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Effects of water and nitrogen availability on nitrogen contribution by the legume, *Lupinus argenteus* Pursh

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ABSTRACT

Nitrogen-fixing species contribute to ecosystem nitrogen budgets, but background resource levels influence nodulation, fixation, and plant growth. We conducted a greenhouse experiment to examine the separate and interacting effects of water and N availability on biomass production, tissue N concentration, nodulation, nodule activity, and rhizodeposition of *Lupinus argenteus* (Pursh), a legume native to sagebrush steppe. Plants were grown in a replicated, randomized design with three levels of water and four levels of N. Additional water and N increased biomass except at the highest N level. All plants formed nodules regardless of treatment, but plants grown without N had the largest, most active nodules. Organic N was deposited into the rhizosphere of all plants, regardless of treatment, indicating that *Lupinus* can influence N availability while actively growing, even under water stress. High tissue N concentrations and low C:N ratios indicate that *Lupinus* also can provide substantial amounts of N through litter decomposition. The ability of *Lupinus* to affect N availability and cycling indicates that it has the potential to significantly influence N budgets and community composition within the sagebrush steppe.

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1. Introduction

Nitrogen (N) often is a primary limiting nutrient in arid and semi-arid ecosystems. Total soil N ranges from 50 to 500 g m^{-2}, but much of this is present in forms unavailable to plants (West and Klemmedson, 1978; Zak et al., 1994). Thus, processes influencing N availability in these ecosystems play an important role in determining plant productivity (Suding et al., 2005), species composition (Huenneke et al., 1990), and successional patterns (Vinton and Burke, 1995; Paschke et al., 2000). Atmospheric deposition is an important source of N in some arid ecosystems (Fenn et al., 2003; Vourlitis et al., 2007). In portions of the California chaparral, atmospheric N deposition is sufficiently high that it influences species composition and plant invasions (Cione et al., 2002). In other arid ecosystems, N fixation is a main contributor of N. Fixation by legumes is known to be important for the N budget of the Sonoran Desert (Rundel et al., 1982; Shearer et al., 1983), the shrublands of Australia (Unkovich et al., 2000) and northern Mexico (Herrera-Arreola et al., 2007), and the Mediterranean region of Europe (Arianoutsou and Thanos, 1996).

In sagebrush steppe ecosystems of the Intermountain West, N fixation by cryptobiotic crusts is often considered the main source of N (Rychert et al., 1978; Evans and Ehleringer, 1993; Belnap, 2002a; Housman et al., 2006). However, the presence of crusts is highly related to soil characteristics and ecological condition (Evans and Belnap, 1999; Evans and Johansen, 1999). In many areas overgrazing by livestock and other human disturbances have severely limited crust abundance (Belnap, 2002b; Housman et al., 2006). In contrast, N_2 fixing plant species, primarily native legumes, are often relatively abundant in sagebrush steppe (Johnson and Rumbaugh, 1986; Crews, 1999; Goergen and Chambers, in press). Many of these species increase following disturbances such as overgrazing by livestock (Ralphs, 2002) and fire (Tracy and McNaughton, 1997; Goergen and Chambers, in press). Despite the importance of N inputs from legumes in arid regions throughout the world, relatively little is known about N contributions by native legumes within sagebrush ecosystems.

Lupinus argenteus (Pursh) is a native legume abundant in high elevation areas of western North America, and is one of the most common native legumes in sagebrush steppe. *L. argenteus* density can range from 3 to 10 plants m^{-2} depending on time since disturbance (Goergen and Chambers, in press). Prior work on high elevation sites indicates that it can fix substantial amounts of N (Rumbaugh and Johnson, 1991) even in disturbed sites (Johnson and Rumbaugh, 1986), and that it can increase extractable

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inorganic soil N (Kenny and Cuany, 1990). This indicates that *L. argenteus* has the potential to influence N availability in sagebrush ecosystems.

N_2 fixation by legume species and, consequently, the potential contribution of fixed N, is influenced by resource availability. In arid and semi-arid ecosystems, soil water availability is generally the primary determinant of plant growth (Comstock and Ehleringer, 1992; Loik et al., 2004) and can affect nodulation and symbiotic N_2 fixation in legumes (Engin and Sprent, 1973; Zahran, 1999; Hendricks and Boring, 1999; Streeter, 2003). Plant growth also is greatly influenced by mineral N, and levels of available N affect initiation of root nodules in legume species as well as nodule activity (Arnone et al., 1994; Chu et al., 2004). In general, as N levels increase, nodule weight, density, and fixation rates decrease and dependence on mineral N increases (Streeter, 1985; Zahran, 1999; Leidi and Rodriguez-Novarro, 2000). Although both water and N influence N contribution by legumes, these resources are often co-limiting. Thus, the interaction of these resources may have additional consequences for legume productivity and fixation.

Soil beneath canopies of N_2 fixers often contains greater amounts of available inorganic N than soil from areas lacking N_2 fixers (Kenny and Cuany, 1990; Thomas and Bowman, 1998; Maron and Jefferies, 1999; Myrold and Huss-Danell, 2003). However, the exact mechanisms responsible for this increase in available soil N have not been characterized for many systems. The potential mechanism most commonly cited is that N is added through decomposition of N-rich litter (Schlesinger, 1991, pp. 175). However, another potential mechanism through which N can be added is via rhizodeposition of amino acids (Rovira, 1969; Cheng et al., 2003; Jones et al., 2004). For example, when grown under N free conditions, the leguminous tree *Robinia pseudoaccaia* exuded 1–2% of fixed N as dissolved organic N (DON) from roots (Uselman et al., 1999). Many crop legumes have been found to exude even higher amounts of N. For example, rhizodeposition of N by unfertilized *Trifolium* amounted to 64–71 g N m^{-2} depending on the species (Hogh-Jensen and Schjoerring, 2001). This suggests that uncultivated legumes also may contribute substantial amounts of N to ecosystems via this mechanism, but information on N rhizodeposition by native herbaceous legumes from uncultivated systems is lacking.

