineralization (respiration)

During the most active months of the second year of soil collection (March–May 2018), we used the soil mineral N incubations to also collect data on soil respiration over a 48 h period. Samples were collected in plastic syringes with O-ring seals and medical-grade stopcocks. We took three initial 5 mL samples from ambient air above the jars before sealing them with gas-tight lids fitted with septa ( $T_1$ ). After a 48 h dark incubation, we injected 5 mL of CO<sub>2</sub>-free air into each jar, pumped the syringe slowly to mix the headspace, and then withdrew a 5 mL sample for analysis ( $T_2$ ). Samples were analyzed on a FoxBox IRMS Flow Analyzer. We calculated the total headspace C at  $T_1$  and  $T_2$ , adjusting for the temperature and pressure in the jars and the small dilution from injecting CO<sub>2</sub>-free air at  $T_2$ . We calculated respiration as  $(T_2T_1)/T$  (days). Data are reported as  $\mu\text{g CO}_2\text{-C g}^{-1}$  dry soil  $\text{d}^{-1}$ .

### Statistical analysis

We fit all models in *R* (R Core Team 2018) with the package *lme4* (Bates et al. 2015), using a restricted maximum likelihood estimation procedure (REML) and performed a Type III ANOVA using the *lmerTest* package (Kuznetsova et al. 2017). Planned contrasts on the model were made using the *emmeans* package (Lenth 2018). The effects of treatments on each group at each date were determined by *a priori* planned contrasts of estimated marginal means, with no *p*-penalization procedure.

### Half-cage control

At each site, three blocks contained a half-cage control in addition to the fully open control and an enclosure. At both sites J and L (Fig. 1), one of the half-cages was excluded from the analysis due to emergent non-*Adenostoma* shrubs, leaving 10 replicate blocks (2 plots/block) across 4 sites with varying numbers of blocks ( $J = 2$ ,  $T = 3$ ,  $K = 3$ ,  $L = 2$ ). We fit a linear mixed-effects model with Enclosure (half-cage vs. open control) as a fixed factor and Site, Block and Plot as random effects. We fit separate models for each response variable: total shrubs, total herbs and two herb functional groups. There was no difference between open and half-cage controls at the conclusion of the 2017 growing season for any of the herb functional groups tested: total shrubs ( $F = 0.438$ ,  $P = 0.518$ ), total herbs ( $F = 0.04$ ,  $P = 0.846$ ), N-fixing ( $F = 1.25$ ,  $P = 0.291$ ) or non-N-fixing ( $F = 0.96$ ,  $P = 0.354$ ) herbs. We also tested the response variable

gravimetric soil moisture, including Month as an interactive fixed factor. There was an effect of Month on percent moisture ( $F = 275.35$ ,  $P < 0.001$ ), but no effect of Enclosure treatment ( $F = 1.14$ ,  $P = 0.314$ ) or Enclosure  $\times$  Month ( $F = 0.98$ ,  $P = 0.410$ ). Based on a lack of half-cage effect, we stopped maintaining half-cage controls after 2017 and dropped them from all subsequent data/sample collection and analysis.

### Effect of herbivore enclosures

After excluding half-cage plots, we fit a linear mixed-effect model to evaluate the main and interactive effects of Enclosure (Exc) and Date on all measured variables in the experiment:

$$Y = \text{Exc} + \text{Date} + \text{Exc} \times \text{Date} + \text{Random}(\text{Site} + \text{Block} + \text{Plot}) \quad (2)$$

We excluded blocks from the analysis that had emergent non-*Adenostoma* shrubs, leaving 17 replicate blocks (3 plots/block) across 4 sites with varying numbers of blocks ( $J = 5$ ,  $T = 5$ ,  $K = 3$ ,  $L = 4$ ). The response variables ( $Y$ ) for vegetation sampling were square-root-transformed biomass for total herbs and functional groups N-fixers and non-N-fixers. These groups were further divided into subgroups based on life history (annual vs. perennial) and origin (native vs. non-native). Response variables for soil were gravimetric percent moisture, total C, total N, C:N ratio, ammonium-N, nitrate-N or total mineral N and carbon mineralization (respiration). This publication addresses the impact of herbivore enclosures across all sites, regardless of the date burning took place at each site (spring or fall prescribed fire); thus, Site was treated as a simple random effect. Despite potential site differences in measured variables, treating Site as a fixed effect would neither yield useful inference nor be statistically valid. The blocks were placed at each site in such a way to take advantage of continuous regions of *Adenostoma*-dominated chaparral, not to representatively sample the extent of the site *per se*. Fitting with the definition of random variables, we selected sample blocks from a larger sample of *Adenostoma* regions, without *a priori* hypotheses of site differences.

