

ARTICLE

Ecology of Critical Zones

Removal of N-fixing vs. non-N-fixing herbs in postfire chaparral: Competition and contributions to soil N and C cycling

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Abstract

As climate change increases fire frequency in Mediterranean-type shrublands, it is essential to understand the links between common postfire plant assemblages and soil nitrogen (N) and carbon (C) cycling during succession. In California chaparral, periodic fire removes shrub cover, deposits ammonium ($\text{NH}_4^+\text{-N}$) on soils, and allows herbaceous assemblages to dominate for 3–5 years. Herbs influence soil biogeochemistry through several mechanisms, including nutrient uptake, litter decomposition, and rhizodeposition. Controlled experimental removal of select plant groups from wild assemblages can demonstrate interactions between plant groups and how plant traits influence belowground processes. In a two-year herb-removal experiment, we investigated the impact of N-fixing and non-N-fixing herbs on soil N and C cycling. Treatments were (1) all herbs, (2) only non-N-fixing species, (3) only N-fixing species, and (4) no herbs. In high-N environments, N-fixers were predicted to compete poorly against non-N-fixing neighbors. N-fixers doubled in abundance when non-N-fixers were removed, but non-N-fixers were unaffected by N-fixer removal. Two years after fire, no-herbs plots had the lowest soil microbial respiration rates, and total accumulated C and N were lower than all-herb plots. Two treatments, no-herb and N-fixer plots, had elevated mineral N concentrations, net N mineralization, and net nitrification in the second year of the experiment. Our findings underscore the importance of fire-following herbs for postfire N retention and organic matter accumulation. A combination of both N-fixing and non-N-fixing herbs maximized total soil C and N, although the accumulation of TC and TN in all-herb plots was not significantly higher than in non-N-fixer plots. Results demonstrated the key role of non-N-fixing herbs in accumulating soil C and herbaceous communities for retaining N. Elevated soil nutrient availability two years postfire may contribute to the long-term recovery of shrubs, even after herbs are no longer dominant. Future investigations should also consider the magnitude of soil

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microbial N retention in plots with different herb functional groups, along with the species-specific contribution of non-N-fixing herbs to postfire C and N cycling.

KEYWORDS

C cycling, chaparral, fire ecology, N cycling, plant–soil feedbacks, succession

INTRODUCTION

As the frequency of wildfires and duration of fire season increase with changing climate (Westerling, 2016), it is urgent to elucidate the mechanisms that drive postfire ecosystem recovery. Nitrogen (N) availability can be an important driver of plant succession after disturbance (Tilman, 1986). At the same time, plants themselves influence soil ecosystem processes through nutrient uptake, symbiotic N fixation, interspecific competition, and litter decomposition, all processes heavily influenced by plant traits and thus subsequent functional groupings (Díaz & Cabido, 2001). There is a growing appreciation for the need to link ecological patterns in aboveground communities to soil nutrient cycling (Bardgett, 2018); this is particularly important in order to achieve restoration and conservation goals (Heneghan et al., 2008; Kardol & Wardle, 2010). California chaparral shrublands cover 7% of the state's land area and are susceptible to high-intensity summer and fall fires (Keeley & Davis, 2007). Mature chamise chaparral generally has an understory bare of herbaceous species and is covered with a thin litter layer of dry twigs and chamise leaves (Haydu-Houdeshell et al., 2017). Chaparral shrubs have been shown to decompose slowly due to high C:N and high lignin content, with the chaparral litter layer actually immobilizing N and P over the first three years of leaf decomposition (Schlesinger, 1985). Thus, N is often limiting to plant growth in chaparral ecosystems (Christensen & Muller, 1975; McMaster et al., 1982), and N availability can influence shrub and herb succession after fire (Guo, 2001; Kimball et al., 2014; Pasquini & Vourlitis, 2010).

Stand-replacing chaparral fires temporarily consume almost all fine living biomass, oxidizing organic N in shrubs, litter, and soils, which results in large net losses of N to the atmosphere (DeBano & Conrad, 1978). At the same time, the residual ash layer deposited on chaparral soils is rich in ammonium ($\text{NH}_4^+\text{-N}$) (DeBano et al., 1979; Homyak et al., 2014). Postfire conditions of increased pH and high available $\text{NH}_4^+\text{-N}$ substrate promote enhanced nitrification, conversion to nitrate-N ($\text{NO}_3^-\text{-N}$), during the early years of recovery (Hanan, Schimel, et al., 2016). This effect is particularly pronounced at the onset of early

autumn/winter rains, which can stimulate the diffusion of $\text{NH}_4^+\text{-N}$ substrate to nitrifying bacteria and rapid nitrification, demonstrated by measured nitrifier activity within hours to days of rewetting (Placella & Firestone, 2013). Nitrate is more mobile in soils than $\text{NH}_4^+\text{-N}$ and vulnerable to leaching and runoff, particularly from the steep slopes where chaparral occurs. Thus, nitrification in postfire chaparral can contribute to even greater soil N losses in the months to years following fire, with enhanced nitrate export to nearby streams (Goodridge et al., 2018; Homyak et al., 2014; Meixner et al., 2006). The plants that immediately colonize after fire may play an important role in retaining mineral N through plant uptake, influencing soil nutrient cycling, and preventing N losses that would slow shrub recovery and threaten water quality in adjacent aquatic habitats (Hanan, D'Antonio, et al., 2016; Rundel & Parsons, 1984). Ammonium is generally preferred over NO_3^- for plant uptake because the incorporation of NO_3^- requires energy expenditure in the plant for reduction to NH_4^+ in tissues (Salsac et al., 1987), but both forms of mineral N are available for uptake by plants and immobilization by microbes. Internal cycling of this retained N through subsequent litter decomposition may provide a supply of N to support plant productivity (Parton et al., 2007) during succession in often N-limited chaparral (Chen et al., 2020).

Sequences of postfire plant succession in chamise chaparral have been well characterized. At maturity, stands contain dense assemblages of native shrubs, mainly *Adenostoma fasciculatum* with interspersed *Ceanothus* spp., *Arctostaphylos* spp., *Quercus* spp., and other taxa, depending on location (Keeley & Davis, 2007). Mature *Adenostoma* chaparral hosts almost no herbaceous understory beneath its dense shrub canopy (Christensen & Muller, 1975; Tyler, 1995). Periodic wildfire (every 30–90 years; Van de Water & Safford, 2011) temporarily eliminates shrub cover and the woody litter layer, and initiates a transient change in plant assemblages. Soon after fire, shrubs re-establish from either a fire-stimulated seed bank or resprouting burls of burned adults (Keeley et al., 2005). At the same time, bare soil is rapidly colonized by a flush of herbaceous plants, which dominate for 3–5 years before the recovering shrub canopy closes (Keeley et al., 2005; Potts et al., 2010).

These herbs are functionally diverse, including non-N-fixing annual herbs (e.g., *Emmenanthe penduliflora* ssp. *penduliflora*, *Calandrinia breweri*), non-N-fixing perennials (e.g., *Chlorogalum pomeridianum*), N-fixing perennials (e.g., *Acmispon glaber*), and N-fixing annuals (e.g., *Acmispon* spp. and *Trifolium* spp.). Short-lived postfire herbs may play an important role in absorbing available N deposited on soils during fire (Goodridge et al., 2018; Hanan, D'Antonio, et al., 2016; Rundel & Parsons, 1984) and in increasing soil N availability through inputs from N-fixing herbs (Guo, 2001; Rundel & Parsons, 1984); however, the impact of these ephemeral plant assemblages, and their functional diversity, on postfire N or C cycling has not been explored experimentally.

Postfire herb assemblages contain a mix of N-fixing and non-N-fixing species (Keeley et al., 1981). These two functional groups are predicted to exert distinct effects on soil N and C cycling, based on their plant tissue chemistry and the energetic costs associated with their resource acquisition strategies. Nitrogen-fixers, which host root bacterial symbionts that convert atmospheric N_2 to NH_3 , tend to have higher tissue N content (lower C:N ratio) than non-N-fixers. When N-fixers die or senesce, they deposit N-rich litter, which can rapidly decompose (Hobbie, 2005; Parton et al., 2007; Scherer-Lorenzen, 2008) and increase rates of soil net N mineralization (Robertson & Groffman, 2007). Plant litter with a comparatively high C:N ratio, such as that from non-N-fixers, may decompose more slowly and even lead to the immobilization of N by microbes during early decomposition (Parton et al., 2007). Ultimately, plant litter decomposition is a major determinant of the accumulation of soil organic C (SOC). Presuming that N-fixers have typically lower C:N than co-occurring non-N-fixers, N-fixers litter would likely promote faster rates of N and C mineralization than non-N-fixer litter. However, patterns of N and C cycling and plant growth are more complex in mixed stands and may depend on soil nutrient availability.

Interactions between N-fixers and non-N-fixers may be mediated by soil N availability and also impact plant biomass and soil biogeochemistry. When non-N-fixers and N-fixers grow together, N-fixers may facilitate the growth of non-N-fixers through the addition of their high-N litter (Maron & Connors, 1996; Menge et al., 2008) and root N exudates (Paynel et al., 2008). While the enhanced growth of non-N-fixers may competitively inhibit the N-fixers themselves, their co-occurrence may lead to enhanced biomass and litter production. Increased litter mass is generally associated with increased soil C accumulation and soil heterotrophic respiration (Xu et al., 2013). However, in the high-N postfire environment, it is unclear whether N-fixers do facilitate

non-N-fixers, leading to such an effect. Non-N-fixers can grow quickly in high-N conditions, absorbing available soil mineral N and ultimately producing more biomass and plant litter than slower growing N-fixers. Furthermore, the N-rich postfire conditions may not favor the growth of N-fixers, which can be poor competitors in an environment with high available N (Haubensak & D'Antonio, 2011; Vitousek et al., 2002; Yelenik et al., 2017), and some N-fixers may not even fix N when soil N is high (Menge et al., 2009). Symbiotic N fixation is costly for host plants in terms of C and mineral resources, which can limit their growth rate (Gutschick, 1981; Vitousek & Howarth, 1991), even if they cease N fixation (Menge et al., 2009). Furthermore, the postfire environment is characterized by rapidly changing N availability, typically with initially high soil ammonium NH_4^+ concentrations, decreasing due to nitrification and hydrological losses (Hanan, Schimel, et al., 2016). Thus, the soil N availability might be higher in the first year after fire than in the second, leading to a different interaction between N-fixers and non-N-fixers in the early postfire years.