Contribution of N by native legumes, either through rhizodeposition or decomposition, can potentially have a large effect on maintaining native species diversity and productivity of sagebrush ecosystems. However, if an exotic seed source is present, N-rich patches created by native legumes can create conditions favorable for invasion by exotic species. Understanding how *L. argenteus* responds to varying water and N availability can provide insights into its influence on species interactions and community functioning under different environmental conditions and following disturbances such as fire or increased N deposition. We examined the separate and interacting effects of water and N availability on N contribution by the native sagebrush legume *L. argenteus*. We addressed two questions. (1) How does *L. argenteus* biomass, tissue N, nodulation, and nodule activity change with resource availability? (2) Does *L. argenteus* exude detectable amounts of organic N and if so, how does exudation change with resource availability?

2. Materials and methods

2.1. Experimental design

We conducted a greenhouse experiment to examine the effects of water and N on *L. argenteus* growth, tissue concentration, nodulation, nodule activity and root exudation. A factorial experiment with three levels of water and four levels of N was used. The study was implemented as a complete randomized design with 12 replications of each treatment combination, although mortality over the course of the experiment resulted in lower sample sizes for some treatments (n = 6–12).

In spring 2006 *L. argenteus* (hereafter *Lupinus*) seeds were obtained from a local Sierran source (Comstock Seeds, Gardnerville, NV). Seeds were scarified with sandpaper to promote germination. Scarified seeds were then coated with a commercial inoculum (Nitragin, Lupine inoculum H; Milwaukee, WI) and allowed to dry before planting. This peat-based inoculum contains 100 million *Rhizobium* bacteria per gram and is composed of bacterial strains selected for their ability to produce effective nodules on *Lupinus* species (Smith et al., 1988). Preliminary studies indicate that this inoculum promotes a similar density of active nodules and biomass production in *Lupinus* as that seen in field inoculated plants. Seeds were planted in 0.1 m diameter by 0.35 m tall (2.7 L) PVC tubes (3 seeds/tube) containing washed sand (#16 mesh size). The bottom of each PVC tube was covered with mesh and pots were placed on an elevated platform to allow free drainage. Pots were watered with deionized (DI) water until the seedlings had emerged and had their first true leaf. Seedlings were then thinned to one plant per pot. Plants were grown in the University of Nevada, Reno greenhouses under natural light with a climate controlled maximum daily temperature of 27 °C and minimum nightly temperature of 7 °C.

Plants were randomly assigned to one of the 12 water and N combinations. Plants received the assigned nutrient solution during each regular watering session. All nutrient solutions were based on a modified 1/4 strength Hoagland's nutrient solution with only N varying. Concentration of N used was based on the range of N (as NH_4NO_3) found in soil extracts under field conditions in semi-arid sagebrush steppe (West and Klemmedson, 1978): 0 N, 5 mM N (end of season conditions), 20 mM N (early season conditions), and 100 mM N (extreme post-fire conditions). To prevent accumulation of nutrients in the dried sand, pots were flushed with 1 L of DI at each scheduled watering and allowed to freely drain prior to receiving 200 mL of fertilizer. The water holding capacity of the sand filled pots was approximately 400 mL, and preliminary tests indicated that flushing pots with this amount was more than sufficient to remove any residual nutrient solution. This method allowed plants to be exposed to a more consistent nutrient environment throughout the experiment.

For water response, preliminary trials were conducted to determine the volume of water needed to bring pots to field capacity and at what frequency of watering plants exhibited different degrees of water stress. Pots were watered to field capacity (400 mL) and allowed to freely drain. The number of days required for plant wilt to occur was recorded and served as the watering interval for the low water treatment. Water stress was verified by comparing stomatal conductance using a LI-6400 (LI-COR Lincoln, NE) between droughted and well watered plants. Based on these results, plants assigned the high water treatment were flushed and received the 200 mL nutrient solution twice a week, plants under the moderate water treatment once a week, and plants in the low water treatment once every two weeks. PVC tubes without any seedlings also were set up and watered with DI water (n = 6) to determine any background N. Plants were grown under their respective treatments for 3 months (March–May 2006) and were randomized every two weeks to reduce edge and neighbor effects. To account for any size bias, height and number of leaves for all seedlings were recorded at the initiation of the experiment (Gibson et al., 1999).

2.2. Root exudation

Before harvesting, all plants were flushed with 2 L of DI water and allowed to drain. Plants were then watered with the 0 N nutrient solution and incubated for 24 h to examine root

exudation. The incubation period was chosen to avoid any possible effect of diurnal fluctuations in root exudation. At the conclusion of incubation, all pots were flushed 3 times with 400 mL DI. The volume of water that drained after each flush was recorded and a 50 mL subsample was collected and immediately frozen for later analysis. The three subsamples for each pot were composited and divided into 2 samples: one for analysis of inorganic N and one for total N. Samples were centrifuged for 2 min at 5000 rpm before analysis (Sorvall RC 5C) to allow any particulates to settle. Amounts of inorganic N ($NH_4^+ + NO_3^-$) were quantified using a LACHAT QuikChem® Flow Injection Analysis System (Milwaukee, WI). Dissolved organic nitrogen was determined by subtracting total inorganic N from total N obtained via persulfate digestion (Sollins et al., 1999). In addition, for plants receiving the 0 N treatment for the duration of the experiment, amounts of organic N present in the rhizosphere were compared to N_2 fixed to determine percentage of fixed N being exuded.

2.3. Plant harvesting and tissue analysis

At the conclusion of the experiment, above and belowground (roots + nodules) plant tissue was harvested, dried at 65 °C for 48 h, and weighed to determine biomass. Above and belowground tissue were milled separately (UDY Corp., Fort Collins, CO) and analyzed for total N and carbon (C) concentrations using a LECO TruSpec CN analyzer (St. Joseph MI). Carbon and N concentrations were multiplied with plant biomass to determine C and N content. A small portion of total plant N originates from seed, thus the average amount of N present in a subsample of *Lupinus* seeds was determined.

