

ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

Are fungal networks key to dryland primary production?

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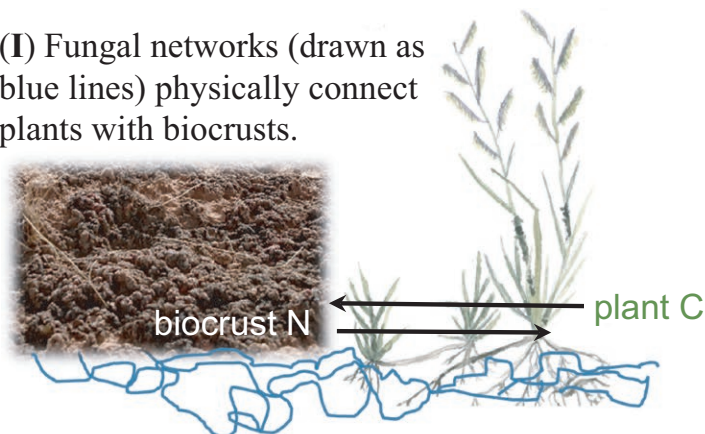
In low-resource ecosystems, competition among primary producers can be reduced through the partitioning of limiting resources in space or time. Partitioning, coupled with species interactions, can be a source of ecosystem stability by retaining resources within a biotic “loop” and slowing losses due to physical processes, such as erosion, gaseous loss, or leaching. Such coupling occurs in marine food webs (the microbial loop), where positive interactions among microbes limit losses of nutrients that would otherwise drop to the ocean floor (Fenchel, 2008). Likewise, mycorrhizal fungi in tropical rainforests may rapidly recycle litter nutrients back to canopy trees, preventing leaching driven by high rainfall (Hattenschwiler et al., 2011). Such dynamics differ from the paradigm for most mesic, terrestrial ecosystems, in which the main source of nutrients for primary production is the decomposition of soil organic matter (Coleman, 1994; Schimel and Bennett, 2004). Thus, in ecosystems that lack large reserves of soil organic matter (e.g., drylands, where sparse litter yields low soil organic matter), biotic retention of nutrients may be particularly critical to primary production and ecosystem dynamics.

The fungal loop hypothesis proposes that microbial networks in drylands link nitrogen-limited plants with nitrogen-fixing biological soil crusts (biocrusts), coupling resource dynamics that would otherwise be disconnected in space or time (Collins et al., 2008). Dryland plants, which produce the bulk of organic matter,

are widely spaced. Interspace soils are often covered by biocrusts, which can include photosynthetic cyanobacteria, lichens, mosses, and heterotrophic bacteria and fungi (Fig. 1, photo). Some of these biocrust members fix atmospheric nitrogen. Spatial separation could limit plant access to the nitrogen (N) fixed by biocrusts or restrict access to plant carbon (C) for biocrust microbes. Temporal separation occurs because only large rain events (>5 mm) increase soil moisture sufficiently to activate plant photosynthesis (Huxman et al., 2004), whereas biocrusts can become active following rains of all sizes (Belnap et al., 2004). Therefore, N fixed by biocrusts during small rains may be lost during times when plants are inactive, unless immobilized by microbes. Fungi are good candidates for connecting plants with biocrusts because they are active at lower soil moistures than plants or bacteria and translocate resources faster than movement through dry soil (Allen, 2007). Fungi are also well known for their capacity to transfer nutrients among producers in other systems (Lindahl et al., 2002).

How could the existence of a fungal loop transform current understanding of dryland biogeochemistry and primary productivity? Fungal interactions in drylands can alter the coexistence of dominant plant species (Chung and Rudgers, 2016); thus, a fungal loop could drive dryland plant community dynamics. Disruption of a fungal loop via grazing, drought, fire, or other disturbances could accelerate nutrient loss and diminish production of dryland

(I) Fungal networks (drawn as blue lines) physically connect plants with biocrusts.



(II) Fungi translocate resources between plants and biocrusts more effectively than movement by physical or other processes.



(III) In the absence of fungal networks that connect plants and biocrusts, primary production (of plants and/or biocrusts) and resource retention declines.



Biocrusts and grasses co-occur at the Sevilleta National Wildlife Refuge in central New Mexico.

FIGURE 1. Necessary conditions for the existence and importance of a fungal loop in drylands.

ecosystems. Ultimately, the fragility of a biotic loop may underlie the frequent state transitions (Petrie et al., 2015) and variable fluxes in annual carbon cycles (Ahlstrom et al., 2015) that are commonly observed in drylands.