### Carbon mineralization (respiration)

After Enclosure was determined not to be a significant predictor of soil heterotrophic respiration, we performed a follow-up analysis to test the hypothesis that soil gravimetric percent moisture (Wat) influences respiration rates (Resp):

$$\text{Resp} = \text{Wat} + \text{Date} + \text{Wat} \times \text{Date} + \text{Random}(\text{Site} + \text{Block} + \text{Plot}) \quad (3)$$

The percent variance explained by the model variables was determined using a modified marginal and conditional  $R^2$  appropriate for mixed-effects models, calculated with the *MuMIn* package in R (Nakagawa and Schielzeth 2013). For all analyses, we considered  $P < 0.1$  as the threshold for a significant effect, a typical threshold for marginal significance that avoids the unnecessary risk of Type II error in ecological models with relatively low sample sizes (Hurlbert and Lombardi 2009).

## RESULTS

In November 2016, soils were sampled across sites (15 cm depth) to characterize differences between non-burned and recently burned chaparral. Burned chaparral soil was higher in  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and net nitrification, but not net N mineralization, which was approximately equal in burned and non-burned sites (Table 1). Block-level pretreatment (late January 2017) bulk density values ranged from 1.0 to 1.5  $\text{Mg m}^{-3}$  in the blocks used. There were wide ranges in soil chemical characteristics pretreatment: TC (16.4–40.9  $\text{mg g}^{-1}$ ), TN (1.2–2.5  $\text{mg g}^{-1}$ ),  $\text{NH}_4^+\text{-N}$  (2.9–23.9  $\mu\text{g g}^{-1}$ ) and  $\text{NO}_3^-\text{-N}$  (0.5–2.6  $\mu\text{g g}^{-1}$ ).

For all variables, we analyzed the interactive effects of Exclosure and Date using linear mixed-effects models to assess treatment effects (Supplementary Table S2; Fig. S2). Planned contrasts are indicated on the graphs or discussed in the text.

### Vegetation monitoring

We measured the response of herbaceous biomass to the exclusion of herbivores and time after fire (annual sampling of peak biomass in 2017 and

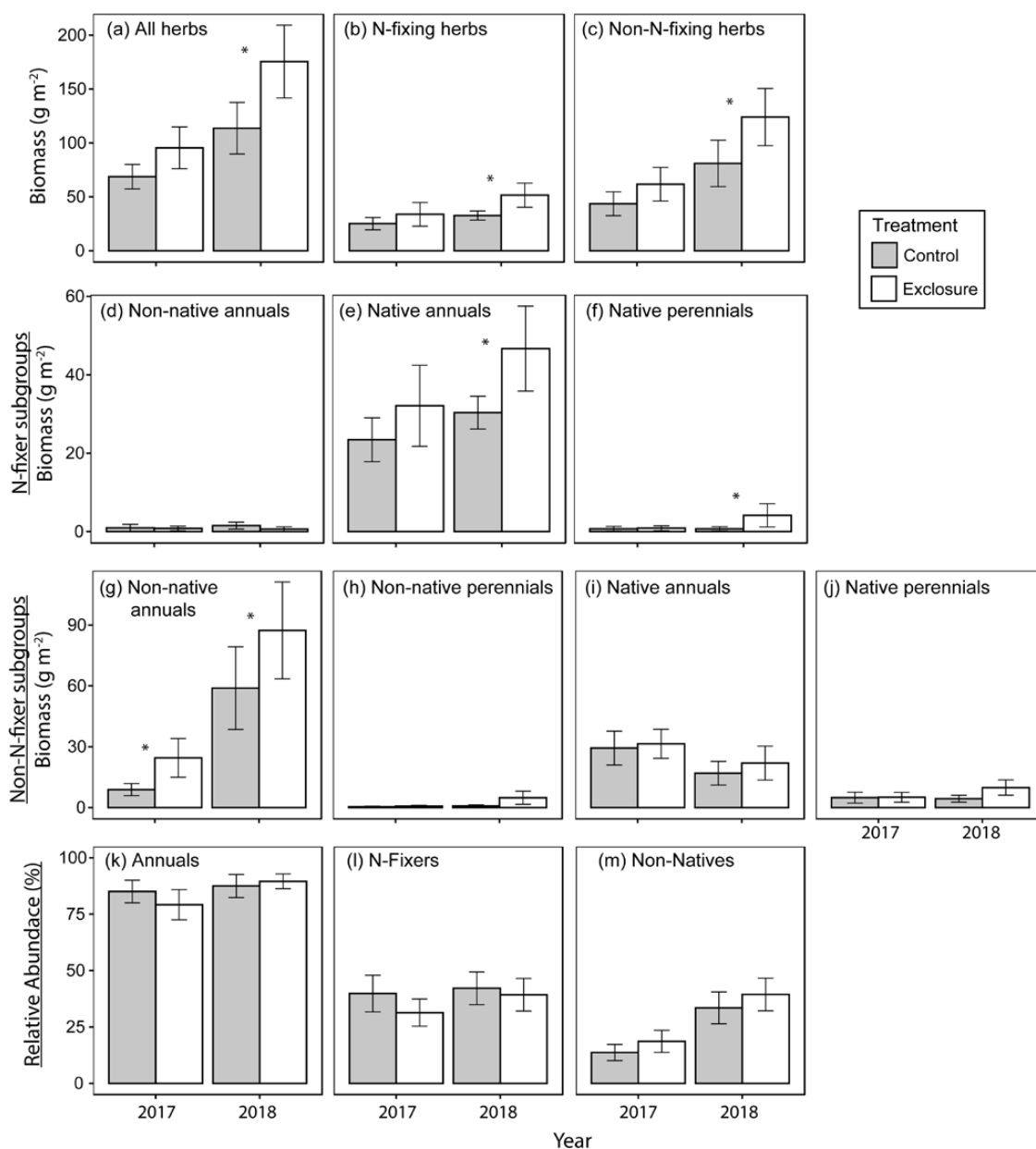
2018). Herbivore exclosures significantly increased the biomass of total herbs, N-fixers and non-N-fixers, but only by 2018 (Fig. 3a–c). All three of these groups also increased in biomass from 2017 to 2018, in both exclosures and controls ( $P < 0.05$ ). Of the N-fixers subgroups (Fig. 3d–f), native annuals were the most abundant and were comprised almost completely of two closely related species, *Acmison brachycarpus* and *Acmispon parviflorus*. Native annual N-fixers were significantly increased by herbivore exclosures in 2018 only (Fig. 3e). The one native perennial N-fixer (*Acmispon glaber*) was comparatively low in abundance but increased significantly in exclosures in 2018 (Fig. 3f). Native annuals increased between 2017 and 2018 in both treatments ( $P < 0.1$ ), while native perennials increased only in the Exclosure treatment ( $P < 0.05$ ). The one non-native annual N-fixer (*Trifolium hirtum*) was not significantly affected by Year or Exclosure (Fig. 3d).