A subsample of aboveground plant tissue from each treatment also was analyzed for natural abundance ^{15}N and ^{13}C (UC Davis Stable Isotope Facility). The $\delta\ ^{15}N$ signature reflects the N acquired over the life of the plant, and is the sum of a fraction of seed N and N obtained from the soil plus any atmospheric N_2 acquired through fixation. Therefore, in addition to analyzing leaf tissue from each treatment for natural abundance ^{15}N, a subsample of *Lupinus* seeds was analyzed for ^{15}N to determine the baseline signature ($\delta\ ^{15}N_{seed} = -0.92‰$). To identify uptake of fertilizer-derived N in the 5 mM, 20 mM and 100 mM N treatments, the signature of the NH_4NO_3 fertilizer also was determined ($\delta\ ^{15}N_{fertilizer} = -0.65‰$).

Tissue ^{13}C was used to verify plant stress according to water treatment, and also to estimate water use efficiency (WUE) under the different treatments (Ehleringer and Osmond, 1983). Leaf $\delta\ ^{13}C$ signature revealed that plants under low water experienced greater water stress than moderate or high water treatments, indicating that our treatment had the desired effect. Further, across all water treatments, plants increased WUE (leaf $\delta\ ^{13}C$ became more positive) as N increased (data not shown), indicating that *Lupinus* responds to water and N as would be expected for a plants from a cold desert environment (Toft et al., 1988).

2.4. Nodulation and acetylene reduction activity

At harvest, the number of nodules was recorded for each plant, and a subsample of nodules was weighed to determine mean specific nodule weight in each treatment. In addition, on a subsample of plants from each treatment, the acetylene reduction technique was used to determine treatment effect on acetylene reduction activity (ARA) as a measure of nodule activity. Although this method does not provide a direct measure of N_2 fixation or nitrogenase activity, it is a valuable tool to assess differences in fixation potential among treatments (Vessey, 1994). Further, whereas natural abundance ^{15}N analysis provides a measure of fixation over the life of the plant, ARA provides a snapshot of nodule activity over a given period of time. A segment of root with intact nodules was cut and placed in a 40 cm^3 vial filled with a 10% acetylene atmosphere. Airtight vials were incubated for 1 h in ambient temperatures and shielded from light. Vials containing nodulated root segments without acetylene and vials with only acetylene also were included to examine any exogenous production of ethylene from root nodules and any contamination of ethylene in the acetylene. After the incubation period, a subsample of gas was removed with a syringe and placed in an evacuated container. 250 μL gas samples were analyzed for ethylene content using a gas chromatograph (Shimadzu GC Mini Z) equipped with a hydrogen-flame ionization detector to determine the amount of ethylene evolved from nodulated root segments. Ultra high purity nitrogen (Sierra Airgas) was used as the carrier gas and was passed through an 8′ column packed with 50–80 mesh Porapak "T". Column and injector temperatures were held at 80 and 100 °C respectively. Dry weight of nodules assayed was recorded, as well as number of nodules present to provide an estimate of acetylene reduced per mean nodule weight.

2.5. Data analysis

The data were analyzed as a completely randomized design with three levels of water and four levels of N treatment. Differences in *Lupinus* biomass, tissue N concentration and content, leaf $\delta\ ^{15}N$, number and weight of nodules, ARA, and root exudation were compared among treatments using a two-way ANOVA with water and N treatments as fixed effects. For the 0 N treatment, percentage of plant fixed N exuded was compared among different water treatments using a one-way ANOVA. All data were transformed as necessary to meet assumptions of normality and equality of variance. For results with significant effects, mean comparisons were Tukey adjusted for multiple comparisons and considered significant at the 95% confidence level. All statistical analyses were conducted using a mixed model in SAS™ ver. 9.1 (SAS Institute, 2004).

3. Results

3.1. Biomass production

At the initiation of the experiment, there was no difference in plant size (mean height of 2.7 cm and 1 leaf). However, at the conclusion of the experiment, there were significant differences in above and belowground biomass among water treatments (Table 1). Biomass was greater with increasing water for all but the 0 N treatment. Above and belowground biomass were both affected by N treatments, with biomass being highest in 5 and 20 mM N treatments (Fig. 1, Table 1). A water by N interaction

Table 1
ANOVA results for effects of water and N treatment on total, above, and belowground biomass.

Source	Total			Above			Below		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Water (W)	2,116	43.14	<0.0001	2,120	108.82	<0.0001	2,116	16.41	<0.0001
Nitrogen (N)	3,116	105.4	<0.0001	3,120	116.17	<0.0001	3,116	89.84	<0.0001
W × N	6,116	5.62	<0.0001	6,120	7.55	<0.0001	6,116	3.85	0.0015

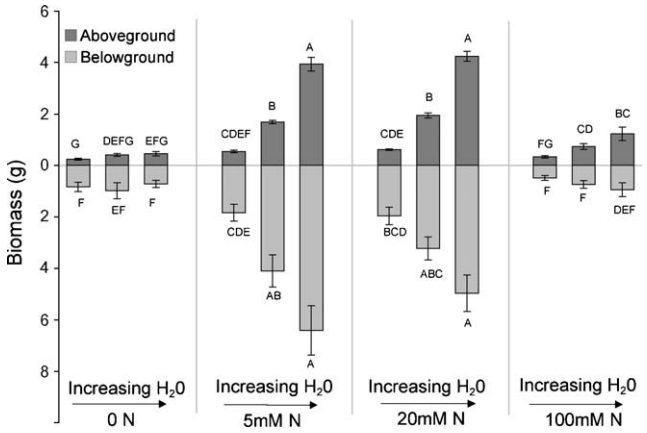

Fig. 1. Above and belowground biomass of *Lupinus* plants in the different water and N treatments. Values are means ± SE. For 0 N, $n = 11$ (L) or 12 (M and H); for 5 mM N, $n = 12$; for 20 mM N, $n = 11$ (L) or 12 (M and H); for 100 mM N, $n = 11$ (L), 9 (M), or 6 (H). Different letters for above or belowground biomass indicate significant differences among N and water treatments ($P < 0.05$).