We propose three criteria for evaluating the existence and importance of a fungal loop in drylands (Fig. 1). These criteria have not been fully assessed for any dryland ecosystem. Here, we briefly review the evidence in support of each criterion, then propose new directions as a road map to advance understanding.

First, fungal hyphae should physically connect plants with biocrusts (Fig. 1, Criterion I). Some evidence supports this criterion, but much remains unknown. Roots of dryland plants are heavily colonized by fungi, particularly melanized Ascomycota (Jumpponen et al., 2017), which are also found in biocrusts. Plant rhizospheres and biocrusts shared ~50% of their fungal taxa in one study (Porras-Alfaro et al., 2011). However, there has been no direct visualization of fungal hyphae connecting a plant to a biocrust, and advances in technology may be required to visualize such connections within soils. Outstanding questions include: How temporally stable are fungal connections, and does the fungal loop vary phenologically with season or climate? Do connections stop at the rhizosphere or extend to endophytes in roots or leaves, which may require fungal specializations to life inside plants? Do metabolically active

fungi differ from the total fungal assemblage? Thus far, work to identify taxa involved in a fungal loop has involved DNA-based sequencing. However, new techniques, such as stable isotope probing could isolate candidate “fungal loop” taxa by identifying the subset of the community that is metabolically active when translocation happens, such as during rain events. Knowing which fungi are key players would enable more focused experimentation as well as inform management. For example, restoration efforts could include microbial inoculations designed to restore functionality of the loop (e.g., Wubs et al., 2016).

Second, substantial translocation of materials must occur via fungal networks (Fig. 1, Criterion II). Isotopic tracer studies have shown that N and C compounds can move between plants and biocrusts over distances up to 1 m (Table 1). However, few studies have directly visualized compounds moving through fungal hyphae. Nanoscale secondary ion mass spectrometry (nanoSIMS), positron emission tomography (PET), and nanoscale semiconductors (quantum dots) show promise for tracking movement (Whiteside et al., 2009). Roots, other soil organisms, or physical processes may also be involved, and deconstructing these pathways of translocation will require linking Criterion II with Criterion I. Statistical approaches such as structural equation modeling of observational data may assist in this partitioning.

TABLE 1. Synthesis of isotopic labeling studies to detect translocation of resources between plants and biocrusts (Fig. 1, Criterion II). The reference number (Ref.) indicates the reference below the table. MAT is mean annual temperature. MAP is mean annual precipitation. Focal taxa include plant species names or biocrust. Biocrust indicates the classification of the biological soil crust type. Tracer identifies the isotopic tracer used; some studies used multiple tracer types. Tracer ¹⁵N/¹³C gives the amount of tracer added, which was highly variable among studies. Diameter (Diam.) is the spatial extent over which the tracer was applied. Location identifies where the tracer was applied in the ecosystem. Distance gives the distance over which the tracer movement was measured. Results show information for the earliest (Early) and final (Late) times since the application of the tracer that the tracer movement was quantified, along with the Time, as duration in days, and concentration of ¹⁵N or ¹³C (% or % recovery) that was detected to move at each time point. Units appear in each column header. N/A indicates that information was not reported in the publication; “~” means data were estimated from information given in the paper.

