Received: 6 August 2020 | Accepted: 11 January 2021

DOI: 10.1111/1365-2745.13610

RESEARCH ARTICLE

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

Evidence for a fungal loop in shrublands

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Funding information
Dimensions of Biodiversity, National Science Foundation, Grant/Award Number: 1840931

Handling Editor: Amy Austin

Abstract

1. Dryland communities may mitigate the loss of limited resources by exchanging nutrients through subterranean fungal connections, termed fungal loops. In arid grasslands, fungal loops can influence community composition and primary productivity, yet their ecological significance across dryland systems remains unexplored. We investigated the functional role of fungal loops in nutrient translocation in a North American shrubland ecosystem.
2. We traced the movement of ^{15}N from moss-dominated biocrusts to the dominant xeric shrub *Larrea tridentata*, and the movement of ^{13}C from *L. tridentata* to biocrusts in plots established in situ in the Sonoran Desert. Measurements occurred at three time points spanning 1 week following a simulated 2.5 mm rainfall event, and at distances up to 1 m from tracer application. We also used ITS sequencing to investigate changes in fungal community composition in soils over the 1-week period.
3. We discovered movement of ^{15}N from biocrusts into *L. tridentata* foliage as well as ^{15}N movement to other spatially isolated moss-dominated biocrust patches, yet this movement did not occur until 4–6 days post-rainfall, when significantly higher $\delta^{15}N$ was observed in *L. tridentata* and biocrusts compared to previous days. We did not observe consistent patterns of ^{13}C movement from *L. tridentata* into neighbouring shrubs or biocrusts, suggesting differential environmental drivers for carbon movement in this system. Fungal communities exhibited a decrease in alpha diversity on the last day of the study, indicative of a delayed community response to rainfall concomitant with nutrient translocation. Fungal endophyte orders Pleosporales and Pezizales dominated all plot soils, and order Pleosporales was significantly more abundant in ^{15}N enriched plots, suggesting that dark septate endophytic fungi were involved in nitrogen translocation. The delay in nutrient translocation may reflect a rainfall-triggered rebuilding of mycelial networks between community members following drought.
4. *Synthesis*. Our results point to fungal-mediated nutrient exchange pathways in a previously uninvestigated vegetation type, shrublands, where nutrients are translocated between moss-dominated biocrusts and nearby shrubs. We provide the first evidence that nutrient transfer may be delayed up to 6 days following rainfall, consistent with pulse-dynamic responses in drylands, and that moss-dominated biocrusts play a role in fungal loops.

KEYWORDS
dark septate endophyte, drought response, dryland, *Larrea tridentata*, moss-dominated biocrust, nutrient exchange, pulse delay, stable isotope tracer

1 | INTRODUCTION

Biological networks that enable resource exchange between plants and microbes are important drivers of primary production (Klironomos et al., 2000; Vogt et al., 1982), trophic dynamics (Averill et al., 2014) and biogeochemical cycling (Fogel & Hunt, 1983; Phillips et al., 2013) in nearly all terrestrial ecosystems. Endophytic fungi often act as the conduits for these relationships, connecting spatially or temporally isolated community members (and their respective metabolic processes) through the exchange of nutrients, water and secondary metabolites via fungal hyphae (Hättenschwiler et al., 2011; He et al., 2003). In dryland systems (e.g. deserts, semi deserts, savannas, and shrublands), similar mycelial networks, termed fungal loops, may enable nutrient exchange between the two dominant producer communities: biological soil crusts (biocrusts) and vascular plants (Green et al., 2008; Rudgers et al., 2018).

Biocrusts are diverse communities of cyanobacteria, mosses, lichens and microbes living in the top several centimetres of soils that fix carbon (C) (Belnap et al., 2016; Chilton et al., 2018), increase water retention (Chamizo et al., 2016; Xiao et al., 2019), stabilize soil (Belnap & Büdel, 2016; Chamizo et al., 2017) and often act as a primary source of nitrogen (N) in the ecosystems they inhabit (Evans & Ehleringer, 1993; Evans & Lange, 2001). Biocrusts can dominate the groundcover of many dryland systems and considering that they can be active following rain events as small as 1.25 mm (Coe et al., 2012), often play a significant role in dryland resource dynamics (Büdel et al., 2009; Elbert et al., 2012; Lenhart et al., 2015; Pointing & Belnap, 2012; Porada et al., 2013, 2014). Vascular plants generate the majority of organic matter in drylands (Aguiar & Sala, 1999), yet often appear in patchy 'resource islands' of biological activity across dryland landscapes (Belsky et al., 1989; Dunkerley, 2014; Garcia-Moya & McKell, 1970; Schlesinger & Pilmanis, 1998; Tiedemann & Klemmedson, 1986; van der Valk & Warner, 2009; Zhang et al., 2016). Dryland vascular plants are primarily limited by water (and secondarily N), and in contrast to biocrusts, usually require rainfall events >5 mm to activate metabolism (Collins et al., 2014; Lauenroth & Bradford, 2012). The spatial separation between dryland vascular plant rooting depth and biocrusts, along with the decoupled nature of their metabolic activities in response to rainfall thresholds, suggests that these communities may operate using relatively isolated nutrient pools (Collins et al., 2014; Dettweiler-Robinson et al., 2020). If present, a fungal loop between biocrusts and vascular plants would enable the retention and exchange of limiting nutrients (such as N and C) that would otherwise be isolated or lost from the system through processes such as volatilization and photodegradation (Adair et al., 2017; Austin & Vivanco, 2006), as well as leaching and erosion (Peterjohn & Schlesinger, 1990; Throop & Archer, 2009). In this way, fungal loops may mediate community responses to pulsed precipitation events in dryland systems, coupling temporally isolated C and N cycling, net primary production and decomposition processes between spatially isolated organisms (Collins et al., 2008, 2014; Dijkstra et al., 2012).

Evidence for the existence and functional role of fungal loops in dryland systems derives primarily from studies conducted in grasslands in North America and Asia (Aanderud et al., 2018; Dettweiler-Robinson et al., 2018, 2019; Green et al., 2008; Hawkes, 2003; Lingfei et al., 2005; Lutgen et al., 2003; Rudgers et al., 2018; Zhuang et al., 2015). These studies have documented the biologically mediated (Collins et al., 2014) movement of the stable isotope ^{15}N from cyanobacterial biocrusts to nearby grasses and forbs (with one study showing the reciprocal movement of ^{13}C from grasses to biocrusts; Green et al., 2008), and have found that dark septate fungal endophytes in orders such as Pleosporales and Pezizales routinely dominate the microbial communities in both biocrust and plant tissues (Allen, 2007; Apple, 2010; Barrow, 2003; Bates et al., 2012; Maier et al., 2016; Massimo et al., 2015), as well as the rhizosphere soils and soil interspaces in these experiments (Aanderud et al., 2018; de Mesquita et al., 2018; Green et al., 2008; Porras-Alfaro & Bayman, 2011; She et al., 2018). Dark septate endophyte (DSE) taxa in the soil can increase in community dominance and abundance in response to the moving concentration gradient of ^{15}N through soil interspaces (Dean et al., 2014; She et al., 2018), and their presence in the soil correlates with improved plant and biocrust fitness (reviewed by Newsham, 2011). Considering current literature relating N translocation through dark septate fungal bodies (Collins et al., 2014; de Mesquita et al., 2018; Green et al., 2008; Rudgers et al., 2018; She et al., 2018; Zhuang et al., 2015), the direct role of DSEs in the exchange of nutrients across drylands is likely. In grasslands, the direct or indirect severing of fungal connections has resulted in decreased primary production in vascular plant communities (Dettweiler-Robinson et al., 2018, 2020), and even changes in plant community composition (reviewed by Zhang et al., 2016), indicating that the presence of fungal networks directly impacted these two phenomena. Evidence suggests the presence of fungal nutrient exchange networks between biocrust and plant communities in grasslands. However, nutrient exchange rate, directionality of nutrient exchange and the community impact of fungal loops likely depend greatly on the climate, precipitation regime and functional group of the organisms involved (Rudgers et al., 2018; Zhang et al., 2016).