The non-N-fixers were further divided into four groups based on life history and origin (Fig. 3g–j). Of these groups, only non-native annuals were impacted by Exclosure, with higher standing biomass in exclosures than controls in both 2017 and 2018 (Fig. 3g). The species most enhanced in herbivore exclosures were the common invasive grasses *Bromus diandrus*, *Bromus madritensis* ssp. *rubens*, *Festuca myuros* and *Gastridium phleoides*, along with common invasive forbs *Centaurea melitensis* and *Torilis arvensis* (Supplementary Fig. S3). Non-native annual non-N-fixers increased between years 2017 and 2018 in both treatments ( $P < 0.001$ ), mainly comprising increases in *Aira caryophyllea*, *B. diandrus*, *G. phleoides*, *F. myuros*, *Hypochaeris glabra* and *Logfia gallica*. One species from this group, *L. gallica*, was only half as abundant in

**Table 1:** Pre-treatment soil sampling (mean  $\pm$  S.E.M). (A) In November 2016, soils were sampled to 15 cm depth across whole burned sites and adjacent non-burned chaparral. Sites were burned in April or October 2016. **Bold** indicates significant differences between burned and non-burned sites in a Type III ANOVA on linear mixed models with a random site ( $P \leq 0.10$ ). (B) In January 2017, soils were sampled to 10 cm depth and pooled across the experimental blocks used in the exclosure experiment.

	Burned	Non-Burned		Burned
(A) November 2016			(B) January 2017	
$\text{NH}_4^+\text{-N}$ ( $\mu\text{g g}^{-1}$ )	<b>5.67 <math>\pm</math> 0.56</b>	<b>2.47 <math>\pm</math> 0.37</b>	$\text{NH}_4^+\text{-N}$ ( $\mu\text{g g}^{-1}$ )	9.01 $\pm$ 1.11
$\text{NO}_3^-\text{-N}$ ( $\mu\text{g g}^{-1}$ )	<b>1.69 <math>\pm</math> 0.36</b>	<b>0.36 <math>\pm</math> 0.09</b>	$\text{NO}_3^-\text{-N}$ ( $\mu\text{g g}^{-1}$ )	1.35 $\pm$ 0.17
Net N Mineralization ( $\mu\text{g g}^{-1} \text{wk}^{-1}$ )	1.06 $\pm$ 0.37	0.78 $\pm$ 0.27	TN	1.8 $\pm$ 0.1
Net Nitrification ( $\mu\text{g g}^{-1} \text{wk}^{-1}$ )	<b>1.51 <math>\pm</math> 0.28</b>	<b>0.06 <math>\pm</math> 0.14</b>	TC	30.0 $\pm$ 1.2





**Figure 3:** Response of herb functional groups to herbivore exclusions. (a–c) Broad functional groups, (d–f) N-fixer subgroups, (g–j) non-N-fixer subgroups and (k–m) relative biomass abundance of each herb functional group compared with total herb biomass. Error bars represent S.E.M. \*Significant effect of exclusion ( $P \leq 0.10$ ) in planned contrasts of treatment within year.

exclusions as open controls. Native annual non-N-fixers decreased between 2017 and 2018 ( $P < 0.1$ , Fig. 3i); fire followers *Emmenanthe penduliflora* and *Gilia capitata* were abundant in 2017 and almost totally disappeared by 2018. A few individual native annual non-N-fixers were negatively impacted by exclusions (*Calandrinia breweri*, *Logfia filaginoides* and *Navarretia melitta*), while a few were enhanced (*Cryptantha muricata* and *Galium californicum*). The perennial non-N-fixers were not significantly affected by Exclusion or Year (Fig. 3h and j).

