Translocation of nitrogen and carbon integrates biotic crust and grass production in desert grassland

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Summary

1. Arid ecosystems are a patch mosaic of plants and biological soil crusts that have been described as islands and mantles of fertility, respectively. To determine whether these patches are metabolically linked by a fungal network of dark septate and arbuscular mycorrhizal fungi (AMF), we measured translocation of nitrogen (N) and carbon (C) in a desert grassland using $^{15}$N-NO$_3$ and $^{13}$C, $^{15}$N-glutamic acid as substrates.

2. Substrates were applied as point sources to either small patches of biotic crust or to a subset of leaves within a bunch grass tussock.

3. Both substrates were translocated over approximately 1 m$^2$ areas (approximately 20 patches) at rates up to 100 cm day$^{-1}$ during a 4-day period following a natural rainfall event. Foliar uptake of $^{15}$N from glutamate was initially more rapid than $^{15}$N uptake from nitrate, and translocation from foliage was initially more rapid than translocation from soil crust. Rates of $^{15}$N translocation between patches were similar to rates of atmospheric N deposition and denitrification.

4. $^{13}$C from $^{13}$C$_5$, $^{15}$N-glutamic acid applied to leaves was translocated to crust suggesting that plant C may provide metabolic support to biological soil crusts during periods of active growth. $^{13}$C from $^{13}$C$_5$, $^{15}$N-glutamic acid applied to soil crusts did not enter plants, in contrast to the $^{15}$N from this substrate, indicating that glutamate was not translocated intact.

5. The dominant fungi of roots, rhizosphere soil and biological soil crusts are dark septate ascomycetes, most classified as Pleosporales, and AMF are rare. Phylogenetic analyses indicate substantial overlap in fungal community composition between roots and crusts, which may facilitate nutrient transfers.

6. Synthesis. This study supports the hypothesis that the spatial structure of semi-arid ecosystems, a patch mosaic of grasses and biological soil crust described, respectively, by the island of fertility and mantle of fertility paradigms, is functionally integrated by exchanges of C and N through a symbiotic fungal network dominated by dark-septate fungi.

Key-words: arbuscular mycorrhizal fungi, biological soil crust, Bouteloua, carbon translocation, dark septate endophytes, desert grassland, islands of fertility, nitrogen translocation

Introduction

The spatial structure of arid ecosystems has been described as a mosaic of plant-centred ‘islands of fertility’ (Schlesinger et al. 1996). These islands, defined by concentrations of organic matter and nutrients that exceed those of surrounding soil, are loci for biogeochemical activity. Because precipitation is scarce, this activity is episodic. The temporal dynamics of these episodes are described by the pulse–reserve model (Reynolds et al. 2004): Precipitation events that increase soil moisture above critical thresholds initiate a pulse of plant growth that produces reserves of biomass and propagules that accumulate until soil moisture is depleted. In their initial formulations, the island of fertility and pulse–reserve paradigms generally neglected microbial contributions, notably carbon (C) and nitrogen (N) fixation by biological soil crusts that develop on exposed soil between plant ‘islands’. These microbial processes not only contribute to soil fertility, but they also respond at different, generally lower, soil moisture thresholds than plants (Austin et al. 2004; Huxman et al. 2004). Collins et al. (2008) have proposed an expanded pulse–reserve model (the threshold–delay nutrient dynamics model) that explicitly includes both plant and microbial contributions to the C and N dynamics of arid ecosystems. However, many details concerning the functional integration of these contributions are lacking.

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Biological soil crusts, dominated variously by lichens, mosses and cyanobacteria, are a prominent feature of arid and semi-arid regions around the world (Belnap & Lange 2001; Li et al. 2003; Maestre et al. 2006). These crusts, described as ‘mantles of fertility’, are sites of C and N fixation and the interface for exchanges between soil and atmosphere (García-Pichel et al. 2003; Hawkes 2003). Crusts are informally classified by their colour and texture. Darker crusts typically include cyanolichens and mosses. Light crusts are often dominated by the cyanobacterium Microcoleus vaginatus and are common in water-limited systems between widely spaced vascular plants (Belnap & Lange 2001; Bowker et al. 2002, Belnap 2006). Nucleic acid analyses of light crusts in regions of the Southwestern United States indicate that bacterial diversity is high (Redfield et al. 2002; García-Pichel et al. 2003) and community composition is distinct from that of the rhizosphere (Kuske et al. 2002). Fungal diversity is also moderately high and dominated by ascomycetes (States & Christensen 2001; Porras-Alfaro et al. 2007, 2008).