One biocrust functional type that may play a particularly important role in nutrient exchange dynamics and fungal loops in drylands are moss-dominated biocrusts. Mosses often dominate mature, late successional crusts (vs. early successional crusts dominated by certain species of cyanobacteria), and biocrust maturity is often correlated with ecological function in biocrusts, most notably nutrient cycling (Barger et al., 2016; Chamizo et al., 2012). While mosses do not fix N themselves, they often form symbiotic associations with N-fixing cyanobacteria (Adams & Duggan, 2008), whereby providing a habitat for these organisms as well as a direct conduit via their rhizoids for N movement to associated soil dwelling organisms. As such, moss-dominated biocrusts have been shown to increase N availability (specifically in the form of NH_4^+) in soils (Hu et al., 2020), and contain substantially varying microbial communities than cyanobacteria-dominated crusts from similar regions (Xiao & Veste, 2017). Therefore, dryland mosses within biocrusts may act as key hubs for N fixation (Su et al., 2011) as well as translocation in dryland systems.

Fungal loops appear to play an important role in the ecology in some dryland systems (Collins et al., 2008, 2014), and are also likely to become increasingly important as conduits for nutrient exchange under climate change-induced fluctuations in precipitation (Gherardi & Sala, 2019) in these already water-limited environments. In spite of their ecological significance, the functional role of fungal loops across dryland systems remains unexplored or incompletely captured. First, the presence and role of fungal loops in shrublands remains undocumented, even though shrublands are rapidly replacing grasslands world-wide due to shifting climate regimes (Van Auken, 2000), and already account for over half of drylands globally (Giri et al., 2005). Second, moss-dominated biocrusts have structural, functional and nutrient cycling roles unique from cyanobacteria-dominated biocrusts (Chen et al., 2019; Reed et al., 2012; Zhao et al., 2010), yet remain largely absent from our understanding of fungal loops (Aanderud et al., 2018). Finally, fungal loops, like other biological processes in drylands, require water inputs from precipitation to operate (Collins et al., 2014), yet the role of rainfall in fungal-mediated resource translocation has yet to be fully represented in fungal loop studies. Field simulation experiments to date have seldom hydrated more than the site of tracer application (Aanderud et al., 2018; Green et al., 2008; Zhuang et al., 2015), and have thus not hydrated the greater surface community likely to be linked by fungal connections following an actual rainfall event. Additionally, nutrient dynamics in drylands are proposed to follow a pulsed and rainfall-mediated Threshold Delay Nutrient Dynamics (TDND) model (Collins et al., 2008, 2014; Goldberg & Novoplansky, 1997; Noy-Meir, 1973), where rainfall events activate different primary producers and heterotrophs depending on timing and volume. However, the impact of TDND characteristics on the functional ecology of fungal loops, including temporal delays in biological activity in response to rainfall, remain incompletely addressed (Collins et al., 2014). To illustrate, the fungal loop study of longest duration spanned 4 days following a single precipitation event (Rudgers et al., 2018). Considering that endophytic connections have been shown to both break down and re-form in less than four days (Jacobson et al., 2015; Staddon et al., 2003), and that these rates can be affected by water availability (Apple, 2010; Apple et al., 2005), previous methodologies could have missed relationships that would occur following an actual rainfall event, or that occur over longer time-scales.

Guided by the patterns of nutrient exchange shown to exist in grasslands, this study sought to determine the existence and functional significance of a fungal loop in a North American shrubland system dominated by creosote bush (*Larrea tridentata*) and moss-dominated biocrusts. *L. tridentata* is estimated to occur over 330 million ha across the arid and semi-arid United States (Gibbens et al., 2005; Kim et al., 2017; Knapp et al., 2008), where it represents the dominant vegetation across 19 million ha of previous grassland (Van Auken, 2000). Centring on these two ecologically important functional types, moss-dominated crusts and *L. tridentata* shrubs, we investigated three related hypotheses: Given a common rainfall event for the season, (a) N will move from patches of moss-dominated biocrusts to *L. tridentata* tissue, (b) C will move from *L. tridentata* shrubs to biocrust tissue and (c) fungal taxa known to contain DSEs will increase in both relative abundance and community dominance in soil interspaces, mirroring the moving concentration of nutrients between the two community members. Finally, while the aforementioned directions of nutrient translocation were the primary target of our study, we also sought to investigate two additional nutrient translocation pathways. First, as nutrients have long been shown to move between spatially isolated vascular plants (Bethlenfalvay et al., 1991; Frey & Schüepp, 1992; Simard et al., 1997; Van der Heijden & Horton, 2009), we examined potential movement of C between *L. tridentata* shrubs. Second, as fungal-mediated nutrient translocation between mosses (including mosses within biocrusts) has never been identified, we also investigated the potential movement of N between spatially isolated moss-dominated biocrust patches.

2 | MATERIALS AND METHODS

2.1 | Site description

This study took place in Estrella Mountain Regional Park, located in the Sonoran Desert in south-central Arizona, USA (33°21′50.0″N 112°18′37.9″W, 3 m), between 25 July and 1 August 2019. Temperatures reached mid-day highs between 36.1 and 45°C and nightly lows between 24.4 and 33.8°C (NOAA, 2020). This site received ~200 mm of rainfall in 2019, and 17 mm of rainfall in the month of July (NOAA, 2020). No precipitation occurred at the field site during the experiment, with the last rain event of 2.5 mm occurring 21 days prior to the start of the experiment (NOAA, 2020). Soil in the region is an Ebon-Pinamt complex, with an A horizon of gravelly loam (Natural Resources Conservation Service, 2020; USGS, 2019) and pH of 6.8 (Allen et al., 2018). Dominant plant flora included the shrub Creosote Bush *L. tridentata*, as well as Palo Verde *Parkinsonia aculeata* and Saguaro Cactus *Carnegiea gigantea*. Biocrust communities contained mosses (*Encalypta*, *Ceratodon*, *Funaria*, *Bryum* and *Syntrichia*), lichens (*Collema* and *Peltula*) and cyanobacteria (*Microcoleus*, *Scytonema* and *Nostoc*) in varying proportions depending on microhabitat. Moss-dominated biocrusts were defined as a biocrust surface with >60% moss cover, and only patches >3 cm in diameter were sampled in this study.