Herb-removal experiments are a powerful tool to aid in our understanding of ecosystem effects of local plant functional types in natural ecosystems (Díaz et al., 2003). For example, the approach has been used extensively to investigate community recovery after plant invader removal (Guido & Pillar, 2015). The removal approach contrasts with the use of synthetically assembled plant communities, which allows more investigator control over initial plant species composition and soil conditions, minimizes differences in soil disturbance between treatments, and may provide clearer tests of isolated mechanistic links between plant diversity and ecosystem function (Díaz et al., 2003). While removal experiments sacrifice this degree of investigator control, they provide an essential complement to artificially assemblage experiments by demonstrating how specific functional groups impact community and ecosystem functioning in real-world conditions and in a local context. Removal experiments test the effects of functional diversity given existing natural abundances of co-occurring plant functional groups and their seedbanks, not necessarily the ratios decided by the investigator. As such, they may provide a more realistic test of functional group interactions and ecosystem effects that are dependent on local environmental conditions (Gundale et al., 2012), with implications for applied conservation and management (Díaz et al., 2003). Furthermore, the artificial disturbance caused by removal experiments can be greatly reduced when they are conducted at early successional stages (such as immediately postfire), when minimal biomass is removed and ground disturbance is low (Wardle et al., 1999).

We implemented a two-year field herb-removal experiment to test the impact of herb functional group (N-fixers vs. non-N-fixers) on postfire chaparral soil N and C cycling. By weeding plants as they emerged from recently burned chaparral, we manipulated the presence of N-fixers and non-N-fixers in postfire herb assemblages. Treatments included (1) control (all herbs, no removal), (2) only non-N-fixing species, (3) only N-fixing species, or (4) no herbs (all herbs removed). This experiment also provided an in situ test of competition and facilitation between N-fixing and non-N-fixing herbs in postfire chaparral, in a realistic natural context with a typical postfire chaparral herb species pool. We predicted that N-fixers would be poor competitors against non-N-fixers when soil available N was high (especially in the first year) due to energetic costs associated with maintaining N-fixing bacterial symbionts. Thus, when N-fixers are removed, non-N-fixer biomass should be unaffected or increase. Removing non-N-fixers should release N-fixers from competition, enhancing their biomass. We predicted the highest levels of mineral N and rates of N mineralization would occur in plots with exclusively N-fixing herbs, due to inputs of N-rich plant litter. However, we predicted the highest rate of total soil N (TN) and C (TC) accumulation from all-herb plots, followed by non-N-fixers, then N-fixers and the lowest from bare plots, following predicted herb biomass trends, and assuming slower decomposition of high C:N litter non-N-fixers.

METHODS

Study location and site selection

We conducted this study at the Hopland Research and Extension Center (HREC, Mendocino County, CA, 39°1'14" N, 123°4'52" W; Appendix S1: Figure S1). Hopland is located in the inner North Coast 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 winter (83.7-cm precipitation). The first season of monitoring had a particularly rainy fall and winter (2016–2017: 117.0 cm) and typical spring and summer (9.1 cm). The second fall and winter were comparatively dry (2017–2018: 18.3 cm), again followed by a typical spring and summer dry season (12.6 cm). 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 National Climate Data Center (NCDC) Hopland Station (elevation 817 m; 39°1' N, 123°4' W) and 30-year normal is from HREC Headquarters (elevation 263 m; 39°0' N, 123°4' W). Soils at this site are derived from weathered sandstone and shale and are classified as a complex (Maymen-Etsel-Snook) of sandy and gravelly loam (Soil Survey Staff, 2020).

Plots were all located in the perimeter of a ~1-ha prescribed fire burned on April 21, 2016 by the California Department of Forestry and Fire Protection (CalFire) (for prescribed fire methods, see Hendricks-Franco et al., 2021). Air temperatures that day were 6–18°C, and windspeeds were 10 with 15 km/h gusts. Humidity was approximately 80% in the morning, with rain beginning in the evening after the fires. Ocular estimates of flame lengths were approximately 6–10 m high, consistent with other spring prescribed burns at the same site (Stephens et al., 2008). Only areas with complete consumption of living plant materials (leaving charred shrub skeletons only) were considered burned and included in the study area. The site contained mature chaparral vegetation (>20 years since last fire) dominated by chamise (*Adenostoma fasciculatum*) shrubs. Elevation ranged from 565 to 635 m, aspect was south-facing, and slopes were steep (75% maximum, 25% average). 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). However, changing precipitation patterns may lead to a future lengthened or altered wildfire season in the coming decades (Molinari et al., 2018), increasingly the relevance of investigations on out-of-season burns. These controlled fires were followed by a flush of herbaceous growth as is typical for wildfires in chaparral. Prescribed fires in chaparral are commonly implemented outside of the wildfire season for increased safety (Stephens et al., 2008).

During the prescribed fire, we measured the maximum temperature at the soil surface using temperature-sensitive paints (Tempilaq, LA-CO Industries, Elk Grove Village, IL) designed to melt at given temperature thresholds. Fifteen color-changing paints with different temperature thresholds were painted in arrays of 1-cm² squares on 10 × 10 cm ceramic tiles (79, 93, 149, 204, 316, 371, 399, 427, 482, 538, 593, 649, 704, 760, 816°C). Before the fire, tiles were placed in a triangle grid throughout the planned burn site with approximately 25 m between each site. The maximum temperature reached at each point was recorded. Soil temperatures can provide a useful proxy for fire severity, especially if nutrients can be directly

volatilized from soils during burning (Janzen & Tobin-Janzen, 2008). At the same points, we measured the height of the *Adenostoma* shrub canopy, an indication of stand age and live fuel loads, and soil gravimetric moisture. Soil moisture during fire can impact rates of soil temperature change and changes to microbial communities during burning (Janzen & Tobin-Janzen, 2008).

Experimental design

We placed six experimental blocks within the site, with blocks 20–40 m apart. Each of the blocks contained four rectangular plots of 2×3 m. The plots in a block were arranged along the hillside contour with a minimum of 1 m between them so that no plot within a block was uphill of another. There were four treatments, randomly assigned to each of the plots in a block. We selectively weeded herbs so that plots included: (1) control (all herbs, no removal); (2) only non-N-fixing species (removal of N-fixers); (3) only N-fixing species (removal of non-N-fixers); or (4) no herbs (full removal) (Figure 1). Plots were monitored weekly or biweekly during the 2016–2017 and 2017–2018 growing seasons (December through May) so that herbs could be weeded as soon as they emerged, minimizing soil disturbance. Roots were typically 1 cm or less in length when plants were removed. In the first year of weeding (2017), all removed herbs were collected, dried, and weighed. Throughout the entire season, minimal biomass was removed, with the vast majority occurring in the beginning of the growing season (late January to early March). In all of 2017, 7.3 g herbs/m^2 were removed from N-fixer plots, 3.2 g/m^2 from non-N-fixer plots, and 7.1 g herbs/m^2 from no-herb (full-removal) plots. Where possible, we weeded by reaching from outside the plots to avoid trampling the interior. To control for the ground disturbance due to weeding, we shallowly penetrated the soil surface with a stick at multiple locations within the control (all-herb, no-removal) plots at each weeding session. Great care was used to place plots in regions dominated by chamise (*Adenostoma fasciculatum*) chaparral. Vegetation sampling (described below) confirmed dominance by *A. fasciculatum*, with an average standing dry biomass of $49 \pm 15 \text{ g/m}^2$ (mean \pm SEM) in 2017 (one year after fire) and $78 \pm 14 \text{ g/m}^2$ in 2018. No other shrub exceeded the average standing biomass of 1.8 g/m^2 in either year (see *Vegetation sampling* below).

Vegetation sampling

We monitored vegetation at peak biomass at the end of each growing season (June–July 2017 and May 2018). We