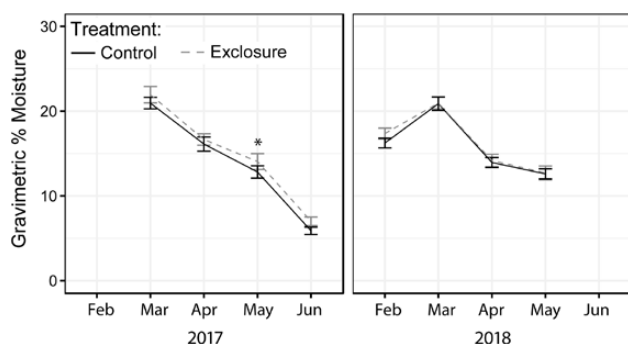
We analyzed the relative abundance of three broad herb functional categories (life history, origin, N-fixation) in response to herbivore exclusions and time since fire (Fig. 3k–m). Most herb biomass was from annuals, at over 79%–90% for all treatments and time points. Between 2017 and 2018, there was an increase in annual relative abundance in exclusions only ( $P < 0.05$ ). N-fixers comprised on average 38.2% of herb biomass and there was no statistically significant effect of Exclusion or Year on their relative abundance. Non-natives were relatively low in

abundance but were significantly increased by both time since fire and herbivore exclusions: non-native herb relative abundance was about 5 percentage points higher in exclusions than in open controls ( $P < 0.1$ ), and there were no significant differences in individual years based on planned contrasts. Non-native relative biomass also increased significantly from 2017 (16.2%) to 2018 (36.4%,  $P < 0.001$ ).

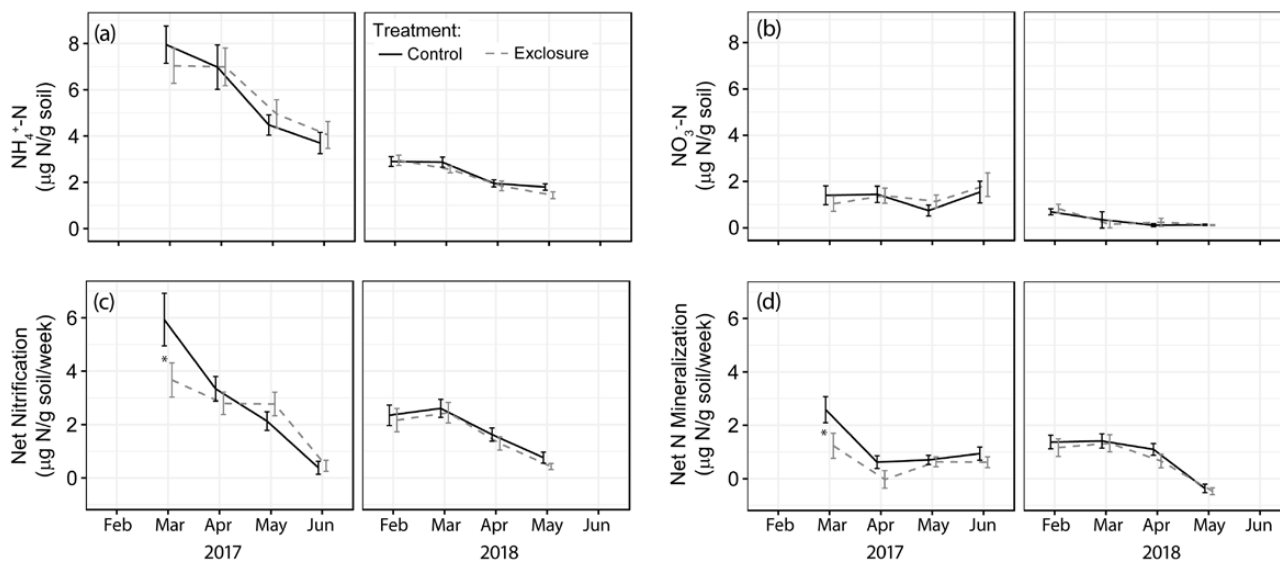
## Soil physical and chemical properties

### Gravimetric percent moisture

The gravimetric percent moisture of the soil samples was measured at each time point. Across all sites and dates, we found a significant effect of Exclusion treatment on gravimetric moisture (Fig. 4). Planned contrasts demonstrate that exclusions were only slightly moister than controls



**Figure 4:** Gravimetric soil moisture over the course of the experiment. Error bars represent S.E.M. \*Significant effect of Exclusion ( $P \leq 0.10$ ) in planned contrasts of treatment within date.



