Sagebrush carrying out hydraulic lift enhances surface soil nitrogen cycling and nitrogen uptake into inflorescences

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Plant roots serve as conduits for water flow not only from soil to leaves but also from wetter to drier soil. This hydraulic redistribution through root systems occurs in soils worldwide and can enhance stomatal opening, transpiration, and plant carbon gain. For decades, upward hydraulic lift (HL) of deep water through roots into dry, litter-rich, surface soil also has been hypothesized to enhance nutrient availability to plants by stimulating microbially controlled nutrient cycling. This link has not been demonstrated in the field. Working in sagebrush-steppe, where water and nitrogen limit plant growth and reproduction and where HL occurs naturally during summer drought, we slightly augmented deep soil water availability to 14 HL+ treatment plants throughout the summer growing season. The HL+ sagebrush lifted greater amounts of water than control plants and had slightly less negative predawn and midday leaf water potentials. Soil respiration was also augmented under HL+ plants. At summer's end, application of a gas-based ^{15}N isotopic labeling technique revealed increased rates of nitrogen cycling in surface soil layers around HL+ plants and increased uptake of nitrogen into HL+ plants' inflorescences as sagebrush set seed. These treatment effects persisted even though unexpected monsoon rainstorms arrived during assays and increased surface soil moisture around all plants. Simulation models from ecosystem to global scales have just begun to include effects of hydraulic redistribution on water and surface energy fluxes. Results from this field study indicate that plants carrying out HL can also substantially enhance decomposition and nitrogen cycling in surface soils.

rhizosphere | flowering | seed production

Hydraulic redistribution of water from deep moist to shallow dry soil, through plant roots, was first demonstrated nearly 25 years ago in northern Utah sagebrush steppe (1). That demonstration of hydraulic lift (HL) by sagebrush (*Artemisia tridentata* var. *vaseyana*) has inspired hundreds of studies of the phenomenon in ecosystems as diverse as Pacific Northwestern forests, Brazillian savanna, and Amazon rainforest (2, 3). Hydraulic redistribution through plant roots facilitates movement of water not only upward (as lift) but also downward and horizontally along moisture gradients within soils, affecting plant physiology, landscape hydrology, and potentially even climate by moving deep water up to dry shallow layers, where it can support enhanced plant transpiration (2, 4–6), and by quickly moving precipitation down into deeper soil layers, where it does not evaporate or run off the landscape (7–9).

Hydraulic redistribution is most commonly detected in terrestrial ecosystems with pronounced dry seasons (2) when water is depleted in heavily rooted upper soil layers as plants transpire. Soil organic matter, the primary source of many plant nutrients, is also present in the greatest concentrations in upper soil layers, so the drying of surface soils during drought may limit nutrient availability to plants through reduced microbial activity, soil hydraulic conductivity, root–soil conductivity, and fine root activity. It has therefore been hypothesized for decades (10, 11) that hydraulic lift of deep water to surface soils could provide a mechanism for ameliorating limitations in both surface soil water availability (12–14) and nutrient availability to plants (10, 11).

Demonstration of an effect of HL on soil microbial activity and associated nutrient uptake by plants in the field has proven elusive, however. Most recently, near Mono Lake, California, decomposition of fine root litter in litter bags was examined in soil cores around sagebrush in the field carrying out HL (15). Despite lower soil moisture in cores with roots compared with cores with very few roots, decomposition of fine root litter was enhanced in the more densely rooted cores; the investigators (15) suggest the effect may have been driven by the influence of HL on soil microbes. Resulting nutrient availability to shrubs was not assessed. In another study, also at the Mono Basin Ecosystem Research Site, uptake of mineral nitrogen (nitrate) injected into soil around the shrub *Sarcobatus vermiculatus* was assessed around individuals carrying out HL (16); nighttime bagging of shoots, meant to stimulate HL and thus possibly uptake of nitrate, unfortunately did not stimulate HL, and the natural variation in HL that remained was not correlated with uptake of the nitrate amendment. Decomposition of native soil organic matter was not assessed.