The contribution of crusts to soil fertility has been demonstrated in disturbed areas where loss of crusts was found to decrease soil N content by 25–75% (Evans & Belnap 1999) and decrease the N and micronutrient content of nearby plants (Harper & Belnap 2001). Other studies have shown that glycolytic, proteolytic and oxidative enzyme potentials in light crusts equal or exceed those of soil from the rhizosphere of grama grass (Stursova et al. 2006; Stursova & Simsabaugh 2008), and that rates of respiration and nitrous oxide production are similar for light crust and soil from the rhizosphere of grass (Maestre & Cortina 2003; Crenshaw et al. 2008).

N fixation in light crusts occurs during moist periods of moderate temperature (1–26 °C; Belnap 2002). At sites on the Colorado Plateau, Belnap (2002) estimated N input from light crusts at 1.4 kg ha⁻¹ year⁻¹, a value approximately equal to the rate of atmospheric N deposition across the Southwestern United States. Rates of C fixation under moist conditions have been estimated at 8 mg C m⁻² year⁻¹ (García-Pichel & Belnap 1996).

The vegetation of semi-arid grassland is dominated by C₄ perennial grasses that grow in association with arbuscular mycorrhizal fungi (AMF) (Corkidi et al. 2002; Johnson et al. 2003) and other diverse fungi that are variously characterized as dark-septate endophytes, saprophytes and facultative pathogens (Davidson & Christensen 1977; Stanton et al. 1981; Barrow 2003; Porras-Alfaro et al. 2007, 2008). In response to AMF infection, Bouteloua gracilis, one of the dominant grasses in arid regions of the Southwestern United States, can increase C fixation by up to 40%; most of this increment is allocated to fungal production (Allen et al. 1981a), leading to increased water (Allen 1982) and P uptake (Allen et al. 1981b). Johnson et al. (2003) found that the development of AMF networks in semi-arid grassland varied in relation to N : P ratios. N addition reduced development when P was relatively abundant and promoted development when P availability was low.

Fungal networks, both saprotrophic and biotrophic, translocate C, N, P and mineral nutrients among resource patches, including hosts (e.g. Frey et al. 2000; Perez-Moreno & Read 2000; Tu et al. 2006). This translocation capacity, considered with recent work on the contributions of biological soil crusts to ecosystem productivity, suggests that phytocentric ‘islands of fertility’ and crust ‘mantles of fertility’ may be functionally integrated by a common fungal network. In this model, fungi supported by biotrophic C from both plants and cyanobacteria act as an intermediate nutrient reserve because of their capacity to mineralize, transform and translocate nutrients at low water potentials (Crenshaw et al. 2008; Collins et al. 2008). As a test of this ‘fungal loop’ model, we examined the connectivity between patches of grama grass and light cyanobacterial crust by measuring rates of N translocation from both crust and foliar point sources using trace quantities of ¹⁵N-N,NO₃ and ¹³C-glutamate as substrates, and by comparing the composition and distribution of fungi in grasses and soil.

Methods

SITE DESCRIPTION

The experiments were conducted at the Sevilleta National Wildlife Refuge (SNWR) in central New Mexico, the site of the Sevilleta Long-Term Ecological Research (LTER) Program. The SNWR contains extensive semi-arid grassland dominated by C₄ perennial grasses (B. gracilis, B. eriopoda, Sporobolis spp., Muhlenbergia spp.). The plots selected for the translocation experiments were located within the grama grassland ecosystem (McKenzie Flats, N 34°24′, W 106°41′; elevation 1630 m). This site receives approximately 250 mm of precipitation annually. However, total annual precipitation and its distribution throughout the year vary widely.

For this experiment, plots were established on fine-grained soils of the Turney loamy sand series, formed by aeolian and alluvial deposition. This soil has a gravimetric water holding capacity (WHC) of 0.2 g g⁻¹ air-dried soil, a bulk density of 1.6 g cm⁻³, and a pH of 8.5 (Crenshaw et al. 2008). Soil C, N and P contents are approximately 0.50%, 0.046% and 0.020%, respectively, with molar C : N and C : P ratios of 13 and 70, respectively (Stursova et al. 2006; Crenshaw et al. 2008). Vegetation cover in this area averages 60%, the open areas between plants are colonized by light cyanobacterial crusts, dominated by M. vaginatus (A. Porras-Alfaro & K. Lipinski, unpubl. data). From 1999 to 2004, annual above-ground net primary production (NPP) was 51 g m⁻² (Muldavin et al. 2008).

ESTABLISHMENT OF STUDY PLOTS

In September 2005, 12 circular plots with a radius of 1.0 m were delineated at least 10 m apart. Six of the plots were centred on a patch of biotic crust and six were centred on a discrete bunch grass tussock. The plots delineated contained only C₄ perennial grasses, dominated by blue and black grama (B. gracilis, B. eriopoda). The spaces between plants supported conspicuous biological soil crust communities, evidenced by surface texture and pigmentation. Each grass tussock and crust patch intersected by one of eight radial transects, extending from the plot centres along N, NE, E, SE, S, SW, W, NW axes, was numbered, tagged and its distance from the centre of plot was recorded. The height and diameter of each grass tussock within the plot was also recorded.