**Figure 5:** Soil concentration of (a)  $\text{NH}_4^+\text{-N}$ , (b)  $\text{NO}_3^-\text{-N}$ , (c) net nitrification, and (d) net N mineralization throughout the experiment. Error bars represent S.E.M. \*Significant effect of exclusion ( $P \leq 0.10$ ) in planned contrasts of treatment within date.

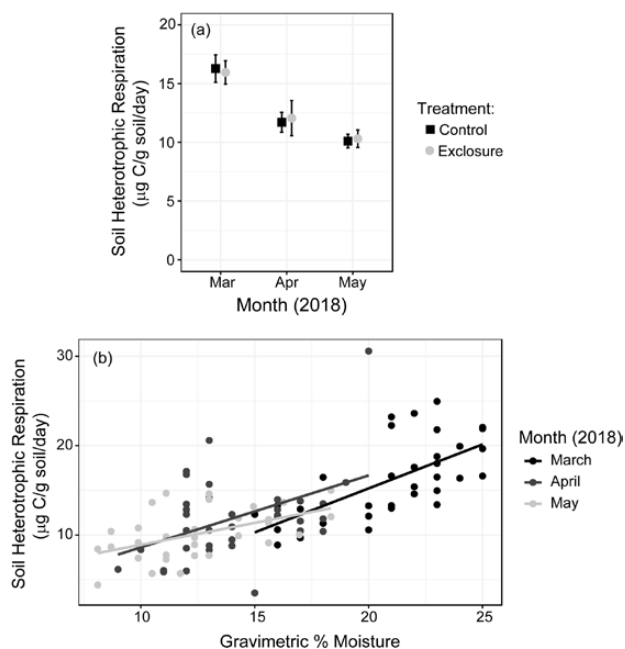
in May 2017 ( $P = 0.084$ ), by a margin of 1.3 percentage points (14.1% vs. 12.8% moisture). Soil moisture varied significantly by Date, ranging from 5.9% to 21.9%.

### Soil mineral nitrogen and mineralization rates

We measured plot-level soil mineral N pools and rates of net N mineralization and nitrification monthly during the 2017 and 2018 spring seasons (Fig. 5a and b). There was no effect of Exclusion on  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_3^-\text{-N}$ . When the monthly values were averaged across each year, all mineral N pools were significantly lower in 2018 than in 2017 ( $P < 0.001$ ). For rates of net N mineralization and nitrification, there was an effect of Exclusion in the first month of sampling only (Fig. 5c and d). Nitrification rates were significantly higher in 2017 than 2018 ( $P < 0.001$ ) and were highly correlated with soil ammonium concentrations ( $F = 31.93$ ,  $P < 0.001$ ), Date ( $F = 1.77$ ,  $P = 0.095$ ) and their interaction ( $F = 4.56$ ,  $P < 0.001$ ). The modified marginal  $R^2$  for Ammonium  $\times$  Date alone was 0.50, and the conditional  $R^2$  (including random Site, Block and Plot) was 0.65. Overall, there was no significant difference in net N mineralization between years 2017 and 2018 ( $P = 0.340$ ).

### Carbon mineralization (respiration)

We measured soil respiration in a lab incubation of soils collected during 3 months of 2018, the second season after fire. There was no effect of Exclusion treatment on respiration; only Month was a significant predictor



**Figure 6:** (a) Monthly soil respiration in the second growing season post-fire and (b) soil respiration as a function of gravimetric percent moisture. Error bars represent S.E.M.

(Fig. 6a,  $P < 0.001$ ), with respiration values decreasing from March through May. We analyzed respiration as a function of gravimetric soil moisture and Month (Fig. 6b). When Percent moisture was included in the model, Month was no longer a significant predictor, either as a main effect ( $F = 0.89$ ,  $P = 0.417$ ) or interaction ( $F = 0.58$ ,  $P = 0.563$ ), but Percent moisture was highly significant ( $F = 100.72$ ,  $P < 0.001$ ). The modified marginal  $R^2$  for Percent moisture alone was 0.51, and the conditional  $R^2$  (including random Site, Block and Plot) was 0.75. Including month in the models improved the marginal  $R^2$  only slightly to 0.54 and the conditional  $R^2$  to 0.83.

#### Total soil C and N

Soil TC and TN were measured at the earliest plot-level sampling point (March 2017) and the last (May 2018) (Table 2). At neither time point were there significant differences between exclosures and open controls in bulk soil TC and TN. Between March 2017 and May 2018, exclosure treatments decreased significantly ( $P < 0.05$ ), but very slightly, in bulk soil TC ( $-4.0 \text{ mg g}^{-1}$ ) and TN ( $-0.22 \text{ mg g}^{-1}$ ), but open control plots did not.