Table 2
R:S ratio and whole plant C:N ratios for all treatments. Different letters within R:S or C:N indicate significant differences across N and water treatments ($P < 0.05$).

		Low H_2O	Medium H_2O	High H_2O
R:S	0 N	3.81 ± 0.49^{a}	2.21 ± 0.33^{abc}	2.12 ± 0.41^{bcd}
	5 mM N	3.32 ± 0.34^{a}	2.41 ± 0.29^{abc}	1.64 ± 0.21^{cde}
	20 mM N	3.13 ± 0.40^{ab}	1.67 ± 0.21^{cde}	1.15 ± 0.11^{def}
	100 mM N	1.44 ± 0.15^{cdef}	1.03 ± 0.18^{ef}	0.74 ± 0.09^{f}
C:N	0 N	24.2 ± 1.25^{a}	22.9 ± 0.79^{a}	22.5 ± 1.30^{a}
	5 mM N	18.2 ± 0.41^{b}	17.5 ± 0.43^{bc}	15.3 ± 0.57^{c}
	20 mM N	10.1 ± 0.28^{d}	9.7 ± 0.24^{d}	10.2 ± 0.28^{d}
	100 mM N	5.7 ± 0.15^{e}	5.7 ± 0.08^{e}	5.6 ± 0.28^{e}

indicated that increases in water level had positive effects on total biomass for all but the 0 N treatments (Fig. 1). *Lupinus* root:shoot ratio (R:S) decreased across all treatments as both water and N increased, with plants in the 100 mM N treatment having the lowest R:S (Table 2).

3.2. Tissue concentration

Aboveground plant tissue N concentrations were affected by both water and N treatment (Fig. 2a, Table 3). N concentration of aboveground tissue in 5 or 20 mM N treatments was significantly reduced under low water conditions, resulting in water by N interaction. Root N concentrations increased with increasing N fertilization but were not affected by water treatments (Fig. 2a).

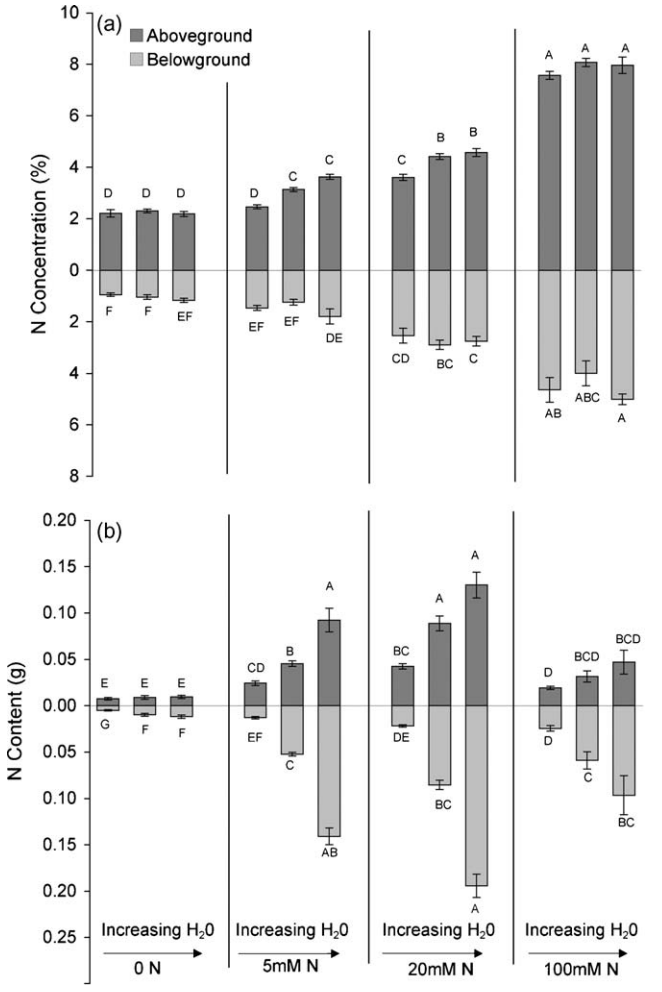

Fig. 2. Above and belowground tissue N concentration and content for *Lupinus* plants in the different water and N treatments. Values are means ± SE. For 0 N, $n = 11$ (L) or 12 (M and H); for 5 mM N, $n = 12$; for 20 mM N, $n = 11$ (L) or 12 (M and H); for 100 mM N, $n = 11$ (L), 9 (M), or 6 (H). Different letters within above or belowground concentration and content indicate significant differences among N and water treatments ($P < 0.05$).

Total plant C:N ratio followed the same pattern as tissue N concentration (Table 2). Total plant N content followed plant biomass responses and was affected by water and N for all but the 0 N treatment, leading to a significant water by N interaction (Fig. 2b, Table 3). Seeds of *Lupinus* are relatively large and N-rich, with an average N content of 0.118 mg N per seed, amounting to 0.04–0.9% of total plant N content depending on treatment.

Table 3
ANOVA results for effects of water and N treatment on tissue N concentration, content, and leaf δ ^{15}N.