2.2 | Establishment of study plots and isotopic tracer application

To investigate the unidirectional movement of N from moss-dominated biocrust patches into the surrounding community, three 2 m diameter plots were each centred on a patch of moss-dominated biocrust (represented as a red star in Figure 1), where ^{15}N-labelled ammonium sulphate was eventually applied. These plots contained three *L. tridentata* shrubs at varying distances from the central moss-dominated biocrust patch, as well as other isolated moss-dominated biocrust patches (accounting for >10% of each plot surface), and sufficient bare soil to collect soil samples for fungal community analysis along three transects from the centre of the plot (Figure 1). Each plot was at least 10 m away from other plots, and each plot did not include substantial variances in microtopography or slope. To investigate the unidirectional movement of C from *L. tridentata* into the surrounding community, three plots were each centred on a *L tridentata* shrub (also represented as a red star in Figure 1), where ^{13}C-labelled glutamic acid was eventually applied. These plots contained the same vegetation cover criteria as the ^{15}N-enriched plots. Three control plots were established to observe naturally occurring concentrations of each stable isotope throughout the experimental period as well as the fungal community response to rainfall without stable isotope enrichment. These control plots received a simulated rainfall event but no isotope enrichment. Control plots were not centred on any particular vegetation; however, they contained the same vegetation cover criteria as the other plots.

Stable isotope application occurred on 26 July 2019 between 10 a.m. and 1 p.m. An atomizer with a 5 cm diameter area of application was used to apply 0.5 g of 99 atom % $(^{15}NH_4)_2SO_4$, dissolved in 5 ml of filter-sterilized water, to the biocrust at the centre of each ^{15}N enriched plot. Ammonium was chosen as a tracer due to its natural presence in Sonoran Desert soil (Hall et al., 2011)—primarily due to N_2 fixation by biocrust cyanobacteria (Belnap, 2003; Welter et al., 2005)—as well as its repeated use in fungal loop studies (Reviewed by Rudgers et al., 2018). $^{15}NH_4$ can be preferentially sequestered by both biocrust constituents such as mosses and lichens, and also by fungal endophytes over $^{15}NH_3$ or other organic N forms (Aanderud et al., 2018; Collins et al., 2014). Ammonium was also chosen due to its aptitude to bond to negatively charged colloids in the soil, thus being less prone to infiltration compared to other commonly used tracers in fungal loop studies (Zhuang et al., 2015). Each ^{13}C enriched plot received 150 mg of $^{13}C_5$, ^{15}N-glutamic acid (99 atom % ^{13}C, 99 atom % ^{15}N) dissolved in 5 ml of filter-sterilized water, which was applied to a 5 cm diameter clump of leaflets on the central *L. tridentata* plant $^{13}C_5$, ^{15}N-glutamic acid was chosen due to its ease of procurement over $^{13}C_5$ glutamic acid, and its use in the only other study documenting C translocation in a fungal loop (Green et al., 2008). Glutamic acid was used solely to track the movement of C from plant to biocrust, and N movement from *L. tridentata* to biocrusts was not investigated in this study. Approximately 7.85 L of filter-sterilized water was then distributed evenly across each plot to complete the simulated rainfall event, which in total was equivalent to a 2.5 mm rainfall event per plot. A 2.5 mm rainfall event was

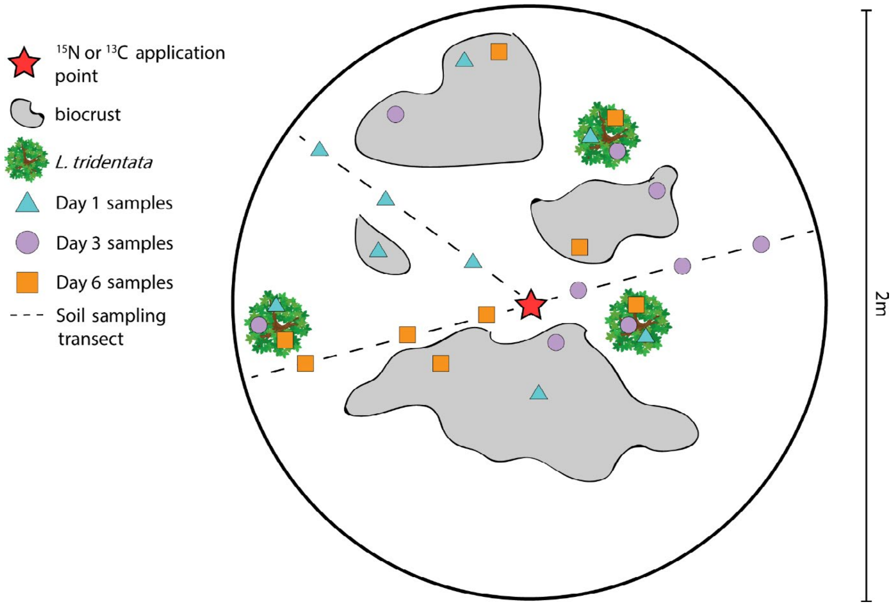

FIGURE 1 Representative treatment and sampling pattern of a plot, where isotope tracers were used to track the movement of N or C from the central cover type into the surrounding community. The red star represents the central cover type (moss-dominated biocrust or *Larreatridentata*) receiving tracer application (^{15}N applied to moss-dominated biocrust, ^{13}C applied to *L. tridentata*). Control plots received no isotope application. Each plot received a single treatment and 2.5 mm rainfall event on Day 0, and there were three plots per treatment (for a total of nine plots in the study). On each sampling day (represented by triangles, circles and squares) three samples were taken from *L. tridentata* plants and moss-dominated biocrust patches within each plot. Three soil samples for fungal community analysis were collected each day along a transect originating from the plot centre

chosen due to small (<5 mm) rainfall events being the most common in the Sonoran Desert (Cable & Huxman, 2004). Approximately 3–5 hr following the simulated rainfall events, biocrust mosses that had received water had returned to a visually desiccated state, and the visible soil surface was dry. To ensure sufficient tracer was applied to track movement over the entire 2 m diameter plot, we used slightly larger amounts compared to previous studies. Specifically, the concentration of ^{15}N tracer applied to biocrust-centred plots was 1.7 times greater than a previous study which suggested that a higher concentration of label may allow for detection of ^{15}N in neighbouring plants, even if some ^{15}N was retained in N deficient moss-dominated biocrust tissue (Aanderud et al., 2018). Similarly, the concentration of glutamic acid applied to *L. tridentata*-centred plots was 1.2 times greater than a previous display of this relationship in grasses (Green et al., 2008).

2.3 | Stable isotope sample collection and analysis

On day 1 (27 July), day 3 (29 July) and day 6 (1 August) following the simulated rain event, samples from three *L. tridentata* shrubs and three moss-dominated biocrusts were collected from each of the nine plots, ensuring that each sample in each plot was a unique distance (between 20 and 100 cm) from the centre of the plot (the central shrub/biocrust patch in the case of tracer-enriched plots, or bare soil in control plots; Figure 1). Samples were not taken within 20 cm from the site of tracer application in order to avoid sampling potential abiotic infiltration of tracers through the soil immediately adjacent to the site of tracer application. For each *L. tridentata* sample, 24 living leaflets were randomly sampled from among three branches of each shrub. For each biocrust sample, 2.5 cm^2 of material was collected to a depth of 1 cm. No root tissue was present in biocrust samples. All samples were dried in an oven at 60°C, for 24 hr following collection. To prepare *L. tridentata* and biocrust samples for elemental analysis, each sample was immersed in liquid nitrogen for 10 s, then pulverized using a mortar and pestle until samples were a homogeneous powder. Samples were then aliquoted into 10 mg subsamples for moss-dominated biocrust tissue and a 3 mg subsamples for *L. tridentata* tissue and analysed for ^{15}N and ^{13}C concentrations as well as %N and %C at the Cornell Stable Isotope Laboratory (Cornell University) using a Finnigan MAT Delta Plus isotope ratio mass spectrometer (IRMS) plumbed to a Carlo Erba NC2500 elemental analyser through a Conflo II open split interface. The resulting isotope ratios were expressed in δ notation as parts per thousand:

$$(\text{‰}): \delta^{15}\text{N or } \delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}}) \times 1{,}000,$$

where R is the molar ratio of the common and uncommon isotope (^{15}N/^{14}N or ^{12}C/^{13}C) for the standard or sample. The standard for δ^{15}N was atmospheric air (0.0036764 ^{15}N abundance), and the standard for δ^{13}C was the Vienna Pee Dee Belemnite (0.0112372 ^{13}C abundance).