Several greenhouse experiments have also attempted to establish whether hydraulic redistribution can affect decomposition of organic material in soil and/or nutrient availability to potted plants. An early greenhouse experiment with potted sugar maples (*Acer saccharum)* used nighttime illumination to maintain high transpiration rates at night, decreasing HL; that decreased HL was associated with altered soil nitrogen cycling (17). However, the illumination treatment may have also induced other plant physiological effects beyond reducing HL (e.g., altered photosynthetic or other circadian rhythms, drawn down soil moisture, etc.), and associated plant nutrient uptake was not examined (17). Two more recent greenhouse experiments each used plants growing in "split pots," in which the root system of each plant was established in an upper and a separate lower pot with only roots connecting the two pots via a gap. Pots were filled either with native soil amended with vermiculite (18) or sand plus fritted clay inoculated with soil (19). The investigators again used nighttime illumination to limit HL, and they examined decomposition of ^{15}N-labeled plant litter inserted via cores into upper pots after the nighttime illumination treatments had been initiated. Both studies found

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increased uptake of ^{15}N from the added litter into plants that were carrying out more HL. Does this effect occur in undisturbed soils under natural conditions in the field? If so, is the increased amount of nitrogen taken up by plants carrying out HL ecologically relevant?

We tested whether plants carrying out HL stimulate microbial activity and N cycling in surface soils, as well as take up more nitrogen, at a field site near Laketown, Utah (N41° 47′ 42.22″, W111° 15′ 10.75″). Mature, deep-rooted sagebrush at this site (*A. tridentata* var. *vaseyana*) carry out HL during early and mid-summer, but at the height of seasonal drought (late July), natural HL slows or stops as even deep soil water is depleted. To prolong HL later into the season in our HL+ treatment, we provided 14 sagebrush plants with 2.4 L/d supplemental deep water beginning May 31 and ending October 17, 2007. (Water was provided via buried pipes at 70-cm depth to ensure it did not reach surface soil layers by capillary rise.) Fourteen interspersed control sagebrush were not provided with deep water. In mid-August, we used injections of isotopically labeled ^{15}N-ammonium and ^{15}N-ammonia gas to measure microbially controlled nitrogen cycling in soil around, and plant nitrogen uptake by, control and HL+ sagebrush. We thus tested the effect of an HL+ treatment in the field over time, avoiding unnatural treatments such as overnight illumination, and we simultaneously examined all linkages from HL to nutrient cycling (decomposition) to plant nutrient uptake, using an in situ technique that does not disturb soil structure, natural soil organic matter content and chemistry, or soil water content.

Results

Beneath each of the 14 HL+ and beneath seven (of the 14 total) control plants, seven screen-cage thermocouple psychrometers had been implanted (at 35- and 50-cm depths) to measure soil water potential, and a polyvinyl chloride (PVC) collar had been permanently inserted 2.5 cm into the soil surface to enable repeated measurement of soil respiration throughout the summer under each sagebrush canopy (*Materials and Methods*). As expected, all HL+ and control plants facilitated HL at night through early August (Fig. 1). The diel sawtooth pattern visible in Fig. 1*B* is diagnostic of nightly HL (soil moistening) followed by transpiration (soil drying) the next day. The effect of the HL+ treatment was to maintain consistently higher (less negative) water potentials measured at the 35-cm soil depth and to maintain HL during even the driest portion of the season.

As summer progressed, soil respiration (comprising both plant root and heterotrophic respiration) decreased under shrub canopies, most strongly under control shrubs (Fig. 2*A*). Repeated-measures ANOVA revealed a significant effect of HL+ deep-watering on soil respiration ($P < 0.001$). Midday (12:00–1:30 PM) and predawn (3:30–4:40 AM) sagebrush leaf water potentials (Fig. 2*B*) were also slightly less negative in HL+ compared with control plants (repeated measures ANOVA: midday, $P = 0.006$, and predawn, $P = 0.001$).