Plant cover within the plots averaged 20% (Table 1). The relatively low cover reflects our non-random selection of monotypic plots
Table 1. Plot characteristics and label recovery on days 1 and 4 following application of either nitrate or glutamate to the surfaces of grama foliage or soil biotic crusts. Values are calculated for a 60 cm radius from the point of label application.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Substrate</th>
<th>Application</th>
<th>Cover (%)</th>
<th>C (g)</th>
<th>N (g)</th>
<th>Day 1-15N</th>
<th>Day 4-15N</th>
<th>Recovery (%)</th>
<th>Cover (%)</th>
<th>C (g)</th>
<th>N (g)</th>
<th>Day 1-15N</th>
<th>Day 4-15N</th>
<th>Recovery (%)</th>
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</table>

Foliage

- Composed of discrete, easily delineated tussocks separated by patches of easily delineated cyanobacterial crust. Crust cover within each plot was approximately 60% of open area. The roots from the bunch grasses do not extend into the open areas colonized by biological crust. Crust samples collected during our study were examined microscopically for fine roots; none were found. All plot marking and plant and crust tagging and measuring were done from outside the plot perimeter to avoid disturbance.

SUBSTRATE APPLICATIONS

On 1 October 2005, nitrate was applied to 6 of the 12 plots (three crust-centred and three grass-centred plots), and glutamate was applied to the other six plots (three crust-centred and three grass-centred). Glutamate was selected because interconversion of glutamate and glutamine is the central regulatory process of cellular N metabolism. For the nitrate applications, 0.58 g of 99 atom% 15N-NaNO₃ was dissolved in 10 mL of deionized water to create a 0.68 mM solution, and a 1.25 mL of solution (12.5 mg 15N) was applied to the centre of each of six plots as a fine spray using an atomizer. A 1.25 mL of solution was sprayed directly onto the surface of the crust over an area approximately 3 cm in diameter in the three crust-centred plots. The 15N addition was equivalent to the total N content of a crust patch 6.8 cm in diameter × 1.0 cm in thickness (calculated using N content data in Table 1). For plots centred on grass tussocks, the 1.25 mL solution was sprayed on a small cluster (5–10) of green leaf tips. The 15N addition was equivalent to the total N content of 755 mg of foliage or one small grass tussock (using data from Table 1). For a plot radius of 60 cm, the 15N additions amounted to 0.2–0.7% of the total N content of foliage and crusts. The applications were done midday under full sun, when surface temperatures exceed 40 °C. We observed no infiltration of water into crust, no runoff of moisture from leaves, and no dispersal of label beyond the intended application point. For the glutamate applications, 1.0 g of 15C, 15N-glutamic acid (99 atom% 15N, 99 atom% 15C), was dissolved in 40 mL of deionized water (0.34 M). A 5 mL solution was applied each of six plots (12.5 mg/15N plot⁻¹, 11 mg/15C plot⁻¹) as described above for nitrate applications, taking care that no label was dispersed beyond the application point.

SEPTEMBER 2005

September is the peak production period for C₄ grasses, coinciding with monsoon rains. Showers on 28 and 29 September 2005 deposited 10.3 and 15.1 mm of water, respectively. At the time of label application on the third day following the rain, the soil surface was dry. No precipitation occurred during the sampling period (1–5 October); mean daily high temperature during this period was 28 °C, mean daily low temperature was 12.3 °C (meteorological data collected at Deep Well monitoring station adjacent to study area).

SAMPLE COLLECTION

Samples of foliage and crust were collected from every tussock and crust patch along each transect of each plot at approximately 24 h intervals for the 4 days following label application. To avoid disturbance, the central grass or crust patches to which tracer substrate was applied were not sampled. Thus, no samples were collected within a 15-cm radius from the application point. Crust samples (1 cm diameter × 1 cm deep (0.8 cm³)) were collected using a cork borer. Foliage was sampled by snipping 2–3 cm of tissue from the tips of 5–8 green leaves using scissors. To avoid disrupting the integrity of the system, no roots or soil samples were collected during foliage and crust sampling and all sampling was done by extension from positions outside the plot. The plots were carefully monitored during the period of the experiment: no animal intrusions occurred, no insect grazing or detritivore activity was observed, and there was no moisture condensation. Crust samples were examined to verify that fine roots did not extend into the biological soil crust. Thus direct plant uptake of crust-applied substrate was unlikely. Conversely, substrate applied to plant foliage that was recovered in the crust soil could not be directly rhizodeposited.