To examine how tracer concentration in samples varied between treatment and control plots, time since application/rainfall event, and distance of sample from plot centre, we used nested two-way analysis of variance (ANOVA) tests with treatment as the main factor and distance nested within day as an interaction term. We conducted individual ANOVAs both for ^{15}N movement from biocrusts to shrubs and from biocrusts to biocrusts, and ^{13}C movement from shrubs to shrubs and shrubs to biocrusts. Tukey post-hoc tests were used to identify differences in means across treatment combinations. To meet standards of homoscedasticity and linearity, three sigma outlier removal was conducted on all treatments (Pukelsheim, 1994), and nitrogen isotope data were log transformed. To examine relationships between distance, sample day and treatment, multiple linear regressions were run for each sample type within each day, within each treatment. ANOVAs and linear regressions were completed using RStudio version 1.1.463 (R Core Team, 2013) using base R as well as tidyverse v1.3.0, stringr v1.4.0, knitr v1.28, kableExtra v1.1.0 and gridExtra v2.3. Holm's correction (Holm, 1979) and the base R p.adjust function were used to address false discovery rates.

2.4 | Soil fungal community sampling, sequencing and statistical analysis

We collected three soil samples from each plot on each day of sampling to investigate potential fluctuations in fungal community composition over distance, and over time (Figure 1). Each day, soil samples (2.5 cm^2 to a depth of 1 cm) were collected along a transect originating from the plot centre. Each transect began at 20 cm from the centre of each plot. No root tissue was present in soil samples. Immediately following collection, soil samples were stored in a portable liquid nitrogen cooler at −29°C before being transported and moved to a −29°C freezer at Middlebury College.

To prepare samples for sequencing, DNA was extracted from 250 mg of homogenized soil using a Quick-DNA™ Fecal/Soil Microbe Miniprep Kit, following manufacturer's instructions (Zymo Research). A Nanodrop was used to assess the DNA concentration (25–195 ng/μl) and purity ($A_{260}/A_{280} < 1.9$, $A_{260}/A_{230} < 0.5$) of samples. Ten microlitre of each sample's extracted DNA was sent to the Integrated Microbiome Resource (IMR Bio) sequencing facility at Dalhousie University for amplification, library preparation, sequencing and bioinformatics analysis. Amplicon libraries were prepared by amplifying the ITS2 region of fungal DNA using the universal primer pair ITS86 and ITS4R (Comeau et al., 2017; op de Beeck et al., 2014). Samples were sequenced using an Illumina MiSeq with paired-end 300 bp reads and at least 50 K raw reads per sample. Reads that had a quality score of less than 30 at more than 10% of positions were removed, and remaining reads were screened of chimeras and assigned into amplicon sequence variants (ASVs) following deblur methods (Amir et al., 2017; Edgar, 2010, 2015). Taxonomy was assigned using the UNITE database version 8.0. For additional details on the bioinformatics workflow used, including PCR thermocycling settings, refer to Comeau et al. (2017). Order-level alpha diversity (Shannon Index, and Simpson Index), was compared between samples using a two-way ANOVA with treatment as the main factor and sample day as an interaction term. In a separate ANOVA, distance from the centre of the plot, included as a binned categorical variable, was not shown to significantly influence diversity (data not shown), so this covariate was removed from subsequent analyses. ANOVAs and post-hoc analyses were generated using the PHYLOSEQ v1.32.0, CARET v6.0–86 and TIDYVERSE packages in R, along with base R functions. Two-way ANOVAs comparing changes in taxon relative abundance as a function of treatment and distance (represented as a continuous variable) nested within sample day were run at the taxonomic levels of phylum, order and family. P values were adjusted using the Benjamini–Hochberg procedure to avoid false discovery (Benjamini & Hochberg, 1995). Indicator species analysis was conducted for the three treatments—^{13}C enrichment, ^{15}N enrichment and control—using the MULTIPATT v1.7.9 package in R. Relative abundance was measured as the normalized proportion of reads of each ASV in the sample.

3 | RESULTS

3.1 | Translocation of ^{15}N

Differences in δ^{15}N between the control and biocrust-centred plots (where δ^{15}N tracer was applied to a central moss-dominated biocrust), were observed in both moss-dominated biocrust and *L. tridentata* samples ($p < 0.001$, Table 1; Figure 2). There was a significant effect of sampling day, and the interaction of treatment and sampling day on δ^{15}N in moss-dominated biocrust and *L. tridentata* samples ($p < 0.05$ for all; Table 1). Specifically, an increase in δ^{15}N was not observed at the first two sampling times ($t = 1$ and 3 days respectively), but was observed at the third sampling time, 6 days after the simulated precipitation event. At $t = 6$ days, both moss-dominated biocrust and *L. tridentata* samples in the biocrust-centred plots had δ^{15}N signatures that were higher than the previous 5 days (Figure 2). The resulting δ^{15}N signatures in the biocrust and shrub samples on day 6 were significantly higher than naturally occurring concentrations for the region (Figure 2; Báez et al., 2007). We also observed a significant increase in δ^{15}N in *L. tridentata* control samples on the last day of sampling, however, this movement fell within typically occurring ^{15}N concentrations for the region (Báez et al., 2007; Figure 2). In biocrust samples from biocrust-centred plots on the sixth day of the study, there was a significant effect of distance from central site of tracer application on ^{15}N concentration ($p < 0.001$, Table 1), and a positive relationship between the ^{15}N concentration and distance from the site of tracer application ($p < 0.001$, $R^2 = 0.51$). Otherwise, δ^{15}N concentration did not vary significantly over distance in the control plots or the biocrust-centred plots. Although the 10 m separation of plots removes the likelihood of tracer movement between plots, pseudo replication could have been introduced by the potential

TABLE 1 Results of nested two-way analysis of variance (ANOVA) tests with treatment as the main factor and distance nested within day as an interaction term. Individual ANOVAs were conducted for ^{15}N translocation from moss-dominated biocrusts to *Larrea tridentata* shrubs and between moss-dominated biocrusts, as well as ^{13}C translocation between *L. tridentata* shrubs and from *L. tridentata* shrubs to moss-dominated biocrusts. Significant ($p < 0.05$) relationships are indicated in bold

^{15}N translocation

Biocrust to biocrust	*df*	SS	MS	*F*	*p*	**Biocrust to *L. tridentata***	*df*	SS	MS	*F*	*p*
Treatment	1	31.847	31.847	49.017	**<0.001**	Treatment	1	21.975	21.975	37.787	**<0.001**
Sample day	2	44.459	22.229	34.214	**<0.001**	Sample day	2	108.913	54.457	93.642	**<0.001**
Sample day/distance	3	10.894	3.631	5.589	**0.003**	Sample day/distance	3	0.918	0.306	0.526	0.667
Treatment × sample day	2	7.236	3.618	5.569	**0.008**	Treatment × sample day	2	12.461	6.231	10.714	**<0.001**
Treatment × sample day/distance	3	4.934	1.645	2.532	0.072	Treatment × sample day/distance	3	0.128	0.043	0.074	0.974