## DISCUSSION

In this study, we established herbivore exclosures in post-fire chaparral to investigate the effect of mammalian

**Table 2:** Soil TC and TN measured at plot level at the beginning (March 2017) and end (May 2018) of the experiment (mean  $\pm$  S.E.M.)

Treatment	C ( $\text{mg g}^{-1}$ )		N ( $\text{mg g}^{-1}$ )	
	2017	2018	2017	2018
Control	$26.8 \pm 1.7$	$26.8 \pm 1.1$	$1.6 \pm 0.1$	$1.6 \pm 0.1$
Exclosure	$28.5 \pm 1.5$	$24.4 \pm 1.6$	$1.7 \pm 0.1$	$1.5 \pm 0.1$

herbivores on herb assemblage composition and nutrient cycling. We hypothesized that the activity of mammalian herbivores would accelerate N cycling, due to relatively high post-fire soil N availability. We hypothesized that exclosures would impact post-fire chaparral herb functional group composition, predicting that (i) due to high soil N availability, herbivores would not demonstrate a strong preference for N-fixing herbs and (ii) that herbivores would limit the growth of non-native herb species.

#### Post-fire N availability

Our post-fire sampling confirmed that the burned sites were higher than non-burned sites in mineral N, especially  $\text{NH}_4^+$  (Table 1), the form typically deposited on soils as a result of burning (e.g. Christensen 1973; Debano and Conrad 1978). We saw a steady drop in  $\text{NH}_4^+$ -N throughout the experiment (Fig. 5a). The steady decrease in  $\text{NH}_4^+$ -N may reflect the time since fire, as demonstrated by others (Fenn *et al.* 1993), although comparison to a non-burned control site throughout 2017 and 2018 would have been needed to support this idea. A key mechanism for rapid loss of ammonium post-fire is nitrification, the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ , which is mobile and vulnerable to leaching (Christensen 1973). As such,  $\text{NH}_4^+$  substrate availability is known to be a key driver of post-fire nitrification in chaparral (Fenn *et al.* 1993; Hanan *et al.* 2016b). Our results support this relationship, with Date and soil  $\text{NH}_4^+$ -N concentrations explaining 50% of the variability in our measured nitrification rates. The rate of net N mineralization, the ongoing supply of mineral N from decaying organic material, did not differ between years (Fig. 5d) or between burned and non-burned plots (Table 1). Thus, differences in available N between 2017 and 2018 or between burned and non-burned plots likely followed from the direct deposition of  $\text{NH}_4^+$  on soils during fire.

#### Herbivore vegetation effects

We hypothesized that the exclusion of herbivores would impact the abundance and composition

of post-fire herbs. We predicted that, due to high available soil N after fire, mammalian herbivores would not preferentially consume N-fixers. In support of this prediction, we found no evidence of the disproportionate consumption of N-fixers, with the fraction of N-fixer biomass not significantly different between treatments and years (Fig. 3l). However, we found that herbivores did reduce overall herb biomass, particularly in year 2, with consumption acutely concentrated on a few functional groups (Fig. 3). The most abundant herb group, non-native non-N-fixing annuals, was also the most affected by herbivory. Brush rabbits (*S. bachmani*) are important herbivores in the chaparral understory, and they typically rely on edible grasses, including *Bromus* spp. (Chapman 1974). Curiously, the biomass of two clovers (*Trifolium* spp.) was not affected by herbivore exclusions, despite members of this genus being a preferred food source for rabbits (Chapman 1974). It is also possible that plant defensive compounds decreased the overall intensity of herbivory on N-fixers; plant defensive chemicals are often N rich, so N-fixing plants might be generally better defended (Vitousek and Field 1999).

Multiple investigations have demonstrated that herbivore effects on plant abundance are not significant in post-fire chaparral (e.g. Tyler 1995), plausibly due to displacement or death of herbivores (Christensen and Muller 1975a). The present study contradicts those findings, with herbivore effects being particularly pronounced in the second year after fire. The relatively small size of these prescribed fires could be responsible for our finding of a strong herbivore impact on post-fire herbs. Although some animal death occurred in these fires (L. Hendricks-Franco, personal communication), the small 1–2 ha burns could have been quickly recolonized by animals from nearby non-burned patches. Mills (1986) found significant effects of herbivore exclusions on shrub seedling assemblages in a small 2.5 ha chaparral prescribed fire, also suggesting that entry of mammals from nearby non-burned could have been responsible. The leaves of resprouting *Adenostoma* are typically enriched in N during the first two post-fire years (Rundel and Parsons 1980), potentially attracting grazers to patches of recently burned chaparral. We found no difference in *Adenostoma* shrub biomass between Exclusion treatments in either year. This is consistent with a similar investigation at the same reserve (Potts *et al.* 2010), which found no Exclusion effect 2 years after fire; however, they did see an effect by year

3. The River Fire in the summer of 2018 burned our experimental plots two seasons after the 2016 prescribed fires. Thus, it was not possible to conduct a third year of vegetation sampling for comparison to results from Potts *et al.* (2010).