Crust and foliage samples were placed in individual plastic bags and returned to the laboratory where they were air-dried. Following crust and foliage sampling on day 4, root samples were collected along the north and south transects of two plots and air-dried. Crust and foliage samples were also collected outside the experimental plots, for analysis of natural C and N isotopic composition.

Crust samples selected for isotopic analysis were mixed, subsampled and sealed in 1.5 mL microcentrifuge tubes. Leaf and root samples were sealed in 1.5 mL microcentrifuge tubes, along with a 5-mm glass bead, and milled using a reciprocating tissue homogenizer.
SAMPLE ANALYSES

Isotope ratio mass spectrometry analyses were performed at the Stable Isotope Facility, University of California, Davis, CA. After preliminary analyses of $^{15}$N distribution within the experimental plots, we focused the analyses on samples collected on days 1 and 4 following substrate application and within a 60-cm radius of the centre of each plot.

Analyses of soil and tissue C and N content were conducted at the Sevilleta LTER analytical laboratory, using high temperature combustion on a ThermoQuest CE Instruments NC2100 Elemental Analyzer. Mean C and N content of foliage (n = 6) was 44.47% (SD = 0.99) and 1.655% (SD = 0.541), respectively (C : N = 31). Corresponding values for the top 1.0 cm of crust soil (n = 6) were 0.304% (SD = 0.08) and 0.0174% (SD = 0.0089), respectively (C : N = 20).

ANALYSIS OF ISOTOPIC DATA

Above-ground biomass was estimated using regressions developed by Sevilleta LTER Program for long-term monitoring of plant biomass and production (Muldavin et al. 2008). For each experimental plot, the standing stock of foliar C and N was calculated for a 60 cm radius (1.131 m²) from the substrate application point, using estimates for foliar biomass and elemental composition described above. The corresponding values for crust C and N within the top 1.0 cm of soil was calculated from cover estimates, a measured bulk density of 2.0 g cm⁻¹ and elemental composition (Table 1).

For each experimental plot, $^{15}$N label recovery from foliage and crust was calculated as follows:

- Total natural $^{15}$N (mg) = (foliar or crust N)(atom% $^{15}$N/100).
- Label recovery (mg) = [(label applied) (mean atom% $^{13}$N/100)] – total natural $^{15}$N.
- % label recovery = (mg label recovered) 100/(mg label applied).

Natural $^{15}$N abundance for crust soil and foliage was 0.36735 atom% ($\delta^{15}$N + 3.7‰) and 0.36618 atom% ($\delta^{15}$N + 0.5‰), respectively, and 12.5 mg of $^{15}$N was applied to each plot. Mean atom% $^{15}$N was calculated as the arithmetic average of all samples analysed within each plot for each sampling date; sample size ranged from 3 to 12.

No $^{13}$C label was detected in foliage. For crusts $^{13}$C recovery was calculated as:

- Total natural $^{13}$C (mg) = (crust C)(atom% natural $^{13}$C/100).
- Label recovery (mg) = [(crust C)(mean atom% $^{13}$C/100)] – total natural $^{13}$C.
- % label recovery = (mg label recovered) 100/(mg label applied).

Natural $^{13}$C abundance for crust soils was 1.09113 atom% ($\delta^{13}$C + 17‰) and 11.0 mg $^{13}$C was applied to each plot.

STATISTICAL ANALYSES

Statistical analyses were done using spss (v11). The covariance of signal strength with distance for crust and foliage samples collected within zones extending from 15 to 60 cm from the application points was quantified by regression using a first order exponential dilution model ($\ln$(N ($^{15}$N or $^{13}$C) vs. distance). Regressions were also used to relate the quantity of label recovered from foliage and crust samples within a plot to standing stocks of foliar N and C and crust N and C.

The fixed effects of substrate (NO₃ or Glu) and application (grass or crust) on the quantity of $^{15}$N recovered per plot from foliage and crust (calculated as described above, Table 1) were tested using MANCOVA models, with foliar $^{15}$N recovery and crust $^{15}$N recovery as dependent variables and foliar biomass N and biological soil crust N as covariates. Recoveries from day 1 and day 4 were analysed separately. The initial analyses included recoveries from all 12 plots. However, label recoveries from plot 3 (Table 1) exceeded those of all other plots by an order of magnitude or more. Consequently, the statistical models did not meet variance assumptions. After excluding plot 3 from the analyses, a full-factorial MANCOVA for $^{15}$N recoveries on day 1 showed no significant substrate × application interaction and no significant covariance effect for biological soil crust N. These components were removed to generate the final MANCOVA model (Table 2). Similar analyses for $^{13}$N recovery on day 4 showed no significant substrate × application interaction.

