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RESEARCH ARTICLE

Fall fertilization during nursery production increases nitrogen status of *Purshia tridentata* seedlings: implications for outplanting

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During container nursery production of *Purshia tridentata* (antelope bitterbrush), we found that three fall fertilization applications successfully loaded plants with nitrogen (N) with little effect on plant biomass. Using ^{15}N-labeled fertilizer to track N movements, we observed that N-loaded seedlings attained luxury consumption and ultimately translocated more N toward roots. In trees, such N-loading efforts have been reported to increase seedling survival and growth after outplanting by improving initial root growth. This leads to enhanced access to water and nutrients that increases overall plant competitiveness and performance. Our results add, in general, to the paucity of information concerning fall fertilization of shrubs, and specifically that this technique offers promise to enhance the quality of antelope bitterbrush seedlings. This could improve overall planting success of this important shrub native to western United States rangelands. Increasing the effectiveness of planting antelope bitterbrush, which can often improve restoration trajectories more than direct seeding or reliance on natural regeneration of this species, could accelerate the pace and scope of critical habitat restoration. Restoration is needed as antelope bitterbrush abundance has been reduced across the landscape because of conversion to agriculture, invasion by nonnative annual grasses, and an increase in fire frequency and intensity, among other reasons. In addition, this species provides browse for ungulates and critical habitat for at-risk species, such as *Centrocercus urophasianus* (greater sage-grouse). Although N loading antelope bitterbrush has potential to improve outplanting performance, the resulting higher nutrient status of this preferred browse species may lead to elevated browsing during seedling establishment.

Key words: antelope bitterbrush, nitrogen loading, nitrogen translocation, nitrogen use efficiency, seedling quality

Implications for Practice

- Outplanting high-quality, nursery grown *Purshia tridentata* seedlings may accelerate the restoration trajectory on disturbed rangelands in the western United States.
- Fall fertilization that promotes luxury consumption (uptake) of nitrogen can be an efficient technique to improve seedling quality.
- Fall-fertilized *P. tridentata* seedlings achieved luxury consumption and most additional nitrogen was subsequently stored in roots.
- Stored nitrogen has potential to promptly support new seedling growth, especially roots, after outplanting and thereby promote water and nutrient acquisition, which in turn can boost photosynthesis and promote more root and shoot growth leading to successful seedling establishment.
- These potential benefits, along with understanding the effects of fall fertilization on browsing, need to be evaluated and quantified for *P. tridentata* through additional outplanting research.

Introduction

Rangeland encompasses about one-third of the land area of the coterminous United States (~238 million hectares; Reeves & Mitchell 2011; USDA FS 2016). In the semi-arid western United States, antelope bitterbrush (*Purshia tridentata*,

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hereafter bitterbrush) is a shrub in the Rosaceae that is widely distributed across ~138 million hectares of rangeland, generally bounded on the west by the mountains of the Cascades and Sierra Nevada, on the east by the Rocky Mountains, and from Arizona and New Mexico northward to southern British Columbia (Hormay 1943; USDA NRCS 2022). Moving from drier to moister sites, bitterbrush associates with sagebrush (*Artemisia*), to piñon–juniper (*Pinus–Juniperus*) woodlands, to forests of Jeffrey (*Pinus jeffreyi*) or ponderosa (*Pinus ponderosa*) pine (Hormay 1943). Bitterbrush is an important browse species among domestic and wild ungulates, such as mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), and pronghorn (*Antilocapra americana*) (Nord 1965; Ngugi et al. 1992), especially during winter (Burrell 1982). It is a notable food source for granivores (Vander Wall 1994), contributes to habitat for the at-risk species greater sage-grouse (*Centrocercus urophasianus*) (Nelle et al. 2000), and as an actinomycete-nodulated, nitrogen-fixing plant, fills an ecologically significant role in nutrient-limited western rangelands (Klemmedson 1979).

By the mid-20th century, Hormay (1943) reported significant loss of bitterbrush on the landscape. Throughout the western United States, loss of shrublands continues to escalate because of conversion to agriculture, invasion by nonnative annual grasses and subsequent changes to fire frequency and intensity, encroachment by trees, improper grazing, climate change, and interactions of all these disturbance factors (Leonard et al. 2000; Connelly et al. 2011; Davies et al. 2011). In particular, invasive annual grasses are increasing fire frequency and intensity far exceeding former events (D'Antonio & Vitousek 1992; Germino et al. 2016). Bitterbrush sprouts infrequently following fire and relies on seedlings for regeneration (Nord 1965; Wambolt et al. 2001; Ziegenhagen & Miller 2009). Although bitterbrush can regenerate promptly from the seed bank following fire (Ziegenhagen & Miller 2009), low seed yield, rodent predation of seeds, and poor competitiveness of small seedlings contribute to poor reestablishment success (Hubbard 1957; Young & Evans 1976; Vander Wall 1994).

For the restoration of native species, three main strategies can be followed: (1) natural regeneration, (2) direct seeding, and (3) planting seedlings. Natural regeneration is, however, impaired by the above-mentioned issues with low seed yield and rodent predation. Direct seeding and assisted natural regeneration efforts have been successful in restoring lands with significantly lower costs than planting seedlings (Monsourian et al. 2005). Although direct seeding has lower cost than seedling establishment, several reviews have showed that it has significantly lower success rates (including poorer seed-to-seedling ratios) compared to planting seedlings (Palma & Laurance 2015; Ceccon et al. 2016; Grossnickle & Ivetić 2017); this is evident in dryland systems as well (Abella et al. 2012; Pérez et al. 2019). Direct seeding into microsites may benefit establishment. Davies et al. (2022*a*) report that bitterbrush direct seeded under burned woody vegetation had improved establishment compared to direct seeded open areas.