A few herbaceous species were negatively impacted by Exclusion treatment: *L. gallica* (non-native non-N-fixing annual) and three native non-N-fixing annuals: *C. breweri*, *L. filaginoides* and *N. melitta*. A likely explanation for this effect is that these species were outcompeted by other herb species that performed especially well in exclusions. Notably, the species negatively impacted by exclusions were low-growing plants, which could have been easily covered by a larger competitor. In particular, *N. melitta* is likely resistant to herbivory and thus would not have benefited directly from exclusions. The plant is sticky, has a strong odor, and is defended by spiny leaves, sepals and bracts. Although we did not detect modified competitive relationships on the level of the functional groups tested, these species-specific results may demonstrate herbivore-mediated indirect competitive effects on the species level.

### Non-native herb invasion

Historically, chaparral has been considered resistant to invasion by non-native herbs, despite its typical proximity to grasslands dominated by invasive grasses and forbs. Southern California chaparral is increasingly falling victim to invasion by non-native herbs from grasslands, with one estimate finding that 34% of historical chaparral vegetation is now covered with herbs (Park *et al.* 2018). The drivers of herbaceous invasion of chaparral remain uncertain, but Park and Jenerette (2019) suggest that mammalian herbivores may consume non-native herbs and limit their success in chaparral. In the present study in northern California, non-native herbs, including grasses, were abundant; we found non-natives representing over a third of the herbaceous biomass, consistent with a similar study in the same reserve, 3 years after prescribed fires (Potts and Stephens 2009). While the present study did demonstrate the role of mammalian herbivores in limiting the biomass of non-native herbs, mammalian herbivory was not sufficient to stop the increase in non-natives in the years after fire (Fig. 3g and m).

Several factors other than herbivory exclusion may contribute to the high abundance of non-native herbs in conditions like those found in this experiment. Chaparral prescribed fires tend to burn at a lower intensity than wildfires (Stephens *et al.*

2008), which may lead to lower mortality rates for shallow non-native annual seedbanks (Ditomaso *et al.* 2006) and lower germination rates for deeply buried fire-germinated native shrubs and forb seedlings (Christensen and Muller 1975b). Furthermore, conditions of high available N, such as those found after fire, tend to favor non-native grasses and disfavor natives adapted to low-N conditions (Fenn *et al.* 2010; Vourelitis 2017). The influence of high-N conditions on plant invasion is particularly relevant in southern California chaparral, where atmospheric N-deposition exceeds critical loads, substantially impacting N cycling and plant composition in otherwise N-limited chaparral. Our northern California site does not currently experience heavy N-deposition (Fenn *et al.* 2010); however, any hypothetical future regional urbanization could increase local N-deposition and further exacerbate non-native grass and forb invasion, even in mature chaparral stands.

This study took place in a noteworthy hydroclimatic period. The first year of this study (2016–17) was uncommonly rainy, while the four preceding years were characterized by a historic drought (Hanak *et al.* 2016). Regrowth of *Adenostoma* could have been compromised by the preceding drought, with implications for nutrient cycling. Despite some resistance to cavitation, *Adenostoma* can die of acute drought-induced xylem dysfunction (Paddock *et al.* 2013). For surviving individuals, carbohydrate stores can become depleted during extended droughts, as shrubs decrease stomatal conductance to conserve water, also reducing photosynthesis (Anderegg *et al.* 2012). Resprouting shrubs, like *Adenostoma*, further deplete carbohydrates to support early post-fire regrowth. These compounding disadvantages may have decreased shrub vitality and allowed for a stronger herbaceous response, particularly from non-natives. The synergy of drought and N fertilization, whether through atmospheric or ash deposition, can promote herbaceous non-natives and increase mortality of native forbs and shrubs (Valliere *et al.* 2017; Vourelitis 2017).

### Herbivore effects on N and C cycling

Overall, the exclusion of small- and medium-sized mammalian herbivores resulted in changes to herbaceous vegetation assemblages that did not translate to detectable changes in soil N or C cycling. We hypothesized that herbivores would accelerate N cycling due to relatively high-N availability after fire (Table 1). Although there was an idiosyncratic

effect of Exclosure on nitrification and net N mineralization in the first month only (Fig. 5c and d), the results over 2 years did not demonstrate an impact of Exclosure on N cycling. There was no effect of Exclosure on any mineral N pool, N mineralization rate or C mineralization rate (Figs 5 and 6). The one possible exception was the interaction between Date and soil C and N. Although there was no Exclosure treatment effect on total soil C and N at initial or final sampling times, soil C and N did significantly decrease in exclosures by the final sampling point; there was no change in open plots. This very slight effect could suggest that herbivore presence contributes to soil C and N storage over time. However, comparing two sampling points is likely insufficient evidence to draw this inference and further investigation would be required. We found that C mineralization varied by Date but not Exclosure (Fig. 6a); soil moisture is known to be a key driver of respiration rates (Moyano *et al.* 2013), as we demonstrated (Fig. 6b). With such variability in short-term C dynamics, it is difficult to draw conclusions about soil organic C accumulation from only two time points.