^{13}C translocation

L. tridentata* to *L. tridentata	*df*	SS	MS	*F*	*p*	***L. tridentata* to Biocrust**	*df*	SS	MS	*F*	*p*
Treatment	1	4.420	4.420	5.170	**0.028**	Treatment	1	10.493	10.493	0.819	0.371
Sample day	2	3.717	1.859	2.174	0.127	Sample day	2	2.096	1.048	0.082	0.922
Sample day/distance	3	2.930	0.977	1.143	0.344	Sample day/distance	3	21.328	7.109	0.555	0.648
Treatment × sample day	2	0.139	0.070	0.081	0.922	Treatment × sample day	2	26.777	13.389	1.045	0.361
Treatment × sample day/distance	3	2.199	0.733	0.857	0.471	Treatment × sample day/distance	3	129.895	43.298	3.379	**0.028**

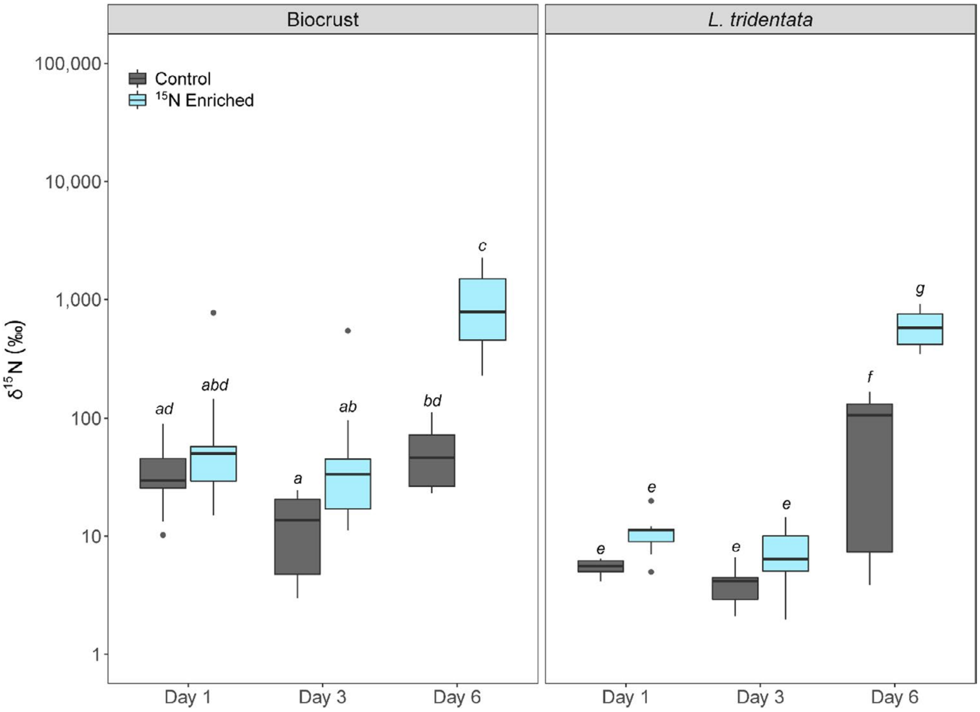

FIGURE 2 δ^{15}N in moss-dominated biocrust and *Larreatridentata* samples over the course of the study. Shared letters indicate no significant ($p > 0.05$) differences between sample means resulting from a three-way ANOVA and separate Tukey post-hoc tests for moss-dominated biocrust (a–d) and *L. tridentata* samples (e–g). The bold line in the centre of each boxplot indicates the median value for the group, the bounds of the boxes represent the interquartile range, and the bounds of the whiskers represent the range of the dataset (excluding outliers, which, if present, are indicated by dots above or below each boxplot)

for shared root systems between *L. tridentata* shrubs within plots (King & Woodell, 1984). To address this, all significant results reported above were rerun after averaging the three ^{15}N concentrations of shrubs within each plot, on each day of sampling. These tests did not change the directionality or overall significance of any result reported above.

3.2 | Translocation of ^{13}C

We found a significant difference in δ^{13}C between *L. tridentata* shrubs sampled from *L. tridentata*-centred plots and control plots ($p = 0.028$), however, δ^{13}C in both *L. tridentata* and moss-dominated biocrusts did not differ significantly between *L. tridentata*-centred and control plots on any given sampling day (Figure S1; Table 1). Additionally, δ^{13}C in both *L. tridentata*-centred plots and control plots never surpassed naturally occurring concentrations for the region (Throop et al., 2013). However, both the control and *L. tridentata*-centred plots saw consistently higher δ^{13}C in moss-dominated biocrust samples over *L. tridentata* samples ($F_{1,106} = 67.916$, $p < 0.001$). For translocation of δ^{13}C from *L. tridentata* to moss-dominated biocrusts, we observed a significant interaction between treatment and distance nested within sample day ($F_{1,106} = 3.379$, $p = 0.028$). This was due to an overall negative relationship between δ^{13}C and distance from *L. tridentata* at the centre of plots and the biocrusts sampled (data not shown).

3.3 | %N and %C

Percent N in samples was higher in *L. tridentata* than in biocrust ($F_{1,16} = 2070.453$, $p < 0.001$) and did not differ significantly between control and biocrust-centred plots, or between sample days ($F_{2,15} = 0.189$, $p = 0.828$). Overall %C in samples was higher in *L. tridentata* than in biocrust ($F_{1,106} = 46{,}532.517$, $p < 0.001$) and did not change significantly between control and *L. tridentata*-centred plots, or between sample days.

3.4 | Fungal community analysis

Comparing the relative abundances of soil fungal communities among samples indicated that the most abundant phylum was Ascomycota accounting for 60%–70% of all reads, the most abundant orders were Pleosporales (~35% of all reads) and Pezizales (~10% of all reads), and the most abundant family was Didymellaceae (~19% of all reads). There was a significantly higher relative abundance of order Pleosporales in biocrust-centred plots over both other treatments irrespective of sample day ($F_{2,26} = 6.376$, $p < 0.01$; Figure 3b), and no other significant changes in fungal taxon relative abundance as a function of treatment, sample day, distance or their interaction. There was a significant reduction in Shannon diversity of fungal orders among all treatment types between days 1 and 6 ($F_{2,42} = 3.140$, $p < 0.05$; Figure 3a), and a similar trend was observed