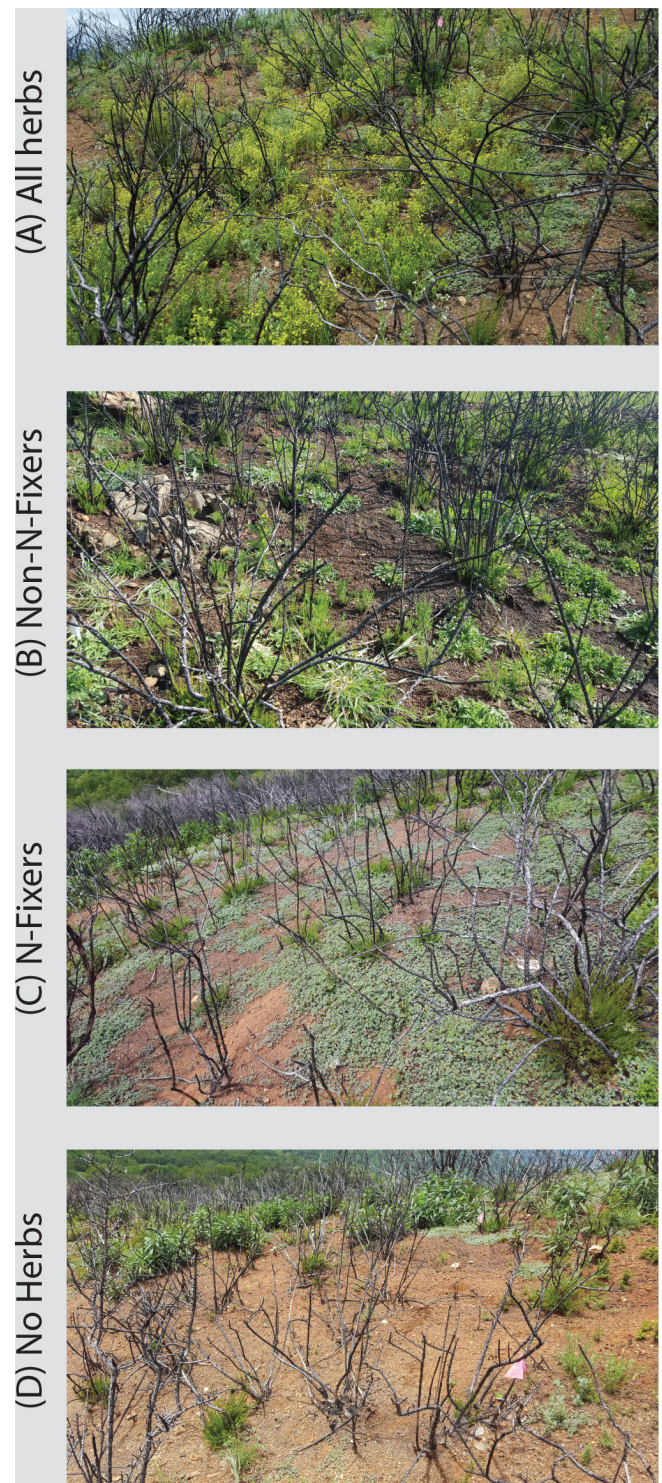


FIGURE 1 Example photographs of each herb-manipulation treatment. Photo credit: L. Hendricks-Franco March 2017.

estimated biomass using the point intercept method with a pin-frame sampler. In each 2×3 m plot, we subsampled three 0.5×0.5 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 pin hit each plant species. We used the total number of pin hits as a proxy for plant dry biomass (Jonasson, 1988), using linear estimates derived in our parallel study at the same reserve (Hendricks-Franco et al., 2021). We related pin hits in each of these groups to dry biomass (in grams per square meter) using a linear regression for each group. To avoid regression estimates of negative biomass values, we set the y intercept to zero and estimated a slope only (β): $\beta_{\text{shrubs}} = 4.56$ ($R^2 = 0.96$), $\beta_{\text{non-N-fixer}} = 3.97$ ($R^2 = 0.91$), and $\beta_{\text{N-fixers}} = 3.78$ ($R^2 = 0.97$). We reported herbaceous biomass values by functional group (N-fixers vs. non-N-fixers) and by species (Appendix S1).

We were unable to analyze tissue samples from the field site, thus estimates of N-fixer and non-N-fixer C and N content come from chaparral at nearby Stebbins Cold Canyon Reserve (with similar climate, vegetation, and topography) following the 2015 Wragg Fire (Wahl et al., U.C. Berkeley unpublished data). The dried standing litter of *Acmispon* spp., the dominant N-fixing herbs from the present study (81%–93% of N-fixer biomass across treatments and years, Appendix S1: Table S1 and Figure S2), had a significantly higher N content (13.6 ± 0.4 mg/g N, C:N 27.8 ± 0.5) than co-occurring typical annual non-N-fixing herbs *Emmenanthe penduliflora* (5.6 ± 0.2 mg/g N, C:N 70.7 ± 2.3 , Wahl et al., unpublished data). The measured N concentrations of *Acmispon* correspond to a previously measured range tissue N in the perennial *A. glaber* (7.6–24.1 mg/g N, depending on tissue type within the same plant, with our estimates falling in the low end of the range; Nilsen & Schlesinger, 1981). In 2017, in our present study, *E. penduliflora* contributed to 13% of non-N-fixer biomass in controls and 43% in non-N-fixer plots. In 2018, *E. penduliflora* constituted only 4% of non-N-fixer biomass in controls and 0% in non-N-fixer plots. However, C:N estimates are in line with the widely variable range of values for non-N-fixing grasses and forbs in non-native California grassland and postfire chaparral (Henry et al., 2005; Pratt, 2022), with our values again on the low end of the range. Thus, we have accepted these values for approximation of non-N-fixer N content at our sites, with the caveat that our N estimates may have a lower bias. While the reported plant N content should not be interpreted as precisely measured values, they provide an important estimate of N stocks at these sites, as well as an indication of likely higher N concentrations for N-fixers than co-occurring non-N-fixers. We estimated the strength of each herb pool as a N sink by multiplying the biomass by estimated tissue N concentration.

Soil physical and chemical properties

Soil collection

For all soil measurements in this project, we collected mineral soils to a depth of 10 cm to capture the dynamics most likely driven by herbaceous growth and litter decay. An initial soil collection was conducted on February 1, 2017, during which we pooled soil cores from across the whole block to capture season conditions and block-level characteristics before the start of the experiment, without respect to a specific treatment. Throughout the 2017 and 2018 spring growing seasons, we collected soil samples monthly from each plot (March–June 2017 and February–May 2018). During these sampling periods, we collected four 2.5-cm-diameter, 10-cm-deep steel tubular soil cores at each plot and pooled the soil to minimize plot-level variation. 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 field-moist soils through a 4-mm sieve. We refrigerated soils at 4°C soon after collection and processed soils for analysis (described below) within 48 h.

We also report results from soil collected in the fall before herb removal began (October 22 and November 28, 2016) to characterize the effect of prescribed fire on soil mineral N concentrations and mineralization rates at these sites. These soils were collected at 15-cm depth (described below) from the burned site ($n = 20$) and adjacent nonburned chaparral control stands ($n = 9$). While the results from these samples cannot be directly compared with shallower soil samples (10 cm) collected throughout the herb-removal experiment (2017–2018), they demonstrate key differences between burned and nonburned soils.

Bulk density

We collected separate soil cores for bulk density analysis during February 1, 2017 block-level sampling. At each block, we collected one soil core (steel tube, 5 cm diameter \times 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 determined as the dry mass of soil divided by the total volume.

Total soil C and N

Total soil carbon C and N were determined from air-dried soils. First, we tested soils for the presence of inorganic C. We finely ground soil samples in a mortar and pestle and moistened them with one drop of deionized water, then added one drop of 4 M HCl. As there was no effervescence, we assumed that no significant inorganic C was present. Thus, we assume that TC is a 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 pretreatment (March 2017) and posttreatment (May 2018) times. Although the pretreatment date was actually shortly after we began weeding, this was the earliest point at which plot-level soils were collected and thus the best indication of pretreatment conditions available. We calculated the change in soil C and N as the difference between levels in May 2018 and March 2017.

Soil mineral nitrogen and nitrogen mineralization

At each monthly soil collection, we extracted soils in 2 M KCl for analysis of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. We mixed 10 g soil samples with 40 mL of 2 M KCl in an acid-washed (1% HCl) 50 mL centrifuge tube. Samples were shaken for 1 h at 200 rpm. The soil slurry was then gravity filtered through KCl-rinsed Whatman no. 1 filters and acid-washed funnels into separate new clean centrifuge tubes. Samples were stored at -20°C until analysis.

We determined the rate of soil net N mineralization and net nitrification using a laboratory incubation. This setup was combined with a soil C mineralization (microbial respiration) incubation described below. After measuring soil subsamples for mineral N at collection (above), soils were incubated at field moisture in 495 mL glass Mason jars. A subsample of approximately 30 g of field-moist soil was gently tamped down into a tared 30 mL specimen cup and weighed. The specimen cup was placed carefully into the base of the jar, which was sealed and allowed to incubate in the dark for one week at 23°C . After the 7 days, we removed each incubated soil sample from its jar, mixed it thoroughly, then measured out a 10 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 $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations using Lachat Flow Injection Analyzer (Hofer, 2003; Knepel, 2003) or the microplate method (Wu et al., 2016). A subset of samples

was analyzed using both methods to ensure consistency between the two ($R^2 > 0.93$). Net nitrification was calculated as the difference in $\text{NO}_3^-\text{-N}$ from the beginning to end of the incubation: $(T_2 - T_1)/T$ (in weeks). Data are reported as microgram of N (per gram of dry soil) per week. Net N mineralization is calculated using the same formula to describe the difference in total mineral N ($\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}$). Negative values are interpreted as microbial immobilization of mineral N.

Soil gravimetric percent moisture was determined for every collected soil. We measured 10 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: $(\text{mass}_{\text{wet}} - \text{mass}_{\text{dry}})/\text{mass}_{\text{dry}}$. All soil measurements were normalized by dry mass.

Microbial respiration (carbon mineralization)

During March–May 2018, we used the soil mineral N incubations to also collect data on soil respiration over a 48-h period. While standard laboratory incubation methods may not capture all field conditions, they allow for a useful comparison of respiration rates as influenced by C substrate and soil microbial communities (Anderson, 1983). Samples were collected in plastic syringes with O-ring seals and gas-tight stopcocks. We took three initial 5 mL samples from ambient air above the jars prior to sealing them with gas-tight lids fitted with septa (T_1). After a 48-h dark incubation, we injected 5 mL of CO_2 -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 carbon at T_1 and T_2 , adjusting for the temperature and pressure in the jars, and the small dilution from injecting CO_2 -free air at T_2 . We calculated respiration as $(T_2 - T_1)/T$ (in days). Data are reported as milligram of $\text{CO}_2\text{-C}$ (per kilogram of dry soil) per day.