Ref.	Environment		Organisms			Study design elements				Results				
	Location	Climate	Focal taxa	Biocrust	Tracer	Application		Early	Late					
	State or Country	MAT (°C)	MAP (mm)	Plant or biocrust	Classification type	Tracer type	¹⁵ N/ ¹³ C (mg)	Diam. (cm)	Location	Dist. (cm)	Time (d)	¹⁵ N/ ¹³ C recovery (% or %)	Time (d)	¹⁵ N/ ¹³ C recovery (% or %)
1	Florida	22.3	1324	<i>Eryngium cuneifolium</i>	cyanobacterial/	¹⁵ NH ₄ ⁺ ¹⁵ NO ₃	0.5/0	<4	biocrust	5	5	~20/ N/A	15	~190%/ N/A
1				<i>Hypericum cumulicola</i>	algal	¹⁵ NH ₄ ⁺ ¹⁵ NO ₃	0.5/0	<4	surface	5	5	~20/ N/A	15	~250%/ N/A
1				<i>Polygonella basiramia</i>		¹⁵ NH ₄ ⁺ ¹⁵ NO ₃	0.5/0	<4		5	5	~30/ N/A	15	~300%/ N/A
1				<i>Paronychia chartacea</i>		¹⁵ NH ₄ ⁺ ¹⁵ NO ₃	0.5/0	<4		5	5	~50/ N/A	15	~400%/ N/A
1				biocrust		¹⁵ NH ₄ ⁺ ¹⁵ NO ₃	0.5/0	<4		5	5	~400/ N/A	15	~250%/ N/A
2	New Mexico	13.2	250	<i>Bouteloua</i> , <i>Muhlenbergia</i> , <i>Sporobolus</i> spp.	light cyanobacterial	Na ¹⁵ NO ₃	12.5/0	3	biocrust	60	1	0.09%/ N/A	4	0.36%/ N/A
2						¹⁵ N, ¹³ C ₅ -glutamic acid	12.5/11	3	surface	60	1	0.13%/ 0%	4	0.36%/ 0%
2						Na ¹⁵ NO ₃	12.5/0	N/A	leaves	60	1	0.09%/ N/A	4	0.36%/ N/A
2						¹⁵ N, ¹³ C ₅ -glutamic acid	12.5/11	N/A		60	1	0.13%/ 0%	4	0.36%/ 0%
2				biocrust	light cyanobacterial	Na ¹⁵ NO ₃	12.5/0	3	biocrust	60	1	0.53%/ N/A	4	0.93%/ N/A
2						¹⁵ N, ¹³ C ₅ -glutamic acid	12.5/11	3	surface	60	1	0.53%/ 3.5%	4	0.93%/ 9.5%
2						Na ¹⁵ NO ₃	12.5/0	N/A	leaves	60	1	0.71%/ N/A	4	0.93%/ N/A
2						¹⁵ N, ¹³ C ₅ -glutamic acid	12.5/11	N/A		60	1	0.71%/ 5%	4	0.93%/ 7.9%
3	China	7.3	80	<i>Erodium oxycorynchum</i>	lichen dominated	¹⁵ N-glutamic acid	12.5/0	3	biocrust	100	1	~6/ N/A	N/A	N/A
3						Na ¹⁵ NO ₃	12.5/0	3	surface	100	1	~4/ N/A	N/A	N/A
3						¹⁵ NH ₄ Cl	12.5/0	3		100	1	~6/ N/A	N/A	N/A
3				biocrust	lichen dominated	¹⁵ N-glutamic acid	12.5/0	3		100	1	~8/ N/A	N/A	N/A
3						Na ¹⁵ NO ₃	12.5/0	3		100	1	~7/ N/A	N/A	N/A
3						¹⁵ NH ₄ Cl	12.5/0	3		100	1	~7/ N/A	N/A	N/A
4	Utah	13.0	269	<i>Achnatherum hymenoides</i>	rugose moss-dominated	K ¹⁵ NH ₄	0.3/0	5	biocrust	100	1	N/A	N/A	N/A
4						(¹⁵ NH ₄) ₂ SO ₄	0.3/0	5	surface	100	1	N/A	N/A	N/A
4				biocrust		K ¹⁵ NO ₃	0.3/0	5		100	1	~10/ N/A	N/A	N/A
4						(¹⁵ NH ₄) ₂ SO ₄	0.3/0	5		100	1	~10/ N/A	N/A	N/A
4				<i>Achnatherum hymenoides</i>	light cyanobacterial	K ¹⁵ NO ₃	0.3/0	5		100	1	~12/ N/A	N/A	N/A
4						(¹⁵ NH ₄) ₂ SO ₄	0.3/0	5		100	1	~12/ N/A	N/A	N/A
4				biocrust		K ¹⁵ NO ₃	0.3/0	5		100	1	~8/ N/A	N/A	N/A
4						(¹⁵ NH ₄) ₂ SO ₄	0.3/0	5		100	1	~10/ N/A	N/A	N/A
5	New Mexico	11.0	290	biocrust	dark cyanobacterial	Na ¹⁵ NO ₃	8/0	3	biocrust	0-20	N/A	N/A	3	64%/ N/A
5				<i>Bouteloua gracilis</i>		Na ¹⁵ NO ₃	8/0	3	surface	5	0.3	leaves: 0%/ N/A	3	leaves: 0.07%/ N/A, roots: 0.5%/ N/A

(1) Hawkes, C. V. 2003. Nitrogen cycling mediated by biological soil crusts and arbuscular mycorrhizal fungi. *Ecology* 84: 1553–1562.
 (2) Green, L. E., A. Porras-Alfaro, and R. L. Sinsabaugh. 2008. Translocation of nitrogen and carbon integrates biotic crust and grass production in desert grassland. *Journal of Ecology* 96: 1076–1085.
 (3) Zhuang, W. W., A. Downing, and Y. M. Zhang. 2015. The influence of biological soil crusts on ¹⁵N translocation in soil and vascular plant in a temperate desert of northwestern China. *Journal of Plant Ecology* 8: 420–428.
 (4) Aanderud, Z. T., B. Smart, N. Wu, A. S. Taylor, Y. Zhang, and J. Belnap. 2018. Fungal loop transfer of N depends on biocrust constituents and N form. *Biogeochemistry* 5: 3831–3840.
 (5) Dettweiler-Robinson, E., R. L. Sinsabaugh, and J. A. Rudgers. Fungal connections between plants and biocrusts improve nutrient transport, resource content, and performance: a test of the fungal loop hypothesis (in preparation).