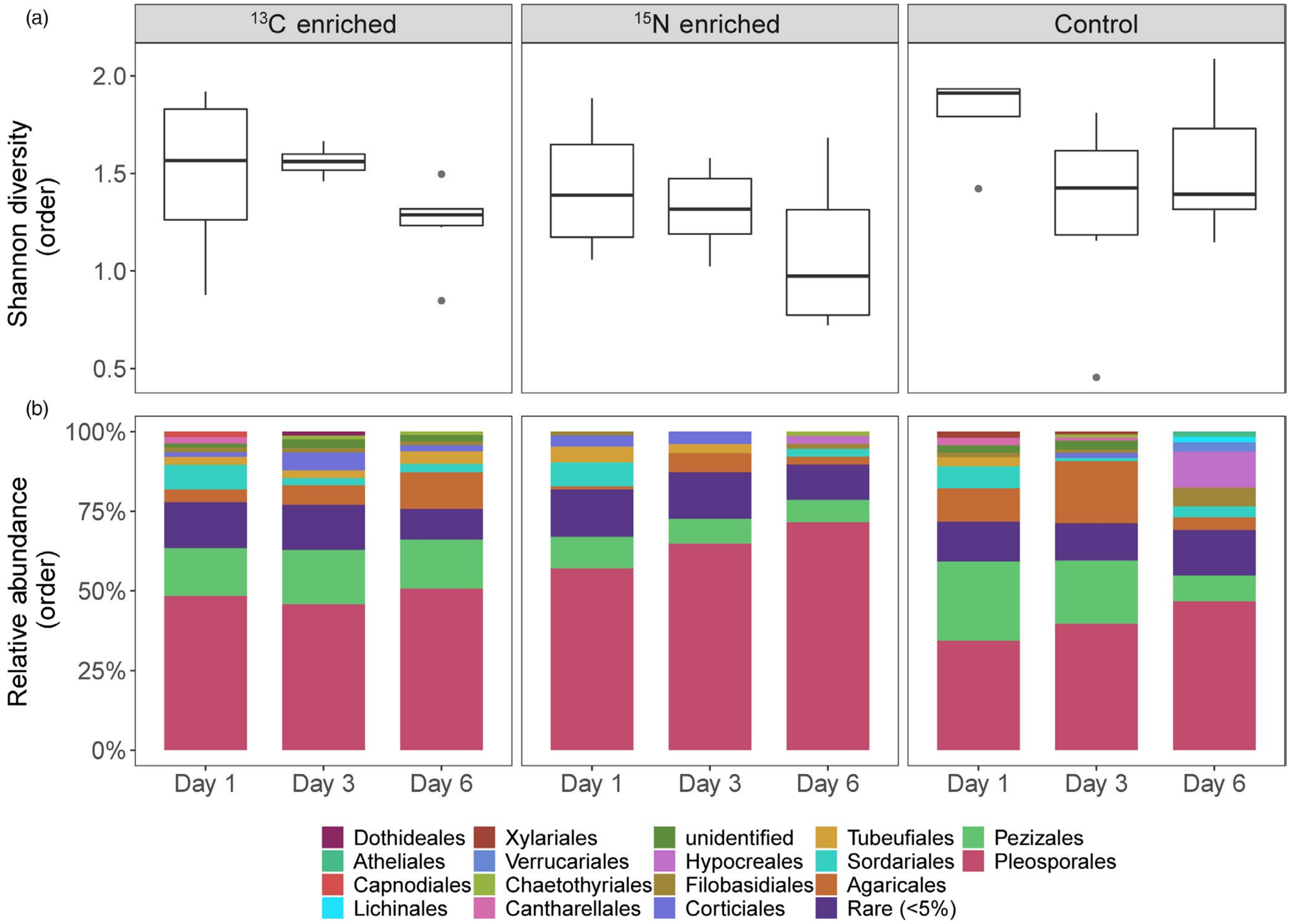

FIGURE 3 (a) Comparison of order-level Shannon diversity in ^{13}C enriched, ^{15}N enriched and Control plots as a function of time since tracer and rainfall event application (Days 1, 3 and 6), and (b) Relative abundances (percentage of total ITS2 reads) of soil fungal orders per sample day per treatment. Taxa with relative abundances of <5% of total reads per sample day per treatment were pooled into the 'Rare (<5%)' category. $n = 6$ for all sampling days within each treatment. Note: Simpson Diversity followed a similar trend to Shannon Diversity. The graphical features of the boxplots in panel (a) are the same as reported in Figure 2

in Simpson diversity ($F_{2,42} = 2.559$, $p = 0.09$). However, no significant changes in Shannon or Simpson diversity were observed in response to treatment. No indicator taxa were found that accounted for more than 1% of ASV reads in a sample (data not shown).

4 | DISCUSSION

4.1 | Translocation of nitrogen

Building upon previous evidence for fungal loops in grasslands (Collins et al., 2014; Rudgers et al., 2018), we provide evidence for a fungal loop in a North American shrubland, where nutrients were translocated between moss-dominated soil biocrust communities and the dominant shrub species, *L. tridentata*. In support of our hypothesis, N was translocated from central patches of moss-dominated biocrusts to shrubs up to 1 m away. However, we did not observe consistent patterns of movement of C in the opposite direction (from central patches of *L. tridentata* shrubs to biocrusts). We also investigated the potential for additional nutrient exchange pathways between community members of the same cover type in this system, and discovered that N was translocated from moss-dominated biocrusts to other moss-dominated biocrust patches within plots. Given the dominance of known DSE fungal taxa in plot soils, the nutrient dispersion patterns of ^{15}N, and the multi-day delay in ^{15}N movement following a rainfall event, our evidence suggests that fungal networks are the most probable conduits for the observed translocation.

Many biological processes in drylands, including nutrient cycling, have been shown to operate in a pulse-dynamic fashion following rainfall events that temporarily stimulate physiological activity (Collins et al., 2014; Loik et al., 2004), and we explicitly addressed this by measuring tracer movement over a 6-day period following rainfall and isotope application. Accordingly, we provide the first evidence that fungal loops can translocate resources following a multi-day delay, where N only moved from biocrusts to the other dominant community members (other biocrusts and shrubs) 4 to 6 days after a precipitation event. No previous work has observed a delay in nutrient translocation that spanned more than a few hours following enrichment.

4.2 | Evidence for fungi as conduits for nutrient exchange

Dark septate endophyte orders Pleosporales and Pezizales were found to dominate soil samples from all plots throughout the week following tracer application, and Pleosporales was observed to be in higher relative abundance throughout the plot when N tracer was introduced. This evidence coupled with similar studies in the region suggest that DSEs were responsible for the nutrient translocation observed in our study. DSE taxa, specifically the order Pleosporales, are most commonly hypothesized to be the vehicle for nutrient translocation in fungal loops due to their dominant role as opportunistic plant endophytes in deserts (Barrow, 2003; Khidir et al., 2010; Jumpponen et al., 2017; Mandyam & Jumpponen, 2005; Porras-Alfaro & Bayman, 2011; She et al., 2018; Usuki & Narisawa, 2007; Valenzuela-Lopez et al., 2018), and their increase in abundance and community dominance in correlation with the distribution of N tracer in desert soils (Aanderud et al., 2018; de Mesquita et al., 2018; Li et al., 2015; She et al., 2018; Upson et al., 2009; Zhuang et al., 2015). We observed an increase in relative abundance of order Pleosporales throughout the plot soil in response to ^{15}N enrichment, which suggests that this fungal order was directly responding to the presence of N as it moved throughout the plots—A phenomenon which has been previously observed in fungal loops (Aanderud et al., 2018; Rudgers et al., 2018; She et al., 2018; Xie et al., 2017). DSE fungi have been shown to colonize roots and vascular tissues of dryland vascular plants in southern North America (Apple, 2010; Massimo et al., 2015) including *L. tridentata* (Apple et al., 2005; Valencia Ontiveros, 2009), as well as bryophyte tissue (Maier et al., 2016; Wang et al., 2011), and can take up and transfer $^{15}NH_4$ given rain events in quantities similar to our study (Aanderud et al., 2018; Green et al., 2008). While we did observe higher relative abundances of DSEs in N-enriched (i.e. biocrust-centred) plots, these taxa did not emerge as indicator species for N-enriched soil samples, likely because of their high abundance in all plots. Therefore, the dominance of Pleosporales and Pezizales throughout plot soils, and the increase in relative abundance of Pleosporales in correlation with N enrichment supports the uptake and subsequent translocation of N through DSE mycelia before its dissemination into other fungal loop community members.