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). We fit the following linear mixed-effects model to evaluate the main and interactive effects of Treatment (Trt) and Date on all measured variables in the experiment:

$$Y = \text{Trt} + \text{Date} + \text{Trt} \times \text{Date} + \text{Random}(\text{Block} + \text{Plot}). \quad (1)$$

The effects of treatments on each group at each date were determined by a priori planned contrasts of estimated marginal means, with no penalization procedure. We considered $p < 0.1$ as the threshold for a significant effect, a typical threshold for marginal significance that avoids unnecessary risk of Type II error in ecological models with relatively low samples size (Hurlbert & Lombardi, 2009). Soil response variables were $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, net N mineralization, and net nitrification, soil gravimetric moisture, TC, TN, C mineralization (microbial respiration), and herb biomass.

The removal of select herb functional groups provided a de facto test of competition and facilitation between N-fixing and non-N-fixing herbs. Competition intensity can be demonstrated by comparisons of competitor growth alone versus in a mix (Grace, 1995). To test the impact of “neighbor removal” (competitor/facilitator removal) on each functional group, we compared the peak biomass of a given functional group, grown with and without neighbors from another functional group. To test the impact of N-fixer presence on non-N-fixers, we compared non-N-fixer biomass in all-herb versus non-N-fixer plots. To test the impact of non-N-fixer presence on N-fixers, we compared N-fixer biomass in all-herb versus N-fixer plots. Differences in total herb biomass and estimates of total herbaceous N were analyzed in the linear mixed-effects model above (Equation 1), but only treatments with herbs were included (i.e., not including no-herb, i.e. full-removal, plots). Planned contrasts were performed with the procedure defined above.

RESULTS

Prefire and pretreatment sampling

During the prescribed fire, the soil surface reached an average maximum temperature of $231 \pm 31^\circ\text{C}$ (mean \pm SE), with a mode of 204°C (8 of 19 burned points). The maximum temperature recorded was 593°C . The mean prefire shrub height was 137 ± 6.8 cm. Gravimetric soil moisture the day before the fire (April 20, 2016) was $10.2 \pm 0.5\%$. In postfire block-level measurements (pretreatment), soil bulk density was 1.32 ± 0.05 Mg/m^3 .

Herb composition effects

We sampled 47 herb species in these plots (Appendix S1: Table S1). Within control plots, the

most common herbs (>5% relative abundance by biomass) in the first postfire year (2017) were annual *Acmispon* spp. (27%), *Logfia gallica* (13%), *Calandrinia breweri* (11%), *Emmenanthe penduliflora* (9%), and *Logfia filaginoides* (9%). In the second year (2018), the most common herbs were *Logfia gallica* (30%), annual *Acmispon* spp. (15%), *Hypochaeris glabra* (11%), *Aira caryophyllea* (10%), *Bromus diandrus* (8%), and *Logfia filaginoides* (7%). The effect of removal treatment on total aboveground herb biomass (excluding no-herb plots) varied by year (year \times treatment, $F = 5.66$, $p = 0.005$; Figure 2A,B). At the end of the first growing season, there was no significant difference in the total biomass between treatments (all herbs vs. N-fixers, $p = 0.333$; all herbs vs. non-N-fixers, $p = 0.388$; N-fixers vs. non-N-fixers, $p = 0.915$). By the end of the second year, all treatments were significantly different in biomass, in descending order from all-herb (control), to non-N-fixer only, to N-fixer only plots (all $p < 0.05$; Figure 2A,B).

Estimates of the aboveground herbaceous N pools varied by treatment ($F = 6.7$, $p = 0.014$) and year ($F = 13.6$, $p = 0.002$), but not their interaction ($F = 2.2$, $p = 0.144$; Table 1). In 2017, there was no significant difference between estimated herb N pool in N-fixer and control (all-herb) plots ($p = 0.468$), while plots with only non-N-fixers were estimated to have less than half of the aboveground herbaceous N found N-fixer plots ($p = 0.023$) or all-herb controls ($p = 0.100$). In 2018, all-herb controls had significantly higher herbaceous N than N-fixer ($p = 0.045$) or non-N-fixer plots ($p = 0.002$), which were not statistically significantly different from each other ($p = 0.190$). Herbaceous N pool estimates were higher in 2018 than 2017 for control ($p = 0.002$) and non-N-fixer plots ($p = 0.078$), but not N-fixer plots ($p = 0.450$). In 2017, herbaceous N in control plots was found approximately equally in N-fixing (53%) and non-N-fixing plant (47%) components, while in 2018 the majority of plant N in control plots came from non-N-fixers (66%).

As a test of the effect of neighbor removal, we compared all-herb controls to plots with either only N-fixers or only non-N-fixers. N-fixer biomass was increased by non-N-fixer removal ($F = 12.99$, $p = 0.005$), with no effect of year ($F = 1.76$, $p = 0.190$) or year \times treatment ($F = 0.06$, $p = 0.805$). N-fixers had nearly twice as much biomass in plots where they grew without non-N-fixers (Figure 2C,D, gray bars). However, different species of N-fixer benefited from non-N-fixer removal, depending on the year (Appendix S1: Figure S2). In the first year (2017), only the annual *Acmispon* spp. were significantly higher in N-fixer plots than in controls (62.4 vs. 31.1 g/m^2 , $p = 0.024$), while in 2018 only the perennial *Acmispon glaber* had much

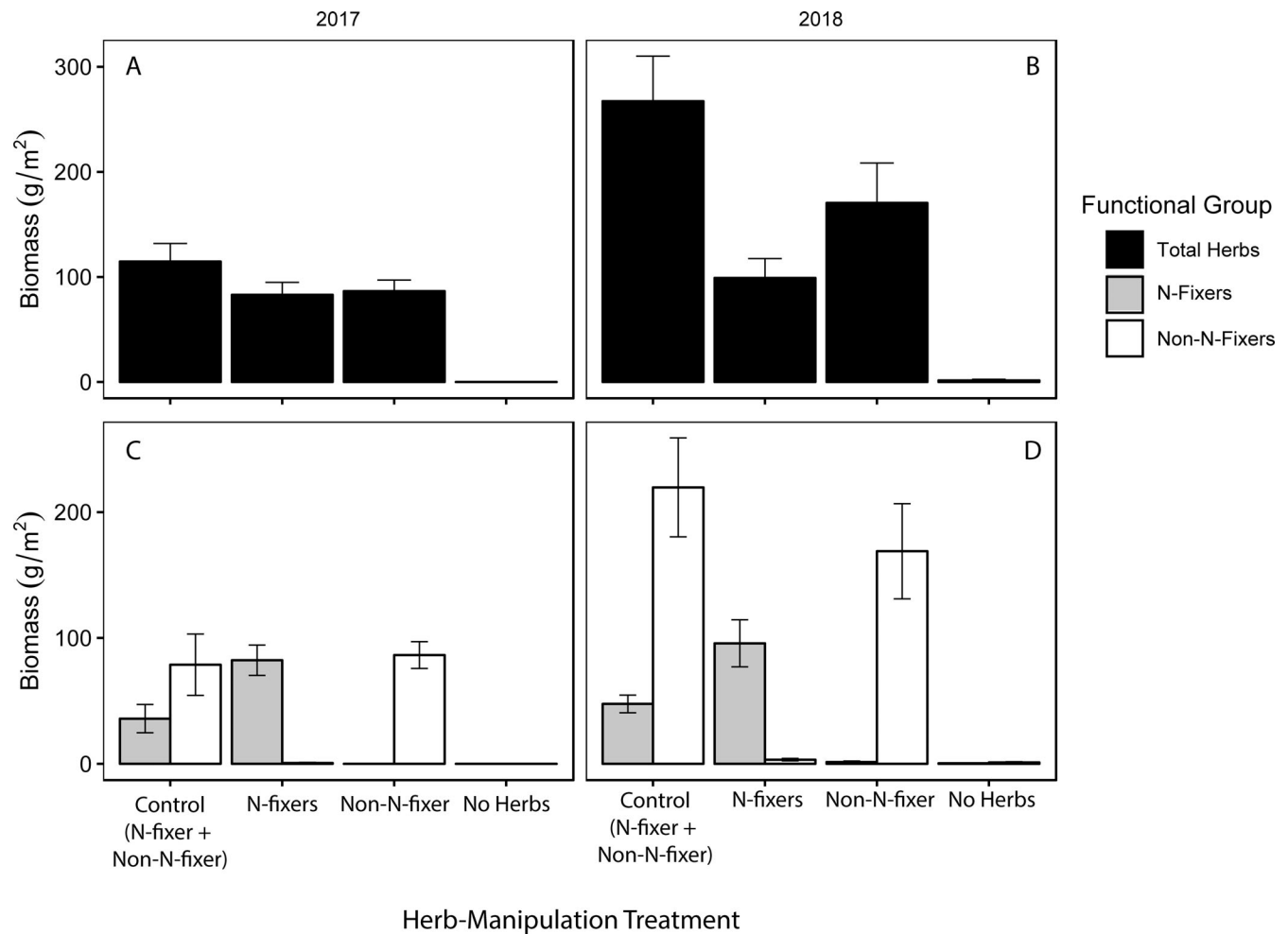


FIGURE 2 Herb biomass by functional group at each herb-manipulation treatment. The x-axis indicates the groups of herbs that were allowed to grow (i.e., not removed). Herbs were measured at assumed peak biomass (June 2017 and late May 2018). Error bars represent SE ($n = 6$). Significance levels are described in the [Results](#).

TABLE 1 Estimated N content in herb biomass by treatment type and plant functional group (mean \pm SE).