N concentration	Source	Above			Below		
		df	*F*	*P*	df	*F*	*P*
	Water (W)	2,114	25.88	<0.0001	2,115	2.09	0.1282
	Nitrogen (N)	3,114	602.05	<0.0001	3,115	166.19	<0.0001
	W × N	6,114	6.59	<0.0001	6,115	0.98	0.4427
N content							
	Water (W)	2,116	185.85	<0.0001	2,114	30.81	<0.0001
	Nitrogen (N)	3,116	202.95	<0.0001	3,114	173.33	<0.0001
	W × N	6,116	10.19	<0.0001	6,114	4.53	0.0004
Isotope δ ^{15}N							
	Water (W)	2,24	11.21	0.0004			
	Nitrogen (N)	3,24	35.71	<0.0001			
	W × N	6,24	0.9126	0.4922			

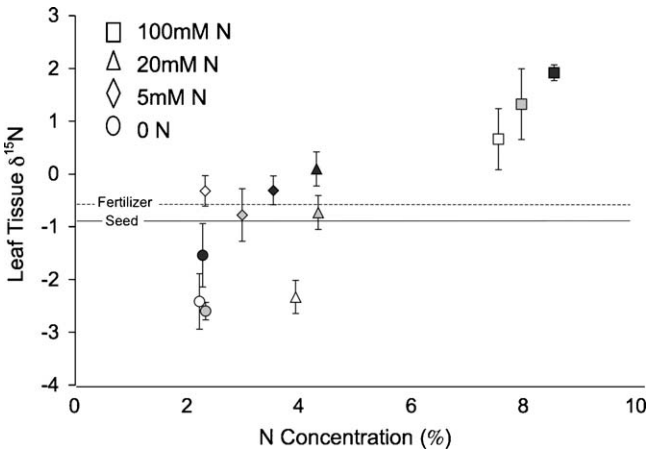

Fig. 3. The δ ^{15}N signature and N content of *Lupinus* leaf tissue in the different water and N treatments. The solid line represents δ ^{15}N of *Lupinus* seeds ($\delta^{15}N_{seed} = -0.92‰$), and the dotted line represents δ ^{15}N of the NH_4NO_3 fertilizer ($\delta^{15}N_{fertilizer} = -0.65‰$). Values below the seed line indicate reliance on fixed N and values above the fertilizer line indicate reliance on mineral N. Within each N treatment, open symbols indicate low water, gray symbols indicate moderate water, and black symbols indicate high water. N = 3 per treatment combination, and values are means ± SE.

The δ ^{15}N signature of *Lupinus* leaf tissue varied by both water and N treatment (Table 3). Although the fertilizer was not labeled, there was sufficient differentiation among treatments to assess relative reliance on fixation versus fertilization. Plants in the 0 N treatment had a lower (more negative) signature than *Lupinus* seeds, whereas plants in the 100 mM N treatment had a higher (more positive) signature than the N fertilizer (Fig. 3). Differential uptake of NH_4 versus NO_3 by *Lupinus* and fractionation of N upon uptake likely contribute to the more positive signature in plant tissue than fertilizer. This difference indicates that plants in the 0 N treatment were relying on fixation, while plants in the 100 mM N treatment were relying more on fertilizer. Plants in the 5 and 20 mM N treatments had intermediate δ ^{15}N values, indicating that the N source was a combination of both fixation and fertilizer N. Across N levels, plants in the high water treatment tended to have the least negative δ ^{15}N values and plants in the low water treatment had the most negative δ ^{15}N values, although differences between low and high water were only significant in the 20 mM N treatment (Fig. 3).

3.3. Nodulation and nodule activity

All plants were nodulated, with number of nodules being affected by both water and N treatment (Fig. 4a, Table 4). Plants grown under 0 or 5 mM N had the highest number of nodules and plants under 100 mM N the least. Additional water did not increase nodule abundance at the lowest and highest N level, leading to a water by N interaction. Although increases in N from 0 to 5 mM increased nodule number, specific nodule weight significantly decreased with increasing N and was not affected by water availability (Fig. 4b, Table 4). Specific nodule activity as measured