In mid-August, to test whether the HL+ treatment stimulated nutrient cycling and nutrient availability to plants, we measured gross N-cycling rates in the intact plant–soil system, using an unusual ^{15}N isotope dilution technique (20, 21). Typically, ^{15}N pool dilution assays require injection of dissolved ^{15}N-ammonium in water into the soil, followed by tracking of the ^{15}N tracer through various soil and organismal N pools (22). However, because the soils at our site were exceptionally dry in August, and the magnitude of HL was quite small at that time, injections of water as part of N-cycling assays would potentially obscure differences between the HL+ and control treatments. Therefore, we isotopically labeled soil ammonium pools by injecting ^{15}N-ammonia gas into the top 10 cm of undisturbed soil around the base of 16 of the 28 shrubs, allowing the ammonia to dissolve in the very thin water films naturally persisting in the soil. For comparison, we also used a more standard, water-based technique, in which soil beneath the remaining 12 shrubs was injected with solutions containing ^{15}N-ammonium in water. The dissolved ^{15}N-ammonium pool was diluted by naturally occurring ^{14}N-ammonium produced during microbial mineralization of organic ^{14}N. This dilution,

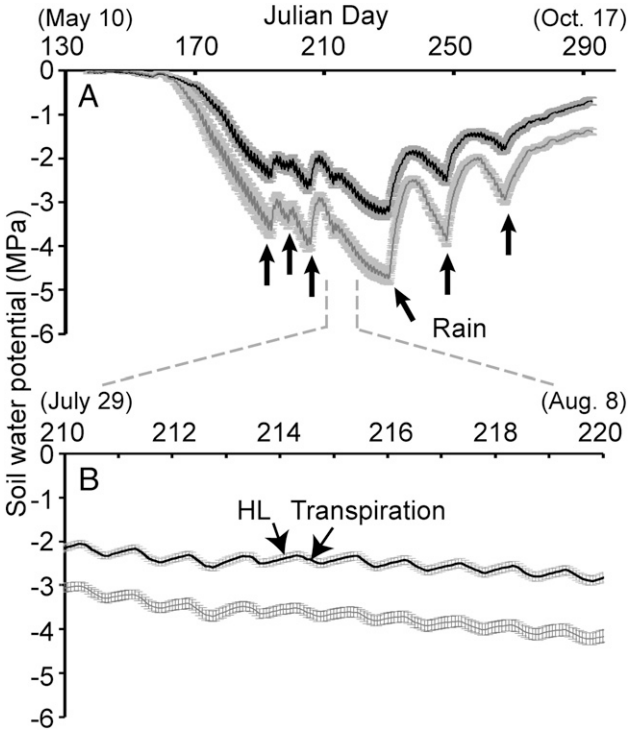

Fig. 1. Soil water potential through time. (*A*) Mean ± SEM of thermocouple psychrometer traces taken every 1.5 h during the summer at 35-cm soil depths beneath HL+ plants (black line) and control plants (dark gray line). Error bars are indicated in lighter gray. Rain events are indicated with black arrows. Variability is maximum at midsummer. (*B*) Zoomed view, showing diel, sawtooth pattern created by hydraulic redistribution of water upward at night and transpirational loss of water during the day.

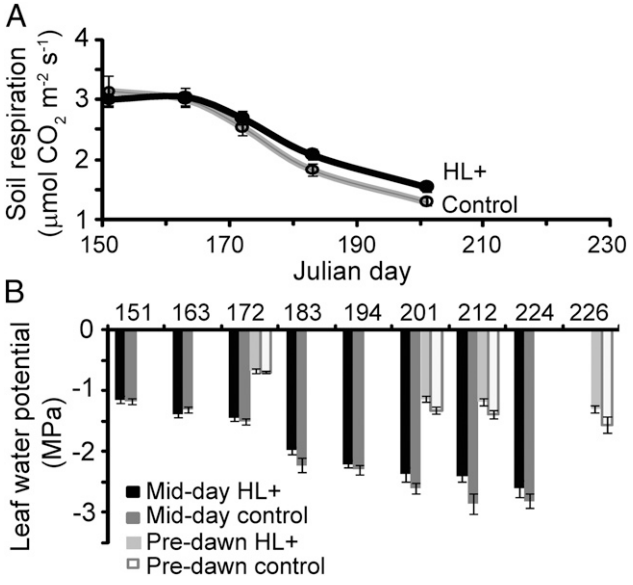

Fig. 2. Seasonal trajectory of (*A*) soil respiration rate under control (gray line) and deep-water-supplemented (HL+; black line) sagebrush, and (*B*) midday and predawn sagebrush leaf water potentials.

combined with the appearance of ^{15}N in soil, microbial, and plant pools during the 48-h in situ incubation, allowed us to quantify net and gross rates of N mineralization and N consumption, as well as rates of plant N uptake from the surface soil layer (0–10 cm) (21–23). This gas-based injection technique enables measurements of gross N-cycling rates in undisturbed plant–soil systems characterized by exceptionally dry soil (21, 24).