Treatment	Estimated herb N pools (mg N/m ²)		
	Total	N-fixers	Non-N-fixers
2017			
Control	929 \pm 98.7^a	488 \pm 153 (53%)	440 \pm 136 (47%)
N-fixers	1123 \pm 162^a	1120 \pm 164 (>99%)	3.7 \pm 2.5 (<1%)
Non-N-fixers	484 \pm 59^b	0 \pm 0	484 \pm 59 (100%)
No herbs	0 \pm 0	0 \pm 0	0 \pm 0
2018			
Control	1877 \pm 276^a	647 \pm 95.1 (34%)	1230 \pm 220 (66%)
N-fixers	1320 \pm 253^b	1302 \pm 254 (99%)	18.5 \pm 5.6 (1%)
Non-N-fixers	966 \pm 215^b	20.0 \pm 10.3 (2%)	946 \pm 211 (98%)
No herbs	11.9 \pm 9.0	5.7 \pm 5.7 (0.5%)	6.2 \pm 3.5 (0.5%)

Note: Superscripts indicate significance grouping in planned contrasts of total herb N in the bolded groups within year, based on a linear mixed model ANOVA. Assumed herb N concentrations based on a concurrent analysis of a nearby postfire chaparral site (Stebbins Cold Canyon Reserve, Solano County, CA).

higher biomass in N-fixer plots (33.0 vs. 2.1 g/m², $p = 0.002$).

On the other hand, the removal of N-fixers did not affect the biomass of non-N-fixers ($F = 0.55$, $p = 0.491$; Figure 2C,D, white bars). Non-N-fixer biomass was much higher in the second year than in the first year ($F = 22.86$, $p < 0.001$), with no interaction of neighbor removal and year ($F = 1.56$, $p = 0.216$). A few individual non-N-fixing species were significantly impacted by N-fixer removal, but the effect was variable in year, magnitude, and direction (Appendix S1: Figure S3). The native annual *Emmenanthe penduliflora* was significantly higher in non-N-fixer plots (without N-fixers) than in control plots, in 2017 only (37.1 vs. 10.6 g/m², $p = 0.005$). In three other native annuals in 2017, there were slight, but significant reductions in biomass when N-fixing neighbors were removed: *Calandrinia breweri* (12.1 vs. 2.0 g/m², $p = 0.051$), *Camissoniopsis hirtella* (0.9 vs. 0.0 g/m², $p = 0.054$), and *Cryptantha muricata* (0.9 vs. 0.0 g/m², $p = 0.098$). Among the non-native annual non-N-fixers, two had significantly lower biomass in the second year (2018), when N-fixers were removed, that is, lower biomass in non-N-fixer plots than controls plots: *Aira caryophyllea* (26.3 vs. 1.8 g/m², $p < 0.001$) and *Logfia gallica* (80.7 vs. 50.5 g/m², $p = 0.033$). In 2018, *Avena barbata* was higher in biomass in non-N-fixer than the control plots (8.6 vs. 0.7 g/m², $p = 0.080$).

Mineral N pools and rates

In October/November 2016, 6–7 months after the spring prescribed fire, NH₄⁺-N and NO₃⁻-N were elevated compared with nonburned sites (Table 2). In general, NH₄⁺-N decreased across all treatments over time ($F = 39.5$, $p < 0.001$; Figure 3A,B) and the effect of treatment varied marginally by date (treatment × date, $F = 1.4$, $p = 0.11$). Although there was a great deal of variation in NH₄⁺-N in the initial months of sampling, a clearer pattern of treatment effects emerged by the end of the first year (June 2017) and throughout most of the second (February–April 2018). In planned treatment contrasts (Figure 3A,B), N-fixer plots were generally elevated in NH₄⁺-N compared with all-herb controls and non-N-fixer plots, with the exception of March 2018, when control plots had intermediate NH₄⁺-N between N-fixers and non-N-fixers. No-herb plots were never significantly different from N-fixers plots in NH₄⁺-N throughout the same period. No-herb plots were higher in NH₄⁺-N than non-N-fixer and control (all-herb) plots in certain months only (June 2017, April 2018). By the last sampling date (May 2018), there were no significant treatment differences in soil NH₄⁺-N concentrations.

The effect of treatment on soil NO₃⁻-N concentrations varied significantly between dates (treatment × date, $F = 1.7$, $p = 0.045$; Figure 3C,D). At the beginning of sampling (March–April 2017), there was no difference

TABLE 2 Pretreatment of soil sampling (mean ± SE). (A) In October and November 2016, soils were sampled to 15-cm depth across the whole burned site ($n = 20$) and adjacent nonburned ($n = 9$) chaparral. Boldface indicates significant differences between burned and nonburned soil in one-way ANOVA ($p \leq 0.10$). (B) In February 2017, soils were sampled to 10-cm depth and pooled across the six experimental blocks used in the herb-removal experiment.

Date and soil characteristic	Burned	Nonburned	F (p)
(A) October 2016			
NH ₄ ⁺ -N (μg N/g soil)	9.65 ± 1.44	3.41 ± 0.38	8.23 (0.008)
NO ₃ ⁻ -N (μg/g)	5.29 ± 1.88	0.11 ± 0.07	3.33 (0.080)
Net N mineralization (μg g ⁻¹ week ⁻¹)	-0.18 ± 0.89	-0.23 ± 0.36	<0.01 (0.972)
Net nitrification (μg g ⁻¹ week ⁻¹)	0.08 ± 0.49	0.26 ± 0.14	0.06 (0.812)
November 2016			
NH ₄ ⁺ -N (μg/g)	6.83 ± 1.19	2.81 ± 0.42	4.95 (0.035)
NO ₃ ⁻ -N (μg/g)	3.97 ± 1.00	0.61 ± 0.26	4.87 (0.036)
Net N mineralization (μg g ⁻¹ week ⁻¹)	0.58 ± 1.24	0.97 ± 0.43	0.04 (0.840)
Net nitrification (μg g ⁻¹ week ⁻¹)	3.17 ± 0.72	-0.13 ± 0.29	8.98 (0.006)
(B) February 2017			
NH ₄ ⁺ -N (μg/g)	6.4 ± 1.3		
NO ₃ ⁻ -N (μg/g)	1.8 ± 0.3		
TN (mg/g)	1.7 ± 0.1		
TC (mg/g)	26.1 ± 2.0		

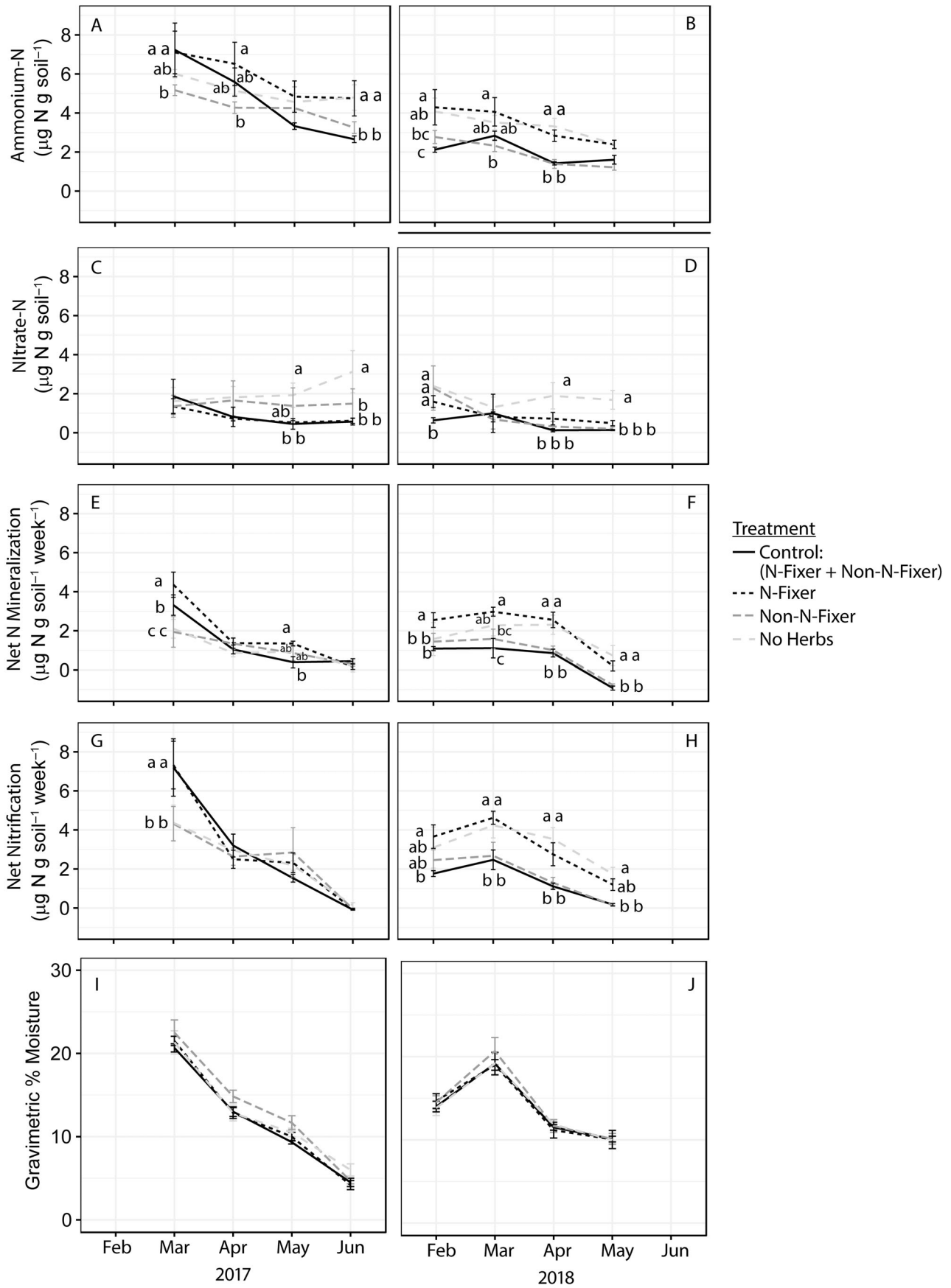


FIGURE 3 Legend on next page.

in NO_3^- -N concentration between treatments. By the end of the first season (June 2017), NO_3^- -N was significantly higher in no-herb plots than in all other treatments. Similarly, by the last two months of the second season (April and May 2018), no-herb plots were higher in nitrate than all other treatments.