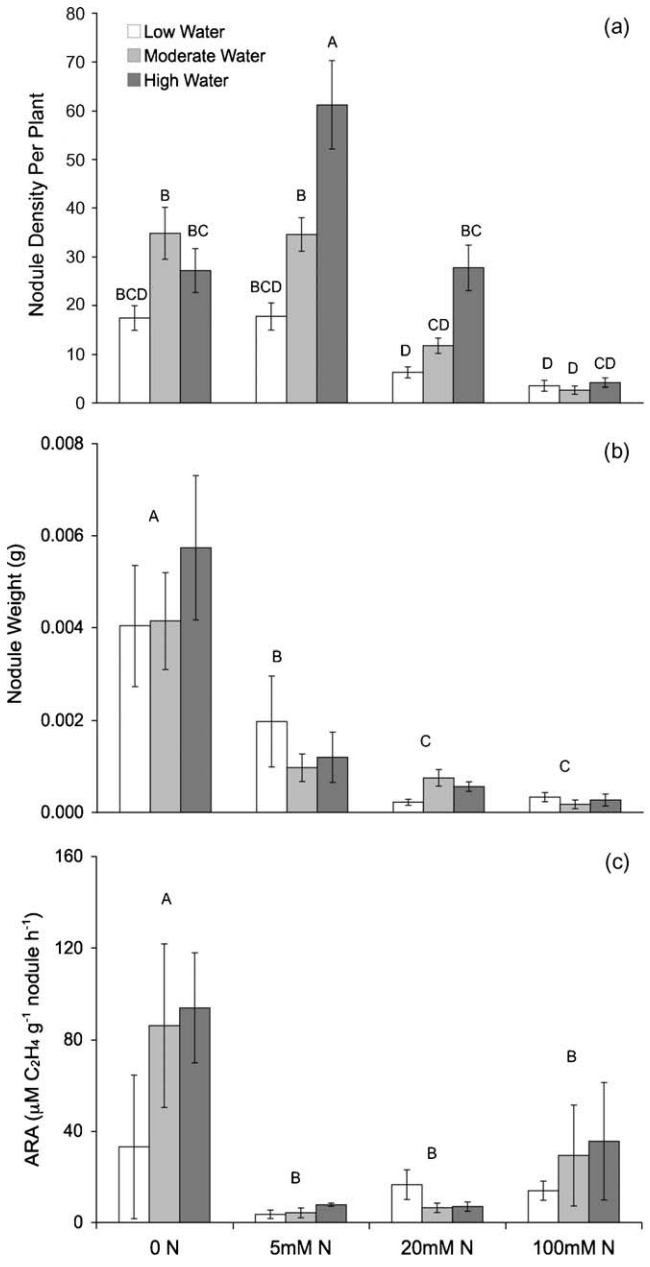

Fig. 4. Number of nodules per plant, specific weight per nodule (mg), and specific activity per nodule weight/h as measured by ARA for *Lupinus* in the different water and N treatments. Values are means ± SE. For nodule density, 0 N, $n = 11$ (L) or 12 (M and H); for 5 mM N, $n = 12$; for 20 mM N, $n = 11$ (L) or 12 (M and H); for 100 mM N, $n = 11$ (L), 9 (M), or 6 (H); for nodule weight and ARA, 0 N, $n = 3$ (L) or 4 (M and H); for 5 mM N, $n = 4$; for 20 mM N, $n = 3$ (L) or 4 (M and H); for 100 mM N, $n = 3$ (L) or 2 (M and H). Different letters indicate significant differences among N treatment (weight and ARA) or among N and water treatments (density) ($P < 0.05$).

by ARA was affected by N but not by water treatment (Fig. 4c, Table 4). Plants in the 5 mM N treatment had the lowest ARA, suggesting that nodules were not very active. In contrast, plants grown in the absence of N produced nodules that were two times

Table 4
ANOVA results for effects of water and N treatment on nodule density per plant, mean nodule weight (mg), and ARA (μM C_2H_4 mg^{-1} nodule h^{-1}).

Source	Nodule density			Nodule weight			ARA		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Water (W)	2,115	10.14	<0.0001	2,24	0.14	0.8709	2,28	1.2	0.3157
Nitrogen (N)	3,114	71.94	<0.0001	3,24	26.69	<0.0001	3,28	8.98	0.0003
W × N	6,114	5.87	<0.0001	6,24	1.95	0.1133	6,28	0.88	0.5256

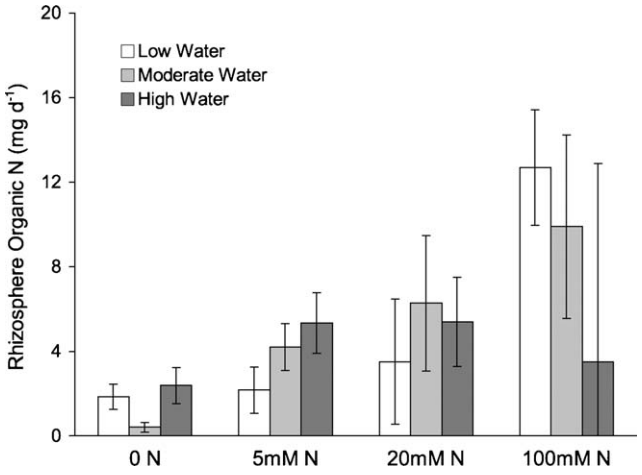

Fig. 5. Organic N exuded into the rhizosphere of *Lupinus* plants grown in the different water and N treatments. For 0 N, $n = 11$ (L) or 12 (M and H); for 5 mM N, $n = 12$; for 20 mM N, $n = 11$ (L) or 12 (M and H); for 100 mM N, $n = 11$ (L), 9 (M), or 6 (H). Values are means ± SE.

heavier than nodules in any other treatment and tended to be most active.

3.4. Rhizodeposition

Substantial amounts of organic N, measured as DON, were deposited into the rhizosphere of all pots (Fig. 5). High variability within treatments resulted in a lack of significance for either the water or N treatment ($P > 0.1$), although plants in the 100 mM N treatment tended to have higher amounts of organic N in the rhizosphere.

Rhizodeposition by plants receiving 0 N was examined in relation to N_2 fixation as assessed by ARA. To convert ARA to estimates of N fixed, we assumed a constant fixation rate over 24 h (Trinick et al., 1976) and an ethylene to N_2 conversion factor of 4 (Hardy et al., 1973). Thus, percentage of N exuded was calculated as root exudation divided by the sum of N fixed over 24 h. Plants in low water exuded 58% of fixed N, high water plants 26%, and moderately watered plant 5%. However, we acknowledge the limitations of using ARA to estimate fixation rates as conversion factors vary greatly with species and condition (Vessey, 1994), resulting in calculation of higher percentages of fixed N being exuded. Thus values of fixation using ARA are at best a rough estimate of fixed N exudation that is likely on the low end. Another approach is to use tissue N content (minus N contribution from seed), which represents fixation over the life of the plant and therefore provides an upper limit of N fixed. Comparing root exudation rates using tissue N content rather than estimates of fixation based on ARA indicates that lower amounts of fixed N are exuded. Plants grown in low water exuded 14%, followed by high water plants (9%) and moderately watered plants (3%). Actual percentages of fixed N being deposited into the rhizosphere likely fall between the values obtained from these two different approaches.

4. Discussion

N_2 fixing species can contribute to the N budgets of ecosystems, but this contribution varies with N and water availability. We examined the entire range of growth responses to N for this species over a range of water availability. *Lupinus* grew best under intermediate N and high water conditions, and exhibited reduced growth at the highest level of N (100 mM) indicating N toxicity (Goyal and Huffaker, 1982). Additional water increased biomass at all N levels although differences were not significant in the 0 N treatment. In a shortgrass steppe field study, additional water led to an increase in legume density and biomass, and addition of both water and N (5 g N m^{-2}) resulted in a small, short-term increase in legume density and biomass, but addition of N alone had no effect (Lauenroth and Dodd, 1979). Lack of similar responses in this field study and our greenhouse experiment are likely attributable to the effect of other limiting resources, the presence or absence of competition or differential responses of adults versus seedlings to water and N availability. Regardless, both studies illustrate the importance of looking at the individual and synergistic effects of N and water on biomass production in legumes.