Because 28 (14 HL+, 14 control) shrubs were to be assayed, we divided the plants into three blocks and conducted the 48-h assays staggered across 5 d (Fig. 3). Unexpected early monsoon rainstorms of sufficient size to affect soil moisture occurred during the second and third assay periods (Fig. 3*A*). As a result, although initial water contents were similar for all assays (Fig. 3*B*, white bars), soil from the three assay periods had widely differing final water contents (Fig. 3*B*, black bars). The effect of liquid vs. gaseous injection of ^{15}N on soil gravimetric water content was also discernible (ANOVA, $P < 0.001$; Fig. 3*B*). Together, the rainfall events and the liquid vs. gaseous ^{15}N injections produced different soil moisture contents, and this enabled our examination of whether the summer-long HL+ treatment influenced soil process rates across a range of soil moistures.

For statistical analyses of these data, we expressed soil moisture content for each assay period as a time-weighted average of the initial and final (48 h) soil moistures (Fig.4*A*), taking into account the timing of rainfall within the assay window. Despite the wetter soil detected by psychrometers at a 35-cm depth around HL+ plants (Fig. 1), our summer-long HL+ treatment had no detectable effect on gravimetric soil moisture content in the surface soil (0- to 10-cm layer) at the time of soil assays (Fig. 3*B*; ANOVA: initial water content, $P = 0.669$; final water content, $P = 0.309$; and time-weighted water content, $P = 0.271$). Nevertheless, nutrient cycling was stimulated in the surface soil layer around HL+ shrubs. Analysis of covariance (ANCOVA) (with time-weighted soil moisture as the covariate) showed that net ammonification was enhanced by the HL+ treatment (Fig. 4*B*; $P = 0.015$). (Net ammonification is the difference between gross N mineralization and ammonium consumption by microbes and plants.) Gross N mineralization was also enhanced marginally by the HL+ treatment ($P = 0.078$), but this stimulation was only clear in the first two assays, when soil was relatively dry (Fig. 4*C*), and was diminished during the third assay, when rainfall approximately doubled soil moisture contents (Fig. 4 *A* and *C*). Gross inorganic N consumption (uptake of soil ammonium and nitrate by plants and microbes) was unaffected by the HL+ treatment ($P = 0.665$; Fig. 4*D*), but incorporation of ^{15}N into developing sagebrush inflorescences was stimulated by the HL+ treatment (Fig. 4*F*, $P = 0.027$). Soil core respiration (measured from two intact soil cores, 0–10 cm, collected beneath each plant and incubated 48 h, comprising respiration from severed roots and soil heterotrophs including microbes) was also enhanced by the HL+ treatment (Fig. 4*E*; $P = 0.043$). Rates in Fig. 4 *B–F* are expressed only for the 17 small volumes of soil injected during the assays; we do not scale up to the full rooted volume of soil available (in the top 10 cm) for each plant because we do not know the lateral extent of the root systems of each plant.