The effect of treatment varied by date for net N mineralization (treatment \times date, $F = 2.4$, $p = 0.001$; Figure 3E,F) and net nitrification (treatment \times date, $F = 2.8$, $p < 0.001$; Figure 3G,H). Both net N mineralization and net nitrification rates were similar across treatments throughout 2017, except for the first sampled date, March 2017, when rates of both processes were elevated in all-herb and N-fixer plots. Throughout most of 2018, N-fixer plots had higher rates of net N mineralization (2.0- to 2.7-fold) and net nitrification (1.7- to 7.4-fold) than control and non-N-fixer plots. Throughout most of the second season (April–May 2018), no-herb plots were also significantly higher in net N mineralization (1.2- to 2.5-fold) and net nitrification (1.6- to 10.9-fold) than all-herb and non-N-fixer plots. Across all treatments and dates, net nitrification rates and net N mineralization rates were positively correlated ($R^2 = 0.68$, $p < 0.001$, $n = 191$), with net nitrification rates almost always higher than net N mineralization rates across sampling points and treatments. Including a treatment interaction did not improve this model (treatment \times net mineralization, $F = 1.73$, $p = 0.115$; Figure 4).

Soil gravimetric moisture content varied by date ($F = 263.39$, $p < 0.001$), but not by treatment ($F = 0.65$, $p = 0.590$) or treatment \times date ($F = 0.75$, $p = 0.774$; Figure 3I,J).

Microbial respiration (soil carbon mineralization)

Soil microbial respiration was measured in a laboratory incubation at three sampling times (March–May) of the 2018 growing season (Figure 5). Both date ($F = 29.40$, $p < 0.001$) and treatment ($F = 3.22$, $p = 0.054$) were significant predictors of soil respiration, with no interaction between the two ($F = 0.06$, $p = 0.999$). Overall, respiration rates were highest in March ($p < 0.001$), with no difference between rates in April and May. Across all timepoints, the no-herb plots were

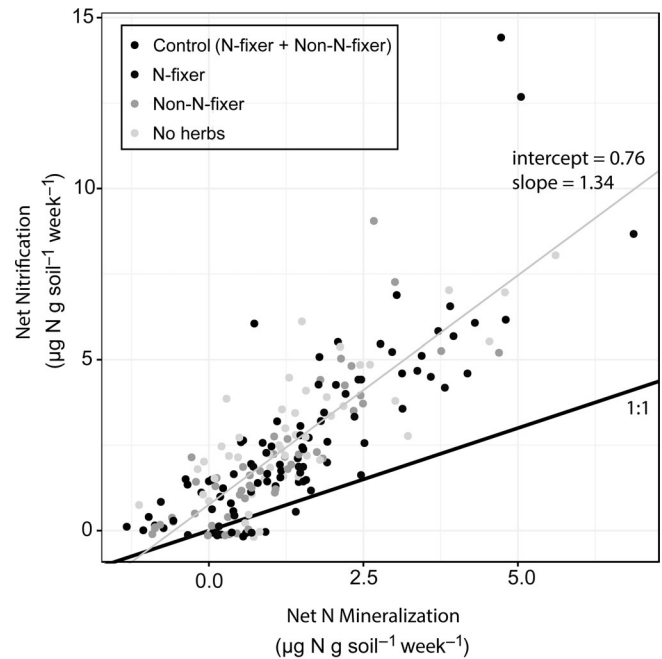


FIGURE 4 Relationship between net nitrification and net N mineralization in May 2018 ($R^2 = 0.68$, $p < 0.001$, $n = 191$).

significantly lower in respiration than all plots with herbs (N-fixer, non-N-fixer, and control), which were not significantly different from each other, with a mean difference of $3.8 \mu\text{g C (g soil}^{-1}\text{) day}^{-1}$ at any month; thus, the effect of full herb removal (no-herb plots) compared with the three treatments with herbs was 23% decrease in soil respiration in March and 32% in April and 37% in May. When May 2018 respiration rates were normalized by soil TC (results below), there was no significant difference between treatments ($3.7 \pm 0.28 \mu\text{g C respired/mg soil C}$, $F_{3,20} = 0.51$, $p = 0.680$).

Bulk soil C and N

Bulk soil C and N were measured at the beginning and end of the experiment. Changes in bulk soil TC, TN, and C:N varied by treatment: TC (treatment \times date, $F = 3.98$, $p = 0.022$), TN (treatment \times date, $F = 2.93$, $p = 0.058$), C:N ratio (treatment \times date, $F = 3.07$, $p = 0.051$; Figure 6). At the beginning of the experiment (March 2017), there were no statistically significant

FIGURE 3 Mineral N pools, net N mineralization, net nitrification, and soil gravimetric moisture over the two years of sampling. Error bars represent SE. Lowercase letters indicate groups with statistically significantly different means ($p \leq 0.10$) in planned contrasts of each treatment at each month. When no letter is shown, there are no statistically significant treatment differences for a particular month. Planned contrasts were performed based on comparisons of estimated marginal means from a linear mixed-effects model (treatment \times date with random block and plot).

treatment differences in bulk soil TC ($p > 0.2$ for all contrasts, mean = 25.0 mg/g soil), TN ($p > 0.2$, 1.6 mg/g soil), or C:N ratio ($p > 0.24$, 15.4). By the end of the experiment (May 2018), control (all-herb) plots were significantly higher than no-herb plots in C ($p = 0.023$, +8.6 mg/g), N ($p = 0.024$, +0.36 mg/g), and C:N (+2.1, $p = 0.076$). The effect of full removal of herbs was substantial, with no-herbs plots 31% lower in C, 22% lower in N, and 13% lower in C:N ratio than all-herb plots. Partial-removal plots, with either N-fixers or non-N-fixers only, were intermediate to the two other treatments, and were neither significantly different from one another or the other two treatments. We also examined the absolute change in

soil C and N from the beginning to end of the experiment (Figure 6D,E). Control (all-herbs) plots gained soil C (4.9 ± 0.9 mg/g), which was a significant difference from N-fixer (-3.6 ± 2.1 mg/g) and no-herb plots (-6.0 ± 3.2 mg/g), which both lost C. Non-N-fixer plots were intermediate and maintained the same soil C values (0.3 ± 2.8 mg/g). Control (all-herbs) plots gained soil N (0.12 ± 0.05 mg/g), which was a significant difference from N-fixer (-0.17 ± 0.06 mg/g) and no-herb plots (-0.29 ± 0.16 mg/g), which both lost N. Non-N-fixer plots were intermediate and delta N values were not significantly different from 0 (-0.07 ± 0.10 mg/g).

DISCUSSION

The drivers of N cycling and retention after chaparral fire have been a topic of intense research interest (e.g., Goodridge et al., 2018; Hanan et al., 2017), including the role of fast-growing herbs in absorbing soil available N that could be lost through leaching, runoff, or denitrification (Hanan, D'Antonio, et al., 2016; Rundel & Parsons, 1984). However, ours was the first study to test the impact of two key herb functional groups, N-fixers and non-N-fixers, on postfire chaparral N and C cycling, through a direct manipulation of germinating herbs. Consistent with other investigations (Hanan, D'Antonio, et al., 2016; Verkaik et al., 2013), our freshly burned plots

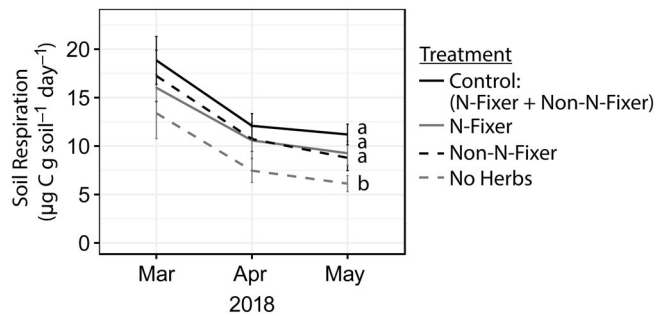


FIGURE 5 Soil microbial respiration over three months of the 2018 growing season. Lowercase letters indicate statistically significantly different means ($p \leq 0.10$) averaged over the three months of sampling. Error bars represent SE ($n = 6$).