Lupinus exhibited luxury consumption of N, with tissue concentrations ranging from 2 to 8% N. Field collections of *Lupinus* from NE Sierra Nevada, the central Great Basin (Goergen and Chambers, in press), and Rocky Mountains (Metzger et al., 2006) have average tissue N concentrations of 3%. Resource availability, especially of N, is extremely variable in sagebrush ecosystems, and the ability to store N in excess of what is required for growth may be a factor that allows this species to persist at relatively high densities in intact, low nutrient systems (Chapin et al., 1986). Luxury consumption also may allow plants to sustain themselves during periods when nutrient resources are low either because of increased competition or reduced uptake caused by water limitation.

High tissue N concentrations combined with low C:N ratios indicate that upon senescence at the end of the growing season, *Lupinus*, like other N_2 fixing species, can contribute substantial amounts of N through litter decomposition. In sagebrush steppe of northwestern Nevada, addition of *Lupinus* litter increased extractable inorganic N in the soil (Goergen, unpublished data). Similarly, decomposition of *L. arboreus* tissue increased both soil extractable NH_4^+ (~3 times) and NO_3^- (~5 times) relative to soil with no lupines (Maron and Connors, 1996). Similarly, litter of the N_2 fixing tree *Morella* (*Myrica*) *faya* decomposed faster and began releasing N sooner than did the native non-fixing tree *Meterosideros polymorpha* (Vitousek and Walker, 1989). Results from our study on biomass and N content suggest that contribution of N to the soil by *Lupinus* tissue decomposition will likely be greatest under intermediate resource availability because plants grown at intermediate resource levels produced the most biomass. Aboveground N content of plants in our greenhouse experiment ranged from 0.04 to 0.08 g N per plant. In lodgepole pine forests of Wyoming, *Lupinus* contributes an average of 0.04 g N per plant (Metzger et al., 2006), and a study in the central Great Basin found an average of 0.18 g N per plant (Goergen and Chambers, in press).

Nodulation of *Lupinus* was reduced but not eliminated under elevated N, irrespective of water availability. Although many studies suggest that increased mineral N reduces nodule size and density, the response of legumes to N level is species specific and depends upon the particular rhizobia-legume symbiosis (Manhart and Wong, 1980; Harper and Gibson, 1983), with some species showing stimulated fixation at moderate levels of N (Peoples et al., 1995; Bado et al., 2006). Nodules of *Lupinus angustifolius* infected with *Rhizobium* sp. 127E15 were unaffected by addition of 15 mM NO_3^-, whereas all other combinations of rhizobia-*L. angustifolius* and rhizobia-*Vigna unguiculata* used in the same study averaged a 30% reduction in nodule weight (Manhart and Wong, 1980). The effect of N on nodulation and nodule activity also depends on the timing of N addition. Consistently high levels of N can prevent root hair infection (Eaglesham, 1989), whereas exposure to elevated N after nodule initiation decreases nodule weight and activity (Arresigor et al., 1997; Voisin et al., 2002). Our plants did not receive any fertilization until after emergence. Thus, it is likely that all seedlings were initially nodulated and that the N treatment

affected existing nodules by influencing the degree (density and size) of subsequent nodulation.

The acetylene reduction activity of nodulated roots in our greenhouse experiment ranged from 3 to 94 $\mu M\ C_2H_4\ g^{-1}$ nodule dry weight h^{-1} depending on treatment. In field studies conducted in high elevation areas of Western North America, *L. argenteus* had ARA ranging from 6 to 35 $\mu M\ C_2H_4\ g^{-1}$ nodule dry weight h^{-1} (Johnson and Rumbaugh, 1986). Similarly, in investigating factors affecting ARA in various *Lupinus* spp. Trinick et al. (1976) found rates ranging from 4.2 to 42 $\mu M\ C_2H_4\ g^{-1}$ nodule wet weight h^{-1} depending on methods and species examined. The higher values found in our study are likely a combination of more favorable moisture and less favorable N conditions than commonly experienced in the field. The use of commercial inoculum also may contribute to observed differences. Some studies indicate that commercially available inoculum has higher and more consistent effectiveness than indigenous inoculum (Pegtel, 1980), although other studies indicate no difference among commercial and indigenous rhizobia (Jensen, 1987; Kelstrup et al., 1996) or even that indigenous rhizobia are more effective (Malek et al., 1998). In our experiment, ARA of *Lupinus* nodules also indicated a greater effect of N on nodule activity than water. In the field, acetylene reduction by *Lupinus* increased with soil moisture and elevation (Rumbaugh and Johnson, 1991). Although fixation tended to decrease with increasing N availability, nodule activity was present under elevated levels of N. A similar result was seen with the *Lupinus* leaf $\delta\ ^{15}N$ data—as N addition increased, plants shifted from relying on only N fixation to a combination of N fixation and fertilizer.

Our results suggest that when N is elevated following fire or other disturbances, *Lupinus* would likely rely mostly on inorganic N to increase growth, reproduction, and tissue concentrations while still maintaining active nodules. Other studies also have found that N_2 fixation is not always completely suppressed by increases in soil N associated with disturbance (Hiers et al., 2003). Casals et al. (2005) found that both seedling and resprouting legume species derived 52–99% of their N from fixation after fire, despite increased amounts of mineral N in burned plots. Similarly, N_2 fixation rates of *Macrozamia riedlei* in southwestern Australia were greatest the year following fire and gradually decreased with time since fire (Grove et al., 1980). In these situations, reduced competition for other limiting resources (light, phosphorus, water) also may contribute to increases in fixation. *Lupinus* is a perennial species, and some work suggests that nodules also may be perennial (Johnson and Rumbaugh, 1981). The ability to retain active nodules during conditions of high N availability would allow this species to return to fixing atmospheric N_2 once N levels decrease or competition for N increases.