While we observed that DSEs were the most abundant fungal taxa in our plot soils, it is worth noting that not all fungal loop studies have observed an increase in relative abundance of DSEs in response to N enrichment as we have (Kwiecinski et al., 2020). To this end, the Shannon diversity of fungal orders in our plots did not change in response to treatment. Instead, Shannon diversity decreased among all treatments in samples between days 1 and 6, likely indicating a lagged change in community composition in response to the simulated rainfall event (Belnap et al., 2005; Meisner et al., 2013, 2015), and potentially also indicating that the selective forces of water limitation are stronger than N limitation in this soil community. Dryland soil fungal communities have been shown to remain metabolically active in dryland soils for over a week following wetting events, (Blazewicz et al., 2014; Saetre & Stark, 2005; Zeglin et al., 2013), and fungal diversity can remain relatively preserved over periods of desiccation and dormancy (Armstrong et al., 2016). Therefore, it is possible that fungal community composition was in flux up until day 6 of sampling, or that the fungal community stabilized following dry down at some point between days 3 and 6 in our study.

While our results implicate DSE fungi as the primary means of N movement observed between community members, other biotic and abiotic methods of nutrient translocation may also occur in this system. One biotic factor could be absorption and transfer of N through lateral root structures. The below-ground root architecture of *L. tridentata* includes lateral root systems which begin to appear at depth of 10–15 cm (Apple et al., 2005; Brisson & Reynolds, 1994; Gile et al., 1998; Guo & Ogle, 2019; Kemp et al., 1997; Marshall, 1995; Nellessen, 2004; Ogle & Reynolds, 2004), and these lateral roots have been observed to enter the upper 10 cm of soil during monsoon seasons (Ogle & Reynolds, 2004). However, small (<5 mm) rain events such as the one we applied rarely lead to sufficient water uptake in *L. tridentata* to trigger increases in plant water potential, or the activation of metabolic activity (Guo & Ogle, 2019; Yan et al., 2000). Additionally, a relatively simultaneous movement of ^{15}N to both *L. tridentata* and biocrusts following a 4–6-day delay in movement suggests that *L. tridentata* root systems could not have been exclusively responsible for the movement of N tracer, since distinct biocrust patches likely cannot take advantage of nutrients found in nearby roots without the mediation of fungal endophytes (suggested by Green et al., 2008). Another potential contributor to the biotic translocation of ^{15}N is the release of nutrients from tissues upon desiccation and rehydration in biocrust mosses. However, as this process occurs on the order of minutes to hours (Slate et al., 2019), not days, nutrient seepage from moss tissues following desiccation is likely to have been limited to time periods on the same scale as the enrichment period—immediately following the rainfall event. Finally, we cannot rule out the possibility that soil-dwelling microfauna played some role in N movement in this study, potentially through the consumption, translocation and/or excretion of N-containing compounds. Microarthropods and nematodes often migrate in response to rainfall in drylands (Whitford et al., 1981), and bacterially sourced N can be incorporated into tissues of *Collembola* and other motile microflora following feeding (Murray et al., 2009).

Abiotic diffusion of nutrients can also occur through desert soils, yet this process is unlikely to have acted as the primary method of ^{15}N distribution in our study. For one, the abiotic diffusion of ammonium sulphate through gravelly loam soil following a 2.5 mm rainfall event has not previously exceeded 3-5 cm from the site of application in similar studies (Aanderud et al., 2018; Ivans et al., 2003; Zhuang et al., 2015), and moss-dominated biocrusts have been shown to have water holding capacities that exceed 21% (gravimetric) soil moisture, retaining the penetration of isolated rainfall events smaller than 9 mm in the top 5 cm of dryland soil (Aanderud et al., 2018; Cantón et al., 2020; Chamizo et al., 2016; Xiao & Hu, 2017; Zhang et al., 2016; Zhuang et al., 2015). Secondarily, the lack of immediate ^{15}N movement in the first 3 days immediately following treatment rules out abiotic capillary dispersion as a potential explanation for the degree of horizontal nutrient transfer observed between days 1 and 6. The positive relationship between ^{15}N concentration and distance from site of application observed between biocrust patches indicates that the highest concentration of ^{15}N was farthest from the site of application. Osmotic nutrient distribution through soils would present the opposite spatial pattern.

4.3 | Translocation of carbon

In contrast to ^{15}N, ^{13}C was likely not translocated from *L. tridentata* to biocrusts or nearby *L. tridentata* shrubs, suggesting that either this direction of nutrient exchange does not readily occur, or that the size of the rainfall event, the length of the study or the quantity and form of the isotopic compound added did not enable the biotic movement of ^{13}C from *L. tridentata* leaves into the surrounding rhizosphere. One relationship we did observe was a significant negative relationship between δ^{13}C and distance from *L. tridentata* and moss-dominated biocrusts (Table 1), indicating that, when treatment and sample day were considered, δ^{13}C was highest in moss tissues closest to isotope-enriched shrubs and lowest in those furthest away. This may indicate that δ^{13}C was being translocated in the direction we hypothesized (from shrubs to moss) but was occurring on much slower time-scales than in the reciprocal translocation of N, however, the lack of an overall treatment effect suggests this relationship may not be occurring consistently in this system.

One potential reason for the lack of translocation of δ^{13}C from shrubs to biocrusts is the possibility that shrubs such as *L. tridentata* require higher moisture thresholds to enable the reciprocal input of photosynthates into fungal networks (Guo & Ogle, 2019; Yan et al., 2000), and that these thresholds are often unmet following rainfall events of less than 5 mm (Collins et al., 2014; Ivans et al., 2003; Zhuang et al., 2015). Given that our study limited precipitation to 2.5 mm to simulate a common rain event for the season, it is likely that this rainfall event was not suitable to fully reinstate metabolic activity in the *L. tridentata* found in our study plots and therefore led to the lack of bidirectional nutrient exchange in this system. Similar to the lack of translocation from plants to biocrusts observed in our study, Dettweiler-Robinson et al. (2020) found that some North American grasses benefitted from a fungal loop connection to cyanobacterial biocrusts, while this relationship had neutral to negative effects on the biocrust communities depending on precipitation regime. Considering that C movement from grasses to cyanobacterial biocrusts has been shown to occur (Green et al., 2008), our results suggest that the drivers for or mechanisms involving C exchange in previous studies were not present in this shrubland system or under these moisture conditions. Reciprocal exchange of nutrients is often observed in fungal mediated connections between primary producers in other terrestrial ecosystems, and our results add to the possibility of more complex systems driving the multidirectionality of nutrient exchange, particularly involving C, in fungal loops.