Table 2. Statistics for $^{15}$N and $^{13}$C recoveries from grama foliage and biological soil crust (Table 1) in relation to substrate applied ($^{15}$NO₃ or $^{13}$C₅-glutamic acid) and point of application (foliage or crust). Data from plot 3 were excluded from the $^{15}$N analyses for day 1. For day 1, the final model for $^{15}$N recovery per plot is a main effects MANCOVA with foliar and crust recovery as dependent variables and foliar N as a covariate (n = 5 or 6 for main effects). For day 4, the final model for $^{15}$N recovery is a main effects MANOVA with foliar and crust recovery as dependent variables. No $^{13}$C was recovered from foliage samples. $^{13}$C recovery on day 1 could not be fitted to a general linear model. For day 4, the final model for $^{13}$C recovery per plot is an ANOVA, comparing recovery in relation to point of substrate application (n = 3). Wilk’s λ is a multivariate statistic generated by models with multiple dependent variables.
significant substrate x application interaction or biomass covariance, which reduced the final model to a MANOVA (Table 2). 

$^{13}$C, derived from glutamate, was not detected in foliage samples. $^{14}$C recoveries per plot from biological soil crust (Table 1) in relation to point of application were compared using ANCOVA with crust C as a covariate. On day 1, $^{13}$C enrichment was found in only three of the six plots and the data could not be fitted to a general linear model ($R^2 = 0.38, F = 1.21, P = 0.387$). On day 4, $^{14}$C recovery per plot did not significantly co-vary with crust C, reducing the final model to ANOVA (Table 2).

DESCRIPTION OF FUNGAL COMMUNITIES

Descriptions of the soil and plant fungal communities at our study site have been reported by Johnson et al. (2003) and Porras-Alfaro et al. (2007, 2008). Collectively, the fungal environmental data base of ITS nrDNA includes 358 sequences from B. gracilis root samples (Genbank accession numbers: EU144389–EU144746), 307 sequences from rhizosphere soil (EU479716–EU480022) and 324 sequences from biological soil crust samples (EU480023–EU480346). The fungal sequences from B. gracilis roots were described by Porras-Alfaro et al. (2008). Analyses of fungal sequences from soil and biological soil crust are unpublished. In this paper, we present a summary of published results and a new analysis of the diversity and distribution of Pleosporales, the dominant order of fungi in this semi-arid grassland. 

Pleosporales account for 52% of the 989 fungal sequences in our environmental data base. Operational taxonomic units (OTU), rarefaction curves and Chao diversity estimators for the Pleosporales were calculated using the program DOTUR, using a 97% similarity criterion for OTU definition (Schloss & Handelsman 2005). After aligning the Pleosporales sequences using the CLUSTALW program, DNA distance matrices were created with PHYLIP using the F84 evolutionary model. A phylogenetic analysis of the dominant Pleosporales OTUs colonizing roots, soil and crust was done using parsimony analysis with RAPU (v4.0b10). A full heuristic search was conducted using tree bisection reconnection (TBR) as the branch-swapping algorithm with all characters equally weighted and unordered. Bootstrap values were obtained based on 1000 replicates with 50% majority rule (Porras-Alfaro et al. 2008) 5.8s rDNA and partial sequences of ITS1 and ITS2 regions of dominant Pleosporales sequences was deposited in Webin-Align <ftp://ftp.ebi.ac.uk/pub/databases/emb/align> under the number: ALIGN_001227. The fraction of Pleosporales OTUs common to root, soil and crust communities was determined by counting shared OTUs and calculating a similarity index using the SONS program (Schloss & Handelsman 2006).

Results

TRANSLOCATION OF $^{15}$N

Approximately 2000 samples of foliage and crust were collected during the 4 days following tracer application. A total of 405 samples, collected on days 1 and 4, were analysed for $^{15}$N signals. Each sample represented a discrete habitat patch situated within a 15–75 cm radius of a substrate application point. Of these samples, 92% had $^{15}$N values that exceeded ambient concentrations (Fig. 1). Only data from samples collected on days 1 and 4 are presented.

On average, the $\delta^{15}$N signals declined exponentially with distance from the application points, but variation within and between patches was high (Fig. 1). After 24 h, foliar $^{15}$N recoveries from grama patches within a 15–60 cm radius of the substrate application points ranged from 0.07% to 0.46% (median 0.25%), excepting an anomalously high value of 15% for plot 3 (Table 1). Four days following tracer application, recoveries ranged from 0.04% to 0.57% (median 0.36%) with a value of 27% for plot 3. Crust recoveries were similar: 0.30–1.2% on day 1 (5.7% for plot 3, median value 0.68%) and 0.31–2.5% on day 4 (8.5% for plot 3, median value 0.93%).