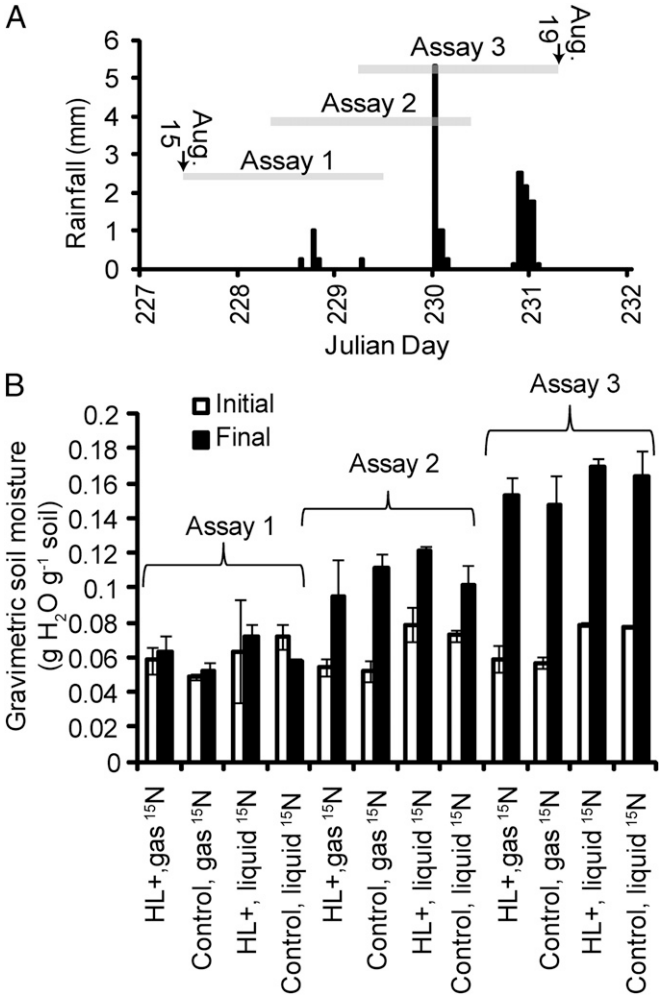

Fig. 3. Sources of soil moisture during ^{15}N assays. (*A*) Mid-August rainfall during the three sets of soil assays that were staggered in time. (*B*) Initial and final soil moisture for each assay period, treatment, and injection type.

Discussion

These results demonstrate that sustained HL of deep water by sagebrush was linked to increased soil microbial activity at 0- to 10-cm depths (Fig. 4 *B–D*) and increased plant uptake of N from this layer (Fig. 4*F*). This enhancement occurred even though the surface (0- to 10-cm depth) gravimetric soil moisture contents were not detectably different around HL+ compared with control plants. The gravimetric approach, however, is likely not sensitive enough to detect HL+-induced, but highly localized, increases in moisture in soil microsites adjacent to plant roots. Because rhizosphere microbial populations exceed bulk soil populations by orders of magnitude (25), localized moisture increases in this densely populated soil region would be expected to have an inordinate effect on measured whole-soil microbial activity, potentially without substantially influencing overall gravimetric moisture contents. More sensitive psychrometric measurements of water potential cannot be made in surface soils because diel temperature fluctuations create unacceptable artifacts (2). However, measurements of predawn leaf water potential (Fig. 2*B*) showed that whole-plant water potentials were slightly more positive in HL+ plants, suggesting that HL+ plants could have maintained a moister rhizosphere microenvironment (undetected in bulk gravimetric soil moisture assays), promoting the greater microbial activity we measured with ^{15}N in surface soils.

Alternatively, we can also speculate that HL+ plants may have maintained a greater biomass of active fine roots and/or mycorrhizas in surface soils later into the season than control plants (12–14, 26, 27). Greater fine root and/or mycorrhizal biomass and specific activity in the surface soil would be consistent with the observed increase in ^{15}N in inflorescences in the HL+ plants (Fig. 4*F*). However, could maintenance of roots and mycorrhizas explain the increased net ammonification and gross mineralization in the HL+ treatment (Fig. 4 *B* and *C*)? Sagebrush support arbuscular mycorrhizas, which are not known for an extensive ability to mineralize complex organic matter (28). Other microbes are more likely responsible for the changes in nutrient