4.4 | Evidence for pulse-delay responses in fungal loops

The most probable explanation for the observed delay in nutrient translocation we observed is the gradual improvement of mycelial connectivity between community members following breakdown due to drought conditions. Regarding nutrient uptake in *L. tridentata*, delayed resource acquisition following a rain event has been linked to the slow recolonization of root tissue by fungal endophytes after droughts (Guo & Ogle, 2019; Yan et al., 2000), which eventually enable plant access to nutrients and water stored in mycelial reservoirs and soil micropores (Allen, 2007, 2011). Access to these resources then enables the repairing of xylem cavitation and hydraulic conductance within first order plant roots and higher vasculature which were damaged due to the desiccation of plant tissues during drought (Guo & Ogle, 2019; Yan et al., 2000). This process has been recorded to occur in *L. tridentata* following small (~5 mm) rain events preceded by droughts of more than 2 weeks, such as the one (21 days) preceding this study. Both the slow colonization rates of fungal endophytes following drought periods and the subsequent repairing of vascular tissues in *L. tridentata* are thought to each contribute to the lagged nutrient uptake response to small rain events by the order of days (Guo & Ogle, 2019; Yan et al., 2000), suggesting that root recolonization and vascular repair may have both played a role in the translocation delay observed between biocrusts and *L. tridentata*. Considering that fungal symbionts are often part of biocrust communities (Kim & Or, 2017), and that moss rhizoids and other gametophytic tissue can be similarly colonized by endophytic fungi (Kim & Or, 2017; Maier et al., 2016; Wang et al., 2011), it is possible that a similar delay in the colonization of biocrust tissues could lead to the delay in nutrient acquisition we observed between discrete biocrust patches.

In addition to delays in host recolonization, it is also possible that the breakdown and subsequent reestablishment of fungal bodies in soil interspaces of our plots led to the delays in translocation we observed. Following droughts, wherein mycelial structures in the soil can break down rapidly (Andreo-Jimenez et al., 2019; Ghimire et al., 2011; Miller et al., 1995; Staddon et al., 2003; Taniguchi et al., 2018; Yang et al., 2014), hyphal turnover and root recolonization by DSE fungi can occur on the order of days after the initial rainfall event (<6 mm/day in one study Jacobson et al., 2015; Staddon et al., 2003; Taniguchi et al., 2018). Month long droughts have also been shown to explicitly lead to delays in the growth response of soil fungal communities that last up to a week from rainfall events, potentially due to the repairing and reanimation of damaged cells that occurred during desiccation, radiation damage or osmolysis during rehydration (Belnap et al., 2005; Meisner et al., 2013, 2015). Both the breakdown of soil fungal structures before the simulated rainfall event, and their subsequent regrowth and reestablishment, fit the time-scales observed in our study. In support of these hypotheses, previous work on fungal loops has shown that nutrient translocation rates increased between initial (Day 1) and secondary (Day 4) sampling days (Green et al., 2008; Rudgers et al., 2018), suggesting that biological connections enabling nutrient uptake and/or translocation improved within that time period. In our study, we observed a decrease in fungal community Shannon diversity 6 days following rainfall, irrespective of treatment, suggesting that fungal community composition in plot soils had a delayed response to the rainfall event. It is therefore probable that either the fungal associations within biocrusts and *L. tridentata*, or the mycelial structures connecting them, required time to improve connectivity before translocating nutrients at significant concentrations.

4.5 | Moss-dominated biocrusts in fungal loops

Biocrusts, particularly those with a moss component, play an array of ecological roles in dryland systems (Collins et al., 2014; Reed et al., 2016) and our study presents evidence that moss-dominated biocrusts can also be implicated in fungal loops. Mosses are involved in nutrient cycling through their ability to form symbiotic associations with N-fixing cyanobacteria (Adams & Duggan, 2008), and their presence in biocrusts influences N availability and form in dryland soils (Reed et al., 2012; Hu et al., 2020). As an extension of this knowledge, we show that moss-dominated biocrusts can act as N hubs for subsequent translocation to nearby shrubs, and that these processes can occur even following very small precipitation events to which other, vascular, community members cannot fully respond. A recent study (Aanderud et al., 2018) was not able to capture the presence of fungal loops between moss-dominated biocrusts and grasses, and it was suggested that this was due to the small rainfall size (2.5 mm) applied only to the site of stable isotope addition, or due to the short time-scale (24 hr) of the tracer study. These authors proposed that N deficient mosses may hold on to greater concentrations of N than cyanobacteria-dominated biocrusts, and that the release of N into the surrounding soil may require larger rainfall events or longer time-scales than cyanobacteria dominated biocrusts. Our study utilized a higher concentration of the same N tracer and applied our 2.5 mm rainfall event over the entire plot area, which may have allowed us to capture N translocation dynamics more completely.

In addition to observing movement of N from moss-dominated biocrusts to *L. tridentata*, we also observed the movement of N *between* spatially separated moss-dominated biocrust patches over distances up to 1 m. While it has been widely documented that vascular plants can be involved in fungal-mediated nutrient exchange (Bethlenfalvay et al., 1991; Frey & Schüepp, 1992; Simard et al., 1997; Van der Heijden & Horton, 2009), this study provides the first evidence of such pathways between isolated patches of moss-dominated biocrusts. This suggests the potential for reciprocal N exchange between disparate biocrusts based on supply and demand, which has not been previously demonstrated. While there is substantial evidence suggesting that active biocrusts represent a net source of N to their surrounding plant and soil communities, it has also been shown that this fixed N released as NH_4^+ can be taken up by nearby soil-dwelling mosses or subsequently taken up (re-uptake) by nearby crusts (Stewart, 1967; Johnson et al., 2007), supporting our observation of N transfer between moss-dominated biocrust patches. This novel biocrust moss uptake pathway also suggests that mosses may represent an additional sink for N once it is taken up by DSE fungi, potentially competing with vascular vegetation based on metabolic demands. To our knowledge, such competition for nutrients has only been demonstrated on a seasonal basis in Antarctic moss and vascular plant communities (Hill et al., 2011).

5 | CONCLUSIONS

Our findings support the hypothesis that fungal loops can enable the exchange of N between biocrusts and vascular plants in drylands, and expand upon previous findings to suggest that: (a) fungal loops also operate in shrubland ecosystems, which are increasing in prevalence globally; (b) moss-dominated biocrusts may play important and understudied ecological roles in fungal loops, especially following small rainfall events; and (c) nutrient transfer through fungal loops may follow a pulse-delay model, where the rebuilding of fungal networks between community members following a rainfall event causes a multi-day delay in translocation. Given the previous experimental support for the existence of fungal loops in grassland systems, and our current findings supporting their presence in shrublands, it is possible that fungal loops play roles in nutrient cycling across many dryland systems, and future work should examine their ecological importance across ecosystem types. Based on their roles connecting plants and biocrusts in nutrient-limited (and water limited) systems, fungal loops may be an important stabilizing force in the face of climate change and future fluctuating precipitation regimes.

ACKNOWLEDGEMENTS

This research was supported by the NSF Dimensions of Biodiversity Award #1840931 (to KKC, Middlebury College). We also wish to thank Quinn Brencher for assistance with field work, Erin Eggleston for assistance with fungal DNA extraction and purification, and Jennifer Johnston and Don Harris for park access and permission to conduct research in the Maricopa County parks system. Mandy Slate, Anita Antonika and Matt Bowker provided valuable feedback on previous drafts of this manuscript.

AUTHORS' CONTRIBUTIONS

N.C.J. came up with the concept for the study and conducted the statistical analyses; N.C.J. and K.K.C. designed and executed plot set-up and data collection, and wrote the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13610.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.0cfxpnw1k (Carvajal Janke & Kirsten, 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Carvajal Janke N, Coe KK. Evidence for a fungal loop in shrublands. *J Ecol*. 2021;109:1842–1857. https://doi.org/10.1111/1365-2745.13610