The between factors comparisons for the MANCOVA of day 1 samples, excluding plot 3, showed that the rate of incorporation of $^{15}$N from glutamate into grama foliage was marginally greater than incorporation of $^{15}$N from nitrate (0.13% recovery vs. 0.09%, respectively, $P = 0.058$, Table 2). Point of application (crust or foliage) had no effect on foliar recovery ($P = 0.256$). In contrast, $^{15}$N recovery from crust patches was marginally greater for plots in which substrates were initially applied to foliage compared to plots where substrates were initially applied to crust (0.71% vs. 0.53%, respectively, $P = 0.052$, Table 2). The type of substrate (nitrate or glutamate) did not affect recovery ($P = 0.338$). By day 4, between factors comparisons from the MANCOVA showed no differences in foliar or crust $^{15}$N recoveries in relation to substrate type or point of application. (Table 2).

For grama foliage, $^{15}$N recovery covaried with biomass N. On day 1, the mean incorporation rate was 27 $\mu$g $^{15}$N g$^{-1}$ foliar N ($n = 11$, plot 3 excluded, $r^2 = 0.59$); by day 4 the specific rate was 45 $\mu$g $^{15}$N g$^{-1}$ foliar N ($n = 11$, plot 3 excluded, $r^2 = 0.75$). $^{15}$N recovery from crusts did not covary with crust N; but specific incorporation rates were about four times greater than foliar rates on average (110 ± 170 (SD) and 210 ± 250 $\mu$g $^{15}$N g$^{-1}$ crust N for days 1 and 4 samples, respectively).
LIMI TATION S ON TOTA L $^{15}$N RECOVERY

After collecting foliage and crust samples on day 4, root samples were collected from two experimental plots at radii > 50 cm from the tracer application points. The mean δ $^{15}$N value for roots sampled from plot 10 (NO$_3$ application to grass), was 2.7 ± 1.5‰ ($n = 4$ grama patches, ambient δ $^{15}$N 0.5‰) with an average distance from application of 77.5 cm. For comparison, the mean δ $^{15}$N for foliage samples in the same plot on day 4 was 5.7‰ ($n = 3$ patches) at an average distance of 45 cm. For roots from plot 5 (Glu application to crust) average δ $^{15}$N was 1.4 ± 0.3‰ ($n = 4$ patches) at an average distance of 50 cm from the application point compared to a mean δ $^{15}$N for foliage samples of 20.3‰ ($n = 8$) at an average distance of 37 cm from the application. The δ $^{15}$N values for outlying roots are consistent with predictions based on the regression in Fig. 1.

The total translocated $^{15}$N recovered from crust and leaf tips accounted for 4.1 ± 0.8% and 7.4 ± 1.3%, for days 1 and 4, respectively, of the tracer applied to each experimental plot. The recovery values are based on conservative cover estimates that account for approximately two-third of the plot surface (Table 1) and are limited to a 60-cm radius. Greater than ambient $^{15}$N signals were found in some crust and plant samples to distances of 100 cm, indicating translocation rates as high as 100 cm day$^{-1}$. Consequently, the total quantity of $^{15}$N translocated from the points of application was probably much greater than that recovered from our foliage and crust sampling.

TRANSLOCATION OF $^{13}$C FROM GLUTAMATE

For experimental plots that received dual-label glutamate, no $^{13}$C signal was detected in foliar samples collected within a 15–60 cm radius of the application points. For crust samples collected at radii of 15–60 cm, $^{13}$C recoveries ranged from 0% to 15% after 24 h and 3.3% to 15% after 96 h (Table 1) with a significant decline in signal strength with increasing radius (Fig. 2). On day 1, no $^{13}$C was recovered from crust samples in three of the six plots. For crust samples collected on day 4, ANOVA indicated that recoveries were higher from plots in which labelled glutamate was applied to a crust patch compared to plots that had foliar application points (9.5% vs. 7.9%, respectively, $P = 0.018$, Table 2). There was weak covariance between $^{13}$C recovery and total crust C (123 μg $^{13}$C g$^{-1}$ crust C, $n = 6$, $r^2 = 0.34$).

The $^{13}$C data show that glutamate was not translocated into plants, even though $^{15}$N from glutamate appeared in foliage more rapidly than $^{13}$N from nitrate. Deamination may have occurred at the point of initial uptake or later at the interface of plant–fungal symbioses. Conversely, $^{13}$C from glutamate did move from plants to crust, implying that plant photosynthetic support of crust metabolism. The mean $^{13}$C : $^{15}$N ratio of label recovered from the plots was 7 ± 4 (the C : N ratio of glutamate is 5) indicating that the results were not skewed by differential losses of C via respiration or N via denitrification over the time course of the study.