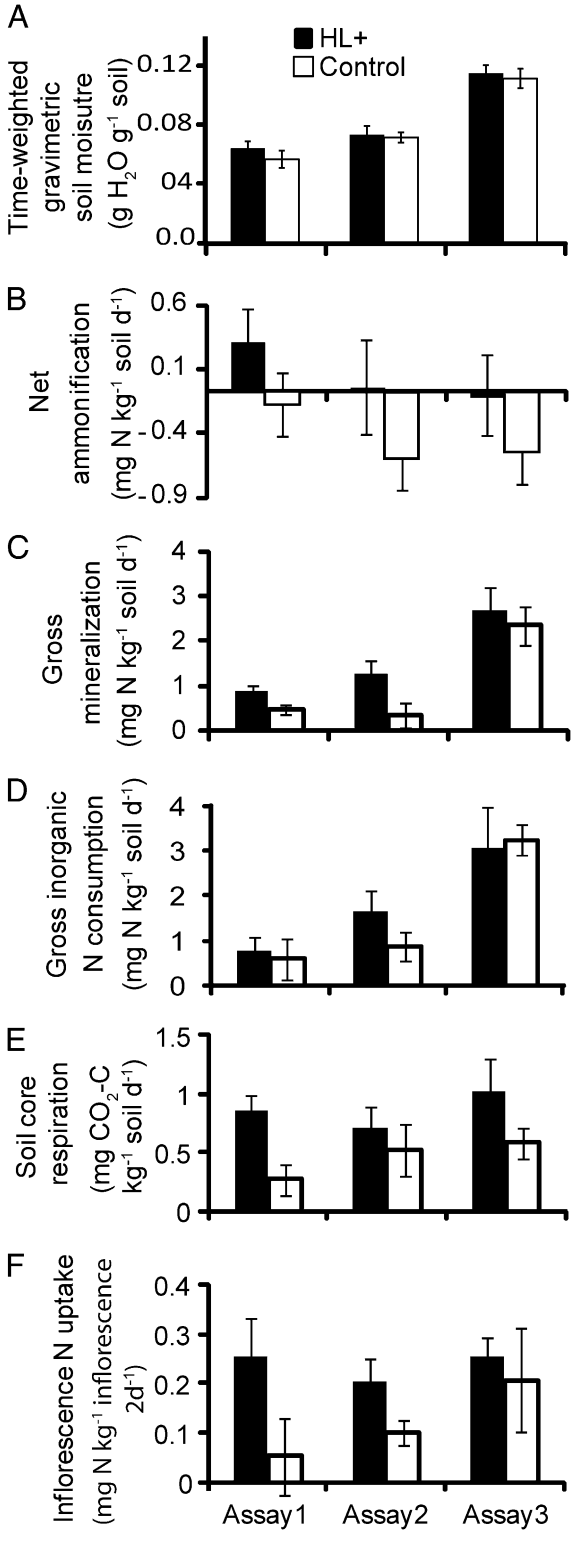

Fig. 4. Soil moisture and plant and soil process rates during ^{15}N assays. (*A*) Time-weighted soil moisture in the 0- to 10-cm soil layer beneath each plant, with plants assayed in groups at the three times shown in Fig. 3*A*. Soil nitrogen (*B–D*) and carbon (*E*) cycling rates assessed in the 0- to 10-cm soil layer, along with rates of plant nitrogen uptake into inflorescences from labeled soil locations (*F*) for HL+ (black bars) and control (white bars) plants. Overall statistical significance of HL+ vs. control treatments on each process was assessed by ANCOVA using data from all three assays combined, with time-weighted soil moisture as the covariate (see *Results* for *P* values). However, to illustrate the effect of rainfall during the assays, means and SEs are presented separately for each assay period.

cycling observed here. However, might those other microbes have been stimulated by root system–derived carbon (rather than water) in surface soil around HL+ plants if roots and mycorrhizas were more active? If temperature and moisture permit, such an increase in the carbon supply to microbes from living roots should increase microbial growth. Such microbial growth would be consistent with the observed increase in soil respiration in the HL+ treatment (Fig. 4*E*). However, immobilization of N would be required for building new biomass, and soil microbial biomass N was not increased in the HL+ treatment ($P > 0.39$; Fig. S1*A*). The simplest explanation consistent with all of the observed patterns is that the HL+ treatment increased water availability locally around plant roots, stimulating microbial (perhaps including some mycorrhizal) activity and N cycling, but we do not dismiss the possibility that enhanced carbon flux from living HL+ roots may also have influenced some of the observed results.

There was no difference in the rate of uptake and translocation of N into foliage ($P > 0.98$; Fig. S1*B*); however, this is not surprising, as our assays were performed during mid-August, when sagebrush inflorescences were maturing and seeds were developing. At this time of year, developing inflorescences are the dominant plant sink for N (29). Given that several studies have shown that N availability limits seed production in sagebrush and other semiarid shrubs (30, 31), our results indicate that HL can provide sagebrush and other shrubs with a way of increasing uptake of a limiting resource at a time that is critical for reproductive success.