Substantial amounts of organic N were released from *Lupinus* roots to the soil, regardless of background resource levels. Another recent study also found that DON exudation by the legume *Trifolium repens* was unaffected by N fertilization even though levels of N fixation decreased (Paynel et al., 2008). The presence of organic N within the rhizosphere could have multiple sources: (1) exudation of fixed N, (2) exudation of N originating from inorganic fertilizer (i.e. fertilizer N taken up by the plant and then released back into the soil, reprocessed), or (3) turnover of root, nodule and rhizosphere microbes. Plants were only grown for 3 months making a large contribution due to root turnover unlikely. Further, regular flushing of the soils over the course of the experiment likely greatly reduced contributions of DON from decomposing plant and microbial tissue. The trend of increasing exudation with increasing N fertilization suggests that exudation likely shifted from being composed of fixed N to reprocessed N. Further, low rates of ARA and high $\delta\ ^{15}N$ values for the higher N treatments suggest that plants were mainly relying on fertilizer N and the majority of exuded N was reprocessed inorganic fertilizer N. However, because the fertilizer was not labeled, it is only possible to distinguish the amount of fixed N being exuded in the 0 N treatment. For those plants, estimates range from 3 to 58% of fixed N exuded depending on water treatment and method used to calculate amount of N_2 fixed. Values of fixed N exuded based on N content (3–14%) fall within the range observed for other herbaceous crop legumes. For example, 13% of *Vicia faba* and 16% of *Lupinus albus* total plant N was added to the soil via rhizodeposition (Mayer et al., 2003).

Rhizodeposition is mainly regulated by passive diffusion, with exudation following a concentration gradient from root to soil (Jones et al., 2004). Although it may appear wasteful for *Lupinus* to be exuding costly N, it may not be an entirely passive process. For example, growth by legumes is often limited by phosphorus (Vitousek and Field, 1999). To overcome this limitation, research suggests that many legumes exude N-rich enzymes such as phosphatase to increase availability of this limiting nutrient (Wang et al., 2007). For example, when grown in phosphorus limiting conditions, *Lupinus* spp. increased acid phosphatase secretion 20 times compared to plants without phosphorus limitation (Bais et al., 2004). In addition, research has shown that root exudates can play an active role in plant–plant and plant–microbe communication (Bais et al., 2004).

Our results suggest that availability of water did not influence rhizodeposition, indicating that exudation may occur throughout the growing season under varying water availability and may potentially provide a novel source of N when this nutrient is most limiting to plant growth. Although some studies suggest that rhizosphere microbes have a greater ability to take up root exudates than other plant roots (Owen and Jones, 2001), microbial turnover of exudates in soil is very fast (Jones et al., 2004), resulting in the quick conversion of this N source to plant-available mineral N. This would suggest that the high levels of N exudation observed in this species may contribute to plant-available N in these soils. Tissue decomposition also can add substantial amounts of N, but contribution from this N source is temporally variable. DON contribution from litter is likely greatest in fall after plant senescence and when rain and snow allow for decomposition. Rhizodeposition, on the other hand, could potentially contribute plant-available N throughout the growing season.

Rhizodeposition has received little attention in desert ecosystems as a source of N to total ecosystem N budgets. Taking the average exudation rate of 5.5 mg N d^{-1} and assuming an average of 3 plants m^{-2} (Goergen and Chambers, in press) and a constant exudation over a growing season of 3 months, our results indicate that *Lupinus* could contribute upwards of 1.5 g of N $m^{-2}\ yr^{-1}$ via organic N rhizodeposition to sagebrush steppe under low N conditions. The contribution of N via decomposition of N-rich tissue is an additional source which, based on results from this study and again assuming a density of 3 plants m^{-2}, can range between 0.04 and 0.9 g of N $m^{-2}\ yr^{-1}$ depending on the resource environment. However, actual contributions of N by *Lupinus* in the field will depend not only on plant responses to the environment, but also on plant density, distribution, and age which can vary greatly over the landscape. Regardless, in comparison to other sources of N such as atmospheric deposition (<0.2 g of N $m^{-2}\ yr^{-1}$, CASTNET, 2004–2006) or fixation by microbiotic crusts (0.1–1.3 g of N $m^{-2}\ yr^{-1}$, Belnap, 2002a; Housman et al., 2006), these results indicate that *Lupinus* can greatly contribute to the N budget of the sagebrush steppe through rhizodeposition during the growing season and decomposition of N-rich tissue after senescence.

Aside from effects on N cycling and budgets in this system, results of our study have implications for community composition and invasion potential. The sagebrush ecosystem is threatened by invasion of numerous exotic species, including both annual grasses and perennial forbs. Differential resource use, growth

rates, and competitive abilities between native and exotic species influence recruitment into these systems and may shift community composition from native to exotic dominance. Modification of the local resource pool by *Lupinus* combined with an exotic seed source may provide an avenue for expansion of highly competitive exotic species by modifying interactions among native and exotic species or by differentially influencing species establishment. Increases in N availability led to invasion by non-native species and resulted in altered disturbance regimes and changes in successional trajectories in Mojave Desert (Brooks, 2003), chaparral (Cione et al., 2002) and sagebrush ecosystems (Chambers et al., 2007). N-rich patches created by *L. arboreus* in California led to a conversion of native shrubland to an exotic annual grassland (Maron and Connors, 1996). Similarly, facilitation by an exotic legume is believed to play a role in invasion by *Pennisetum cetaceum* in Hawaii (Carino and Daehler, 2002). With exotic species introductions on the rise, it is important to understand factors that may increase invasion by exotic species. Local N enrichment due to *Lupinus* may promote changes in species composition and incorporation of exotic species into the native community matrix. However, the effect of *Lupinus* presence ultimately will depend on the composition and abundance of native perennial herbaceous species and the nature of the competitive interactions.

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