FUNGAL DIVERSITY AND DISTRIBUTION

Analyses of fungal clone libraries produced from Sevilleta samples show that Ascomycota are the dominant fungal colonizers of soil and grass tissues accounting for 83.3% of sequences (Table S1 in Supplementary material). Of the 989 sequences from the NCBI data base, 528 sequences (53.4%) are Pleosporales with 71 OTUs and a Chao diversity estimate of 157 OTU (confidence interval 108–207). Analyses of community overlap using the program SONS show that nearly half (6 of 13) of the Pleosporales OTU that comprise root endophytic communities are also found in rhizosphere soil and biological soil crust (Fig. 3), and rhizosphere communities share 40% (24 of 60) of Pleosporales OTU with biological soil
crust. The most common OTU found in roots accounts for 12% of all fungal sequences and 17% of Pleosporales sequences. Phylogenetic analysis showed that this dominant fungal clade is also found in rhizosphere and biological soil crust (Fig. 4). This fungal clade seems to be ubiquitous and closely related to endophytes isolated from another semi-arid grass (*Stipa hymenoides*, AY929107), a coastal grass (*Ammophila arenaria*, AM921730), and several cupressaceous trees (Fig. 4). It is also found in other dominant grasses at the SNWR (e.g. *B. eriopoda* and *Sporobolus* spp.) (Herrera & Porras-Alfaro, unpubl. data). Although described as an endophyte, this organism grows readily in pure culture (Porras-Alfaro et al. 2008).

**Discussion**

**N translocation**

Our results show that both light cyanobacterial crust and the leaf surfaces of grama grasses can be loci for nutrient translocation, and that point sources of organic and inorganic N can be dispersed over approximately 1 m$^2$ areas (equivalent to a mosaic of approximately 20 grass or crust patches) at rates up to 100 cm day$^{-1}$ during periods of active growth. Because N translocation between grasses and crust was bidirectional for both nitrate and glutamate, the proximate control on flow was apparently fungal metabolism rather than plant or cyanobacterial demand.

The quantity of $^{15}$N applied (12.5 mg) to each plot was equivalent to approximately 0.5% of the N stock of biotic crust and grama foliage in the surrounding 1 m$^2$ area. The quantity of $^{15}$N translocated into foliage and crust at radii $>$ 15 cm from the application points averaged 223 $\mu$g day$^{-1}$ (based on standing stock data in Table 1 and incorporation rates of 27 and 110 $\mu$g g$^{-1}$ day$^{-1}$ for foliage and crust, respectively, presented above). This value underestimates total translocation because it does not include incorporation into root biomass. Nonetheless, these translocation rates are comparable to measured rates of N input and output from the Sevilleta grassland ecosystem. Atmospheric N deposition at the site averages 2 kg ha$^{-1}$ year$^{-1}$, equivalent to 550 $\mu$g m$^{-2}$ day$^{-1}$ (Baez et al. 2007). Denitrification, measured by Crenshaw et al. (2008) at 30% and 70% soil WHC was equivalent to 200 and 500 $\mu$g N m$^{-2}$ day$^{-1}$, respectively (assuming bulk soil density of 1.6 g cm$^{-3}$, crust depth of 1 cm, rhizosphere soil depth of 5 cm and cover estimates in Table 1). The similarity of N translocation rates to rates of N input and output, combined with data showing that fungal metabolism is responsible for 85% of N$_2$O production (Crenshaw et al. 2008), provides increasing evidence that a fungal network closely integrated with plant and cyanobacterial producers is responsible for a large fraction of the N cycling in desert...
grassland. There is extensive literature on plant to plant N translocation via ectomycorrhizal fungi (EMF), demonstrating, for example, that N fixed by actinorhizal roots can move via EMF to the roots of non-N-fixing plants (Arnebrant et al. 1993), that N assimilated by sun-exposed plants can move to shaded plants (Simard & Durall 2004), and that N from EMF plants can be translocated to AMF plants (Ek et al. 1996; He et al. 2006). In some cases, fungal translocation can provide a substantial portion of plant N demand.

Nutrient translocation through AMF networks has received less study (Brachmann & Parnisiki 2006). AMF are generally noted for their ability to translocate P (Ezawa et al. 2000; Pfeffer et al. 2004), but they also contribute significantly to N dynamics (Cliquet & Stewart 1993; Simard & Durall 2004; Johnson et al. 2003). Hawkes (2003) measured rates of N translocation into cyanobacterial crusts and AMF-colonized herbs in a Florida scrubland ecosystem, concluding that fungal connections between N-limited plants and N-fixing crusts are integral to ecosystem function. Other studies have shown that AMF infection rates are higher in areas where biological soil crusts develop (Harper & Pendleton 1993; Evans & Ehleringer 1993; Pendleton & Warren 1996).