We can explore whether the enhanced uptake of N under the HL+ treatment is substantial enough to be ecologically relevant using the values for mixed ^{15}N and ^{14}N uptake in Fig. 4*F*. These values reflect uptake only from the very small volume of soil around each plant that we labeled with injections; we did not scale up the numbers for the full rooted area of each plant because we did not measure that area. However, for the sake of argument, as the plants were, on average, 1 m in diameter and 0.7 m in height, we will assume for this calculation that radial extent of the roots' footprint extends 1 m from the main trunk. We base this assumption on two papers in the literature that examine the lateral and depth distribution of *A. tridentata* roots in the field and found that the lateral extent of roots out from the main trunk of plants was approximately equal to (32) or 1.5 times (33) the overall height of the plants.

There were 17 injection points around each plant, and six points were cored immediately at time 0. Over the next 48 h of incubation, the labeled ammonium pool (i.e., mixed ^{15}N and ^{14}N) from the remaining 11 injection points was available for plant uptake. From our previous experience with these soils, we know that the spread of ^{15}N from the injection site is likely less than the 2.5 cm that we used for our coring. However, conservatively using a 2.5-cm diameter for each point, the total labeled area around each plant was 11×0.00049 m^2 or 0.0054 m^2. If we assume that our uptake rates are representative of the full 1-m radius circle centered around each plant (area = 3.14 m^2), then the 0.25 mg $N{\cdot}kg^{-1}{\cdot}2$ d^{-1} uptake from injected soil, shown in Fig. 4*F* for HL+ in assay 1, is the equivalent of 0.145 g N taken up per kilogram inflorescence per 2 d from that full 1-m radius rooted area. Using the known mean of 1.66% N by mass in inflorescence tissue, 0.145 g $N{\cdot}kg^{-1}$ is 0.88% of the N in the inflorescence. Over the course of 1 month (30 d), 13% of inflorescence N could be obtained at this uptake rate by HL+-treatment plants. In contrast, the uptake rate for the control (0.05 mg $N{\cdot}kg^{-1}{\cdot}2$ d^{-1}) scales to 0.029 g $N{\cdot}kg^{-1}$ over the course of 2 d (following the same logic), or only 0.18% of the N in the inflorescence; over the course of 1 month (30 d), 2.6% of inflorescence N could be obtained at this uptake rate. These uptake rates would be even higher if nutrients were taken up from more than just the 0- to 10-cm depth or from outside the 1-m radius area used in these calculations. However, even the very conservative estimate

developed here shows that N uptake by the HL-treatment plants could provide a quarter of the inflorescence N content over the very dry period from day 190 through day 250 (Fig. 1). Over that same 60-d period, control plants would take up only 5% of inflorescence N. The difference in nitrogen availability between control and HL+ treatments is considerable at this critical time of reproduction.

Mathematical models at the ecosystem (34, 35), regional (36), and global (8) scales have just begun to incorporate the effects of hydraulic redistribution, as its importance for plant physiology and landscape hydrology has become more widely acknowledged. However, only the direct hydrologic effects of hydraulic redistribution on evapotranspiration, energy flux, and plant carbon gain (via increased shallow soil moisture and stomatal opening) have been included. Our data indicate that HL can also affect plant fitness and/or ecosystem productivity through a soil biogeochemical pathway by promoting nutrient availability.

Materials and Methods

Field Site. We examined whether plants carrying out HL influence microbial activity and N cycling in surface soils, and whether increased plant N uptake results, at a field site just east of Laketown, UT (N 41° 47′ 42.22″, W 111° 15′ 10.75″), at 2,130 m elevation. Mean annual temperature is 3.4 °C, and mean annual precipitation is 30 cm. The soil is a Kearl Loam characterized as coarse loamy, mixed, frigid, Calcic Haploxerolls (US Department of Agriculture, http://soildatamart.nrcs.usda.gov/manuscripts/UT604/0/rich.pdf). Soil pH was 7.05 (1:1 ratio of soil and water). We used mature, deep-rooted sagebrush (*A. tridentata* var. *vaseyana*) at this site. Plants averaged 1.04 ± 0.02 m diameter by 0.69 ± 0.02 m height (mean ± SE).

Under each plant, 1 year before measurements, four screen-cage thermocouple psychrometers (Westcor) were installed at a 20° angle from horizontal from four cardinal directions, angling in under the plant canopy and ending near the plant's central axis at a 35-cm depth. Three more were implanted at a 50-cm depth by installing at 27° from the north, south, and east sides. Psychrometer wires ran aboveground through white PVC pipes to two solar-powered CR7 Campbell Scientific data loggers equipped with multiplexers and storage modules.