Observations on the natural abundance of stable isotopes, for example, recently demonstrated that biocrusts near C_3 plants had a depleted $\delta^{13}C$ signature, consistent with the hypothesis that biocrusts use plant-derived carbon (Dettweiler-Robinson, 2018). However, experiments that directly track translocation will be essential to evaluating Criterion II.

It remains difficult to speculate which dryland ecosystems or taxa may be most likely to support a fungal loop, because studies have not yet tackled a broad diversity of drylands. Inconsistent methodology among isotopic tracer studies (Table 1) has impeded comparison of transfer rates across plant functional groups, biocrust types, ecosystems, or environmental conditions. Variation in methods includes the timing, location, and areal extent of tracer application, as well as the timing of sample collection to track tracer movement (Table 1). Although prior research has spanned a broad geographic range, thus far, all studies were conducted in sandy soils with bulk densities of 1.5–1.6 (Table 1). While a diversity of biocrust types has been studied, spanning light cyanobacterial crusts to lichen-dominated and rugose moss-dominated crusts, no microbial or plant taxa have been compared in more than one context (Table 1). Furthermore, studies thus far have focused primarily on grasses and forbs, and a lack of data precludes the ability to generalize patterns over plant functional groups. However, an observational study on naturally abundant stable isotopes was consistent with the hypothesis of C transfer to biocrusts from a C_3 shrub, but not from a C_4 grass (Dettweiler-Robinson, 2018). Tracer movement has been observed in most studies of cyanobacterial or lichen biocrusts (Table 1), but long-term retention (>15 d) has not been investigated. Only one study has looked for bidirectional C and N transfer, and only one reported soil moisture at the time of label addition (Table 1, Study 2), limiting the ability to model reciprocal transfers or predict effects of water limitation. Altogether, little is known about how soil characteristics may influence transfer rates. Soil nutrients may be important factors by creating stoichiometric gradients. For example, the role of N limitation in nutrient transfer could be investigated via fertilizer additions. A better understanding of the endogenous factors (e.g., plant or microbial taxa involved) and exogenous factors (e.g., climate, soil type, nutrient supply) that control translocation rates may come from characterizing the stoichiometric requirements of plants, fungi, and biocrusts and quantifying the potential concentration gradients for N and C (Zechmeister-Boltenstern et al., 2015).

Third, for evaluating the functional importance of the fungal loop, the elimination or reduction of fungi that link plants and biocrusts should reduce primary production and resource retention in biotic pools (Fig. 1, Criterion III). In mesic ecosystems, mycorrhizal fungi can redistribute resources between competitor plant species (e.g., Hart et al., 2003). A fungal loop could similarly alter interactions between plants and biocrusts. However, the ecological consequences of fungal connections for production and resource retention are not yet resolved for any dryland. Experiments that manipulate fungal networks with barriers or fungicides have potential to directly test Criterion III. For example, we have successfully used 0.45- μ m mesh to exclude fungi in field settings (Dettweiler-Robinson et al., unpublished data). Indirect approaches could also remove plants or biocrusts to isolate the contribution of each producer to production (Dettweiler-Robinson et al., 2018). Indeed, some prior work suggests that the disruption of biocrusts can reduce plant productivity and alter plant species composition (reviewed by Zhang et al., 2016).

Experiments spanning multiple drylands will help to generalize the ecological importance of a fungal loop across plant and biocrust functional groups, soil textures, and ecosystem types. Whether the fungal loop is context-dependent on climate, nitrogen-deposition, grazing, soil disturbance, or fire remains unknown.

There is substantial work ahead to demonstrate the importance of a fungal loop in drylands. While fungi likely transfer nutrients among organisms in dryland soils, as has been shown in other ecosystems, the extent of the transfer and its importance to primary production has not been established. It will be necessary to combine traditional approaches, such as isotopic labeling, with new genetic techniques to identify the microbial taxa involved and to determine whether the fungal loop is widely relevant across ecosystems. If supported, the role of fungi in regulating nutrient supply and transfer could be key to understanding ecological dynamics in drylands soils with low organic matter worldwide.

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