Classical theories predict that herbivores impact soil N cycling through changes in resource inputs: the addition of dung/urine and changes to plant litter composition (Ritchie *et al.* 1998; Schrama *et al.* 2013b). Indeed, plant litter quality is the primary determinant of the soil net N mineralization rate (Parton *et al.* 2007); mammal dung and urine contain substantial C and N and can be a major source of soil enrichment (Clark *et al.* 2005).

In this experiment, herbivores decreased the total abundance of herbs, but not the balance between N-fixers and non-N-fixers: the fraction of total herbs that were N-fixing was unaffected by Exclosure treatment or Year. In a laboratory litter-soil incubation, Rossignol *et al.* (2011) demonstrated that litter from plants selected by herbivores did accelerate N cycling, while the litter from non-preferred plants slowed it. However, natural plant composition never reached these extremes. Likewise, we found no effect of Exclosure or Date on the relative biomass of N-fixers. The overall quality (C:N ratio) of plant litter may have not shifted enough to impact N cycling, even if the abundance of litter did.

The physical (non-consumptive) effects of herbivores can impact N cycling (Schrama *et al.* 2013b), potentially counteracting the effects of changing litter supply. Larger herbivores, like deer, can compact soil, leading to lower soil moisture, decreased rates of N cycling (Schrama *et al.* 2013a) and losses of

soil C and N (Gass and Binkley 2011). Although it was not explicitly measured in this experiment, it is possible that herbivore trampling exerted a physical effect that counteracted the increases in N cycling potentially caused by dung/urine inputs. However, a direct measurement of extremely high deer density would be needed to support the hypothesis of soil compaction effects, and those data were not collected in the present study.

It is worth noting that this was a short-term experiment. Effects of herbivore exclusions on nutrient cycling might have presented themselves after a decade-scale exclusion experiment. In other short-term exclusion experiments (fewer than 10 years), effects on soil properties were not detected (Andrioli *et al.* 2010; Lu *et al.* 2015). After a 3-year rodent-exclusion experiment in a post-fire grassland, herb biomass was higher in exclusions than open plots, but N mineralization was not significantly different (Moorhead *et al.* 2017). A study in the Canary Islands showed no change to soil chemical characteristics 4 years after goat grazing removal (Fernández-Lugo *et al.* 2009). Relva *et al.* (2014) found no effect of deer exclusion on soil nutrient cycling in a Patagonia conifer forest, despite changes to plant composition.

At the same time, other studies have demonstrated an effect of short-term herbivore exclusion. While one investigation in Swiss grasslands found no effect of short-term grazing on soil total N, C or P (Haynes *et al.* 2014), general mammalian herbivore exclusion in the same study increased net N mineralization after 5 years (Risch *et al.* 2015). Stritar *et al.* (2010) found that elk decreased soil total N and organic C in the first 2 years after fire, but increased nitrate concentration. In other short-term experiments, the impacts on C cycling (Chang *et al.* 2018) and N cycling (Liu *et al.* 2018) depended on the diversity of herbs and/or herbivores.

As previously discussed, the first year of this study (2017) was carried out in a very wet season following a historic drought. The drought could have compromised the recovery of *Adenostoma* shrubs, namely by depleting the carbohydrate stores necessary for rapid regrowth (Anderegg *et al.* 2012). If shrub growth were compromised, this could have also reduced N uptake by plants and intensified N leaching, especially in such a rainy year. Rapid N losses could have promoted low-N conditions that would no longer support the acceleration of N cycling in response to herbivory. In drier post-fire conditions, herbivory may have had a stronger effect on N cycling. Further research should

address the effect of herbivory on N cycling, given different climate conditions.

## CONCLUSIONS

In post-fire chaparral, the exclusion of herbivores led to increased herb biomass but did not result in a dramatic change in relative N-fixer composition or monthly nutrient-cycling measurements. Non-native invasive annual herbs were the most abundant functional group and the most heavily grazed by herbivores; this finding suggests mammalian herbivores may play a role in limiting post-fire herbaceous invasion from grassland into chaparral, even though the consumption of non-native herbs was not sufficient to prevent dominance by this group in post-fire herb assemblages. Investigations that connect patterns of herbivory to N and C cycling have shown varying and contradictory results. This experiment adds to the pool of findings that will contribute to an evolving predictive framework of herbivore impacts on nutrient cycling.

### Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: Relationship of dry plant biomass to pin hits using a 0.5 m × 0.5 m pin frame with 20 pins.

Figure S2: Response of N-fixing herb species to herbivore exclusions.

Figure S3: Response of non-N-fixing herb species to herbivore exclusions.

Table S1: Plant species list.

Table S2: Results of Type III ANOVA on linear mixed-effects models of herb vegetation biomass and soil characteristics.

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*Conflict of interest statement.* The authors declare that they have no conflict of interest.

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