Saprotrophic fungi also translocate N, directing N from areas of net mineralization to facilitate decomposition of fresh litter (Frey et al. 2000). Tiaka et al. (2002) measured rates of amino acid flow up to 50 mm h$^{-1}$ in Phanerochaete cords, with mass transfer of 5–50 pmol h$^{-1}$ per cord. Even higher rates (up to 250 mm h$^{-1}$) have been reported for cytoplasmic flow through hyphae in vitro (Allen 2006). Nutrient translocation has also been demonstrated for DSF (Jumpponen & Trappe 1998; Jumpponen et al. 1998; Addy et al. 2005; Usuki & Narisawa 2007).

Our measurements of rates of N translocation across mosaics of grama and crust patches are comparable to those reported for fungal translocation in other systems with maximal values of approximately 40 mm h$^{-1}$, but the relative contributions of AMF, DSF endophytes and saprotrophic fungi to this process are unknown. Across the mosaic, the fate of C and N from glutamate was consistent with AMF transport. AMF translocate C and N through hyphae, but unlike EMF, little or no C is transferred from hyphae into plants (Bago et al. 2000; Pfeffer et al. 2004). We did not detect N in leaf tissue, even though rates of $^{15}$N incorporation into foliage were similar for both NO$_3$ and Glu. However, AMF are rare at our study site (Porras-Alfaro et al. 2007). The translocation capacities of DSF are not well characterized but their abundance in grama roots and soil crust suggest they may be the principal agents of translocation. Direct plant to plant transfer of $^{15}$N via roots was unlikely because grama is a bunch grass and roots do not extend into the crust or connect adjacent plants. Also, we selected study plots with low cover where each plant was clearly isolated from all others by intervening crust patches.

Fungal community composition

Molecular and microscopic analyses of fungal community composition at our study site were conducted concurrently with the translocation studies. These analyses show that AMF diversity in grama roots is low (a single OTU closely related to Glomus intraradices accounted for 65% of the sequences) and only 1% of live roots showed morphological structures (arbuscules and vesicles) associated with AMF (Porras-Alfaro et al. 2007). In contrast, endophytes are both abundant and diverse (Chao estimate: 91 OTU; Porras-Alfaro et al. 2008). Pleosporales account for 50% of the fungal sequences found in libraries cloned from roots, followed by Sordariales (22%), Agaricales (14%) and Xylariales (12%). Some of these root symbionts have been previously described as coprophilic fungi and were not known to be endophytes, suggesting that herbivory may play a role in their life cycle and in the establishment of hyphal connections between habitat patches (Porras-Alfaro et al. 2008). Fungal diversity is highest in rhizosphere soil and BSC exceeds that of grama roots by a factor of three but like roots, most of OTUs (55%) are Pleosporales.

For both total fungi and Pleosporales, approximately half of the OTUs found within the cortical tissue of grama roots are also present within the soil crusts (Fig. 3). Of the 15 most abundant OTUs, five (30% of all sequences and all but one Pleosporales) are found in grama roots; these five include the two most common fungi, both Pleosporales, that together accounted for 21% of the sequences (Table S1). Although the evidence is circumstantial, the overwhelming dominance of DSF in both roots and crusts and the extent of overlap in the distribution of fungal OTUs in rhizosphere, biological soil crust and root communities suggest that these little studied fungi, rather than comparatively rare AMF, are the principal agents for N transformation and translocation within this system.

Ecosystem perspective

On a global basis AMF consume about 20% of plant photosynthate (Brachmann & Parnisiki 2006). For grasses, estimates of below-ground C allocation range from 25% to 50% (Liljeroth et al. 1994; Warenburg & Estelrich 2001). Our data showing that $^{13}$C derived from glutamate can be translocated from plant foliage to adjacent crust patches indicates that a portion of this below-ground C allocation may reach biological soil crusts, potentially supplementing C inputs from cyanobacteria and thereby contributing to the growth and development of surface crusts.

From the plant perspective, crusts are N sources in an N-limited ecosystem: sites of atmospheric N deposition and biotic N fixation that respond rapidly to wetting, with cyanobacterial photosynthate to support the mineralization and mobilization of N (Evans & Ehleringer 1993; Hawkes 2003). In desert grassland at Sevilleta, respiration rates for crusts are nearly twice that of rhizosphere soil (60 vs. 34 nmol h$^{-1}$ g$^{-1}$ at 30% WHC, Crenshaw et al. 2008) with comparable differences in potential rates of amino acid hydrolysis by extracellular enzymes (Stursova et al. 2006). For crusts, C inputs from plants via fungal translocation may supplement crust metabolism during growth periods when plant nutrient demand is high.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Most common fungi found in semi-arid grassland at the Sevilleta National Wildlife Refuge (New Mexico, USA).  

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2745.2008.01388.x  
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