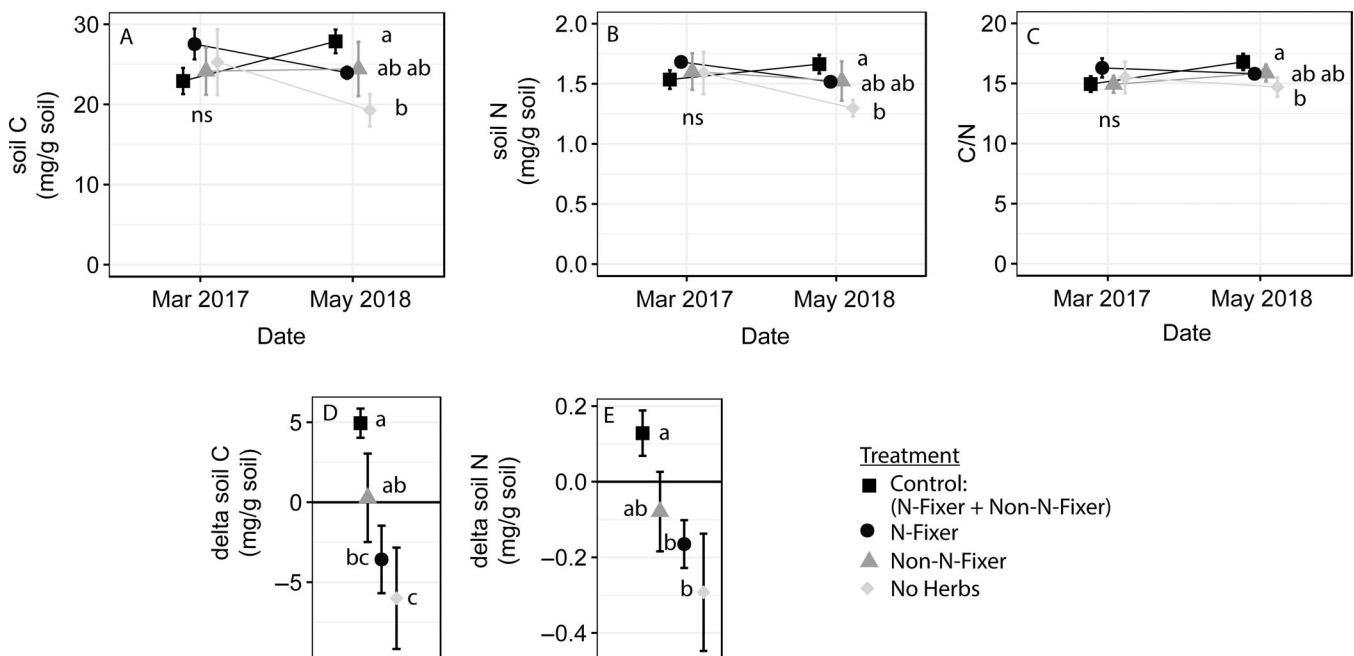


FIGURE 6 Bulk soil (A) total C, (B) total N, and (C) C:N ratio and the change in bulk soil (D) C and (E) N from March 2017 to May 2018. Lowercase letters indicate statistically significantly different means ($p \leq 0.10$) in planned contrast at each date ($n = 6$). Planned contrasts were performed based on comparisons of estimated marginal means from a linear mixed-effects model (treatment \times date with random block and plot). Error bars represent SE.

had elevated soil mineral N concentrations (particularly NH_4^+) compared with nonburned adjacent sites, and those concentrations dropped rapidly throughout the two-year study. As hypothesized for this high-labile-N environmental, N-fixers were competitively inhibited by non-N-fixers in both years of the study (Figure 2). By the second year after fire, there were clear differences in N and C cycling between treatments. As predicted, N-fixer plots were higher in net N mineralization and net nitrification than all-herb and non-N-fixer plots, but N cycling was also elevated in no-herb plots, which we did not predict (Figure 3). No-herb plots were also consistently elevated in mineral N (NH_4^+ and NO_3^-) from the end of the first year and throughout the second, likely due to a lack of plant uptake. N-fixer plots were also elevated in NH_4^+ from the end of the first year, likely due to soil inputs from symbiotic fixation and/or due to reduced mineral N uptake from soil. Rates of microbial respiration were significantly higher in all treatments with herbs (controls, N-fixers, and non-N-fixers) than in no-herb plots, and were positively correlated with soil total C (in May 2018), regardless of treatment (Figure 5). At the end of the first year, plant N pools were highest in N-fixer plots, followed by controls, then non-N-fixers. However, by the second year, controls retained the largest plant N pool ($1877 \pm 276 \text{ mg N/m}^2$), with most of the contribution coming from non-N-fixers (Table 1). All-herb controls were also the only treatment to accumulate soil C and N by the end of the experiment. N-fixer and no-herb plots lost C and N (Figure 6). These results point to the importance of both N-fixing and non-N-fixing plants for maximizing soil C accumulation and N retention after fire, with non-N-fixers ultimately contributing to more N immobilization and biomass production. Specific results and implications are discussed further below.

Herb functional group interactions and N pools

Soil nutrient conditions can influence the interaction between N-fixing and non-N-fixing herbs, which can in turn impact plant functional group abundance and soil biogeochemistry. These plant–soil feedbacks can drive plant and soil successional patterns (Bonanomi et al., 2005; Koffel et al., 2018; Matson, 1990). In our study, the selective removal of N-fixing or non-N-fixing herbs allowed us to assess the impact of postfire conditions on these herb functional groups alone or together, lending insight into how these functional groups might interact over the first two years of postfire succession. Our results suggest that N-fixers are competitively suppressed by non-N-fixing herbs, growing to twice their biomass when non-N-fixers were removed (Figure 2). This finding

is consistent with predictions for environments with temporarily elevated N, such as postfire chaparral, where the ability to fix N is less advantageous (Haubensak & D'Antonio, 2011; Koffel et al., 2018; Vitousek et al., 2002; Yelenik et al., 2017). Symbiotic N fixation is costly for host plants, in terms of C and mineral resources (Gutschick, 1981; Vitousek & Howarth, 1991), and there is a cost of N-fixation ability whether the plant symbiont is an obligate or facultative N-fixer (Menge et al., 2009). On the other hand, non-N-fixer abundance was unaffected by N-fixer removal, implying that the former was neither competitively inhibited nor facilitated by the latter. Nitrogen-fixers can facilitate non-N-fixers through the addition of low C:N litter (Maron & Connors, 1996; Menge et al., 2008) and exudate from their roots (Paynel et al., 2008), which may ultimately competitively inhibit the N-fixers themselves. It is likely that in the high-available-N postfire environment, non-N-fixers were not N-limited and thus not facilitated by N-fixers. It is also possible that N-fixers were not fixing N in this system; however, nearly all legume roots (Fabaceae) were covered with red nodules at removal and NH_4^+ -N was elevated in N-fixer plots, both suggesting that symbiotic N fixation was occurring. Future investigation could address whether N-fixers facilitate growth of non-N-fixing chaparral herbs and shrubs beyond the first two postfire years, when soil available N becomes more limiting.

We predicted that, assuming higher availability in the first year than the second, non-N-fixers would contribute to higher biomass and tissue N in the first year, while N-fixers would be a larger contributor in the second year. Curiously, our results did not follow this trend, even though soil mineral N concentrations and net N mineralization generally decreased in controls between years (see below). At the end of the first year after fire, there were no differences in biomass abundance between all-herb control, non-N-fixer, and N-fixer plots, so the ranking of herbaceous N pools mirrored estimates of tissue N concentrations: N-fixer and all-herb controls had significantly higher herbaceous N than non-N-fixers (Table 1). However, the results we predicted for the first year did emerge by the end of the second year. The plant N pool at the end of two years was maximized by a mix of N-fixers and non-N-fixers. Additionally, non-N-fixers grown alone produced more biomass (Figure 2) and tissue N than N-fixers grown alone, which could lead to greater C inputs to and accumulation in soil, especially if non-N-fixing litter has higher C:N and thus a longer residence time in soils (see below). However, the estimated herbaceous N should be interpreted cautiously, because C:N values were derived from a different neighboring site for a few select species. Common N-non-fixing grasses and forbs can increase

dramatically in percent in high-N conditions (Henry et al., 2005), while N-fixers can reduce N-fixation rates (Regus et al., 2017), potentially decreasing the difference in tissue N between these groups. At the same time, our results suggest important patterns, even if the N contents of non-N-fixers were underestimated. In 2018, the herbaceous N in control plots was contained mostly in non-N-fixers, supporting our hypothesis that non-N-fixers are better able to respond to N fertilization with increased growth.

The response of non-N-fixers to N-fixer removal varied by species and functional group of herbs (Appendix S1: Table S3). Of particular note is that a common non-N-fixing native annual, *Emmenanthe penduliflora*, grew substantially greater biomass when N-fixers were removed, implying competitive release (in 2017 only). On the other hand, several non-native grasses and forbs grew greater biomass in the presence of N-fixers in 2018, suggesting that some non-N-fixers can be facilitated by N-fixers, at least in the second year when soil N concentrations are lower. Thus, the response of non-N-fixers to N-fixer neighbor removal may vary across sites, particularly based on whether those sites are dominated by natives or non-natives. There were 42 non-N-fixing herbs found among the plots, of which 20 were non-native annuals (Appendix S1: Table S1).

Our results also clearly suggest the importance of an assemblage including N-fixers for maximizing herbaceous N pools: all-herb controls accumulated the greatest herbaceous N and the end of the study (Table 1). Additionally, reduced competition with non-N-fixers may impact the persistence of N-fixers in chaparral, with long-term implications for N-supply. Other studies have suggested a key role for N-fixers in postfire N-supply (Guo, 2001; Rundel & Parsons, 1984), with one common native perennial found at our site (*Acmispon glaber*) capable of fixing up to 10–15 kg ha⁻¹ year⁻¹ of N and dropping 83% of its annual leaf production in the litter layer (Nilsen & Schlesinger, 1981). In the second year of our study, *A. glaber* was released from competition with non-N-fixers and grew significantly greater biomass in N-fixer-only plots than in controls (N-fixer and non-N-fixer) (Appendix S1: Figure S2). *A. glaber* seeds germinate prolifically in the first year after fire, then becomes established as perennial by the second year, often persisting in mature chaparral (Guo, 2001). This result suggests that reduced competition with non-N-fixers in early postfire years could impact the abundance of this persistent N-fixer in mature chaparral. Curiously, annual *Acmispon* spp. were only released from competition by non-N-fixer removal in the first year, although they comprised the majority of N-fixer biomass in both years. The diversity of N-fixers in our sites was much lower than that of non-N-fixers (Appendix S1: Table S1), perhaps suggesting

less resiliency in the case of local random variation in species distribution or local extirpation of N-fixers (Biggs et al., 2020), resulting in decreased litter N inputs in the critical years after chaparral fire.

C and N cycling

We investigated the impact to N and C cycling of removing N-fixing and non-N-fixing herbs, two groups predicted to exert distinct effects on soil biogeochemistry. Our prediction was that N-fixers would support faster net N mineralization, along with greater loss and/or slower accumulation of organic soil N. We predicted that the contribution of higher C:N litter from non-N-fixers would result in slower net N mineralization over the course of the experiment, perhaps resulting in greater N and C accumulation in soil organic matter. Our results supported the idea that N-fixing herbs promote higher rates of net N mineralization, net nitrification, and higher soil NH₄⁺-N concentrations. Even in the first postfire season, NH₄⁺-N was elevated in N-fixer plots, even though there were almost no treatment differences in N mineralization or net nitrification in this first year (Figure 3A,C,E,G). Nitrogen-fixers can exude NH₄⁺-N into soils through rhizodeposition (Fustec et al., 2010) or simply allow greater NH₄⁺-N accumulation in soils due to lower demand for mineral N uptake. It is likely that elevated net N mineralization rates in the second year were supported by the decomposition of N-fixer litter from the first year. Our parallel study of decomposition at a nearby postfire chaparral site supports the idea of rapid decomposition in N-fixers (Wahl et al., in prep.), finding that the N-fixer (*Acmispon glaber*) decayed more rapidly than two non-N-fixers (*Emmenanthe penduliflora* and *Chlorogalum pomeridianum*) in the second year after fire.