Four 1.25-cm diameter PVC watering tubes also angled into the soil from outside the canopy drip-line (two tubes on opposite sides of each plant), with lower ends at 70 cm depth directly below the plant. Water was delivered to plants through 0.25-inch (6.35 mm) irrigation tubing threaded down the PVC pipes. Water was provided by a solar- powered Masterflex peristaltic pump (Cole Parmer) with timed switching valves, pumping 2.4 L water d^{-1}·$plant^{-1}$ from a 1,600-L storage tank. During our final statistical analysis, it became clear from thermocouple psychrometer data that one control plant was tapping into the HL+ water being added to its neighbors, so that plant was switched from the control to the HL+ group for subsequent statistical analyses.

Soil Respiration and Plant Water Potential. Soil respiration was measured over the summer season, using a LiCor 6400 Photosynthesis System (Licor Inc.) equipped with soil temperature probe and soil respiration chamber, which slipped onto a section of 10-cm diameter, 5-cm tall PVC ring pushed 2.5 cm into soil, at 15 cm from the central axis of each plant. Midday (12:00–1:30 PM) and predawn (3:30–4:30 AM) leaf water potentials were measured using a Model 600 pressure chamber (PMS Instrument Company).

^{15}N Assays and Soil Core Respiration. For ^{15}N assays, 3 mL ammonia gas (9.9 mmoles NH_3/L at 99 atom% ^{15}N; Sigma-Aldrich) or 3 mL ammonium in water [4.8 mmoles N/L as ammonium sulfate $(NH_4)_2SO_4$ at 99 atom% ^{15}N; Sigma-Aldrich] were injected to a 10-cm soil depth using a syringe and side-port needle, withdrawing the needle as the gas or liquid was injected to distribute the ^{15}N throughout the 10-cm soil layer. Injection was at 17 of 18 points in a 30-cm diameter circle centered on the base of each of the 28 sagebrush. At the eighteenth location, an intact 4.8-cm-diameter by 10-cm-deep soil core was collected and incubated in the laboratory for 50 h at 23 °C in a 1-L canning jar to determine soil respiration (CO_2 production) rates by gas chromatography. At 180 degrees across the plant axis from this soil respiration core, another was taken slightly outside the injection ring and treated identically.

Ammonia gas injections were made around 16 of the 28 shrubs. To enable comparison with the standard technique, ammonium in water was injected in the same manner around the remaining 12 of the 28 shrubs. For all shrubs, immediately after injection, soil cores (2.5 cm in diameter, 10 cm deep) were collected from six of the 17 injection locations and the soil was extracted (in 0.5 M potassium sulfate K_2SO_4) for inorganic N and ^{15}N analyses (21–23). After 48 h, cores were collected from six more injection locations. Soil collected at 48 h was also analyzed for microbial biomass ^{15}N, using chloroform fumigation and extraction, and using a k_{EN} of 0.624 determined previously in soils from this site (21). Plant leaf and inflorescence tissue was collected before injections and at 48 h. Plant, soil, and extract samples were analyzed for ^{15}N on a Europa Scientific 20–20 continuous flow isotope ratio mass spectrometer (CF-IRMS) interfaced with the Europa ANCA-SL elemental analyzer (PDZ Europa) in either the Stable Isotope Laboratory at the Marine Biological Laboratory or at Utah State University. Calculations of microbial process rates followed Herron and colleagues (21). Briefly, gross mineralization was calculated from the dilution of isotope in the injected pool of ammonium and the change in the pool size during the incubation. Net mineralization was calculated from the change in nitrate and ammonium pools. Net ammonification was calculated from the change in ammonium pool, and net nitrification was calculated from the change in nitrate pool. Gross inorganic N consumption was calculated from the difference between gross mineralization and net mineralization. Plant ^{15}N was converted to N uptake on the basis of mean soil $^{15}NH_4^+$ enrichments during the 48-h assay (22).

Statistics. Statistical analyses were performed using Systat 12. Treatment effects were analyzed by ANCOVA, with time-weighted gravimetric soil moisture as the covariate. After accounting for soil moisture, rates determined from ^{15}N gas vs. solution injections were indistinguishable, and thus results from the two techniques were combined in statistical analyses.

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