Interannual weather variability could have impacted our findings. Although temperatures were consistent between the two years of the study, the first year of the study was substantially wetter than the first, with more than four times the precipitation falling in the first year than the second. Increase in precipitation is predicted to accelerate decomposition and hydrological losses of N. Therefore, while losses of NH₄⁺ and NO₃⁻ may have been steeper in the first year (Figure 3A,C), the interannual weather variability does not account for the elevated net N mineralization in control and N-fixer plots in the second year (Figure 3F,H), when conditions were actually drier. The interannual weather conditions do not remove support from the idea that increased N-rich plant litter supported decomposition in the second year.

Plots with all herbs (N-fixers and non-N-fixers) showed similar N dynamics to plots with only

non-N-fixers. By the end of the first year and throughout the second, these treatments were lower in soil mineral N concentrations, and in the second year, they were lower in net N mineralization and net nitrification than N-fixer plots (Figure 3A–H). Based on mineral N results alone, it is difficult to deduce whether N-fixers are contributing N to their non-N-fixing neighbors. Although there was no evidence of facilitation for non-N-fixers by N-fixers, based on vegetation abundance, it is noteworthy that all-herb control plots did not have higher soil mineral N concentrations than non-N-fixer plots, despite the presence of N-fixers (Figure 3A–D). This could reflect the direct transfer of rhizodeposited NH_4^+ -N from N-fixers to non-N-fixers, which has been demonstrated in other ecosystems (Paynel et al., 2008), or the increased immobilization of N by soil microbes, given that litter from non-N-fixers is likely higher in C:N. Both of these treatments had low or even negative rates of net mineralization (Figure 3E,F). While we did not measure decomposition in these sites, a possible explanation is that mineralization rates are influenced by the decomposition of high C:N plant litter, which would decompose more slowly and even lead to the immobilization of N by microbes during early decomposition (Parton et al., 2007). Even without confirming an underlying mechanism, it is clear that the presence of non-N-fixers helps maintain low soil concentration of mineral N and low rates of mineral N resupply in the early postfire years. This result supports the hypothesis that non-N-fixers are essential for absorbing N and potentially preventing runoff from an unprotected soil mineral N pool.

Patterns of N cycling in bare plots may have indicated the potential for N losses during the postfire years, particularly given that net nitrification can be a key factor influencing postfire chaparral soil N export (Goodridge et al., 2018; Hanan et al., 2017). Soils in no-herb plots had the highest rate of net nitrification (similar to N-fixer plots) and also the highest soil concentrations of NO_3^- -N of any plots (Figure 3C,D,G,H). Abundant NH_4^+ -N substrate, typical of postfire soils, is known to be a key driver of postfire net nitrification in chaparral (Fenn et al., 1993; Hanan, Schimel, et al., 2016; Homyak et al., 2014). Without plant uptake of mineral N, it is plausible that accumulated NH_4^+ -N supported a robust soil microbial community of nitrifiers. Other postfire conditions, which would have been present in all treatments, could have further promoted net nitrification. For example, inputs of charcoal (pyrogenic carbon), which is common after chaparral fire (Santin et al., 2020), stimulate and maintain the abundance of nitrifying ammonium oxidizing bacteria (AOB) (Ball et al., 2010); increased soil pH enhances net nitrification (Hanan, Schimel, et al., 2016). At the end of each growing season, NO_3^- -N peaked in

non-herbs plots, but not in any of the plots with herbs. Even N-fixer plots, which had similar rates of NO_3^- -N supply (net nitrification), did not demonstrate an increased accumulation of NO_3^- -N in soils (Figure 3C,D,G,H). Although NO_3^- -N is rarely a preferred nitrogen source for plants (Salsac et al., 1987), it is plausible that NO_3^- -N accumulated in bare plots due to a lack of herb uptake, which would have been particularly noticeable at the end of the growing season, when decreased precipitation is associated with decreased N export (Homyak et al., 2014; Valeron & Meixner, 2010).

The high second-year net N mineralization and net nitrification rates in no-herb plots are a curious result that is difficult to interpret with certainty (Figure 3F,H). One possibility could be decreased C availability for soil microbes, in the absence of herbaceous litter inputs, as microbes decomposed resident soil organic matter. Microbial C limitation could lead to increased net N mineralization by soil microbes, ultimately depleting total soil N. In support of this mechanistic hypothesis, no-herb plots were lower in C mineralization (microbial respiration) than all other plots (Figure 5). Final soil C:N was lower in no-herb than all-herb plots, but not non-N-fixing plots, so soil C:N ratio alone cannot explain the elevation of net N mineralization in these plots, at least at the timescale measured (Figure 6).

Across all timepoints and treatments, net nitrification was correlated with and consistently faster than net N mineralization, suggesting that mineralized N, as well as NH_4^+ -N deposited with ash, was quickly nitrified to NO_3^- -N (Figure 4). Gross rates of nitrification could have been much higher, potentially masking microbial uptake of NO_3^- -N (Stark & Hart, 1997; Verchot et al., 2001). The labile litter inputs (low C:N and low lignin) from fire-following herbs can stimulate microbial biomass and N turnover (Hart et al., 2005), thus serving as an important N retention pool that also synergistically supports the ongoing growth of postfire herbs (Goodridge et al., 2018). Soil microbes can immobilize substantial pools of mineral N, in some cases even greater than the herbs themselves (Hart et al., 2005; Stark & Hart, 1997).

A key objective of this study was to investigate not only how herb growth impacted short-term mineral N cycling patterns, but also what implications this would have for the retention of N and C in soils. Our findings generally support the hypothesis that the presence of herbs resulted in higher soil N and SOC, although differences between functional groups were not statistically significant at this timescale. Only all-herb plots gained soil C and N throughout the experiment, although the gains were not significantly different from non-N-fixer plots (Figure 6). The reduction in soil C in no-herb plots was dramatic, with no-herb plots more than 30% lower in

soil C than control (all-herb plots) two years after fire. The SOC pool also seemed to influence soil microbial respiration, with no treatment differences between the specific soil respiration (normalized for total soil C). A high availability of plant litter can stimulate microbial activity and increase microbial biomass (Plante & Parton, 2007). Although we predicted a difference in total soil C and N based on herb functional group, there was no significant difference in these soil pools between any of the herb plots. Our results do suggest the importance of non-N-fixers in accumulating soil C, as predicted. While there was no difference in delta soil C between no-herb and N-fixer plots, non-N-fixer plots had higher C accumulation than no-herb plots by the end of the experiment. Similarly, control plots (non-N-fixers with N-fixers) had significantly higher C accumulation than N-fixers grown alone. In contrast, the presence of N-fixers to non-N-fixer assemblages (i.e., N-fixers vs. no herbs or controls vs. non-N-fixers) never resulted in increased C accumulation.

Conclusions

It has been repeatedly proposed that herbaceous communities in chaparral play an important role in postfire N retention when soil mineral N is elevated, shrub cover is temporarily removed, and potential for ecosystem N losses is high. While the idea of fire-following herbs as “pyrogenic dams” (Goodridge et al., 2018) has been investigated through modeling (Hanan et al., 2017) and measurements of plant uptake (Hanan, D’Antonio, et al., 2016; Rundel & Parsons, 1984), the present experiment directly tested the impact of herb removal on postfire soil N and C cycling. Our findings underscore the importance of fire-following herbs for soil N and C accumulation after fire. At this postfire chaparral site, soils were rich in mineral N when both N-fixing and non-N-fixing herbs were abundant. Plots with no herbs growing after fire resulted in substantially lower soil C and N than those unmanipulated all-herb plots. In no-herb plots, there were also lower rates of C mineralization, but higher rates of N mineralization, potentially due to depletion of soil C and N in these plots. While N-fixers drove higher rates of net N mineralization, this did not lead to greater N storage in soils than in the other herb treatments, and even lower than all-herb control plots. The presence and identity of postfire herb assemblages impacted soil C and N dynamics, even over the fairly short two-year timescale of this study. The presence of non-N-fixing herbs increased soil C, and all-herb plots accumulated more soil N than no-herb and N-fixing plots; this elevated soil nutrient availability may contribute to the long-term recovery of shrubs, particularly those that

are not N-fixers, even after herbs are no longer dominant. Future research should also consider how the soil microbial N pool is impacted by the functional group of plant litter decomposition inputs.

Future investigation is needed to address the management implications of these findings. Land managers and restoration ecologists are concerned with the effect of localized species loss on postfire chaparral recovery, although discussions have typically focused on seed loss by chaparral shrubs species (Allen et al., 2018). In the early 20th century, freshly burned chaparral was routinely treated for erosion control by aerial spraying with non-native grass seeds. While this method was generally not effective to reduce erosion, non-native grasses did reduce the abundance of native shrubs and fire-following herbs (Beyers, 2004). Thus, human activities have altered the seedbanks of some chaparral areas, even leading to areas persistently dominated by an understory or invasive grasses (Park & Darrel Jenerette, 2019). Chaparral restoration plans that involve the removal of non-native grasses should also consider the importance of promoting a native pool of non-N-fixing herbs, when optimizing N and C soil storage is a goal.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and R code (Hendricks-Franco, 2023) are available from Zenodo: <https://doi.org/10.5281/zenodo.8015418>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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