In arid landscapes, outplanting nursery-grown seedlings during periods of high soil moisture (typically late fall or early spring) can be more effective in establishing plants than direct seeding (Abella et al. 2012). Although outplanting bitterbrush can be effective (Johnson & Okula 2016; Davies et al. 2017), competition and drought remain obstacles to success (Davies et al. 2017, 2022*b*). Producing seedlings to have an advantage when confronted with competition after outplanting could increase seedling survival and growth. One production technique is nutrient loading, which has been shown to improve competitiveness of seedlings against existing vegetation (Timmer 1997). Nutrient loading, or luxury uptake, or luxury consumption, occurs when plants increase their nutrient concentrations with little or no additional growth (Landis et al. 1989; Timmer 1997; Dumroese 2003*a*). In nursery culture, this can be accomplished by applying fertilizer at an exponential rate throughout the growing season, or by applying fertilizer late in the growing season, known as fall fertilization (Rikala et al. 2004; Islam et al. 2009; Oliet et al. 2013). Both techniques can deliver large doses of nutrients, especially nitrogen (N) late in the growing cycle with little effect on the amount of biomass.

These stored nutrients translate into better seedling physiological status and are allocated for growth after outplanting (Salifu & Timmer 2001; Uscola et al. 2015). During a seedling's field establishment period, root growth is crucial to access water and nutrients to sustain new growth. At establishment, however, roots are limited to those formed during nursery production and cannot readily access nutrients and water due to initial poor root–soil contact (Pinto et al. 2016). Thus, immediately after outplanting, seedlings rely on nutrients stored during nursery production and their remobilization to sustain new growth (Burdett 1978; Grossnickle 2005). Nutrient-loaded seedlings generally present better root development than non-loaded seedlings (Timmer 1997; Salifu & Jacobs 2006), leading to enhanced maintenance of water potential and photosynthetic rates during the subsequent summer after outplanting (Villar-Salvador et al. 2012). This improved physiological status is likely responsible for observed improvements in survival and growth compared to non-loaded plants, especially for species that grow in Mediterranean and semi-arid climates subject to expected summer drought (Villar-Salvador et al. 2004; Cuesta et al. 2010; Watkinson et al. 2021).

Nutrient loading, either through exponential or fall fertilization, has been extensively evaluated for a variety of tree species and stocktypes, including bareroot conifer (Islam et al. 2009) and angiosperm (Birge et al. 2006) species, container deciduous (Li et al. 2012; Zhu et al. 2020) and evergreen (Montville et al. 1996; Dumroese et al. 2005; Timmer & Munson 2011) conifers, and a container angiosperm (Oliet et al. 2009). In contrast, for shrubs, few studies have examined exponential fertilization to nutrient load (Dumroese 2003b; Watkinson et al. 2021) and to the best of our knowledge, no studies have explored fall N fertilization. In this study, we examined the effects of fall N fertilization on the status, movement, and storage of N in bitterbrush. With the purpose of discriminating between N uptake from substrate and remobilization within the plant, we used ^{15}N as a label, which is considered more informative and accurate to quantify nutrient influx and afflux in plant tissues than differences in concentration (Millard & Proe 1991). We

hypothesized that fall fertilization would (1) increase N concentration in leaves, stems, and roots, (2) N would undergo translocation after absorption, and (3) total seedling biomass would be unaffected. Producing nutrient-loaded bitterbrush seedlings through fall N fertilization could benefit restoration work by increasing seedling quality through improved nutritional status that promotes survival and growth, including improved competitiveness with neighboring vegetation after outplanting.

Methods

General Propagation

The experiment was conducted in a greenhouse (U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Moscow, ID, U.S.A.; 46.7232°N, 117.0029°W) under natural day-length. In mid-May, we sowed stratified (28 days at 2–3°C) bitterbrush seeds (Montana source, Lawyer Nursery, Plains, MT, U.S.A.) into 5 trays each containing 98 containers (Ray Leach Cone-tainer Super Cells: 164 cm^3, 3.8 cm top diameter, 21 cm depth, and 528 seedlings·m^{-2}; Stuewe and Sons Inc., Tangent, OR, U.S.A.) filled with a peat: vermiculite (1:1, v:v) medium (Sunshine Special Blend Forestry #1, Sun Gro Horticulture, Bellevue, WA, U.S.A.). Containers were thinned to one seedling 3 weeks after sowing. One month after sowing, we began a 10-week fertilization program (mid-June through August) that delivered 2.5 mg N·seedling^{-1}·week^{-1} (25 mg N·seedling^{-1} total) using 20–20–20 (20 N, 20 P_2O_5, 20 K_2O) general purpose fertilizer (Peters Professional, The Scotts Company, Marysville, OH, U.S.A.). Frequency of irrigation (water only) or fertigation (irrigation with soluble fertilizer added once each week) was determined gravimetrically and applied when container mass reached 65% of container capacity (manager's technique; see Dumroese et al. 2015). Day: night temperatures averaged 28:16°C. Edge effects were minimized by rotating trays, and containers within trays, every 2 weeks. During September, seedlings were exposed to ambient temperatures (18:7°C day: night) and irrigated when container capacity reached 65%.

Fall N Fertilization Treatments

Using a completely randomized design with five replications, fall N fertilization treatments began in October. Seedlings were randomly assigned to one of four N application rates (0, 5, 10, and 15 mg N·seedling^{-1}; hereafter N0, N5, N10, and N15) supplied as KNO_3 enriched to 95 atom % ^{15}N (Sigma-Aldrich, St Louis, MO, U.S.A.). Seedlings were irrigated as needed when container capacity reached 65%. Once each week for three consecutive weeks seedlings were fertigated with a third of the desired N treatment total delivered in 15 mL of the appropriate N treatment (N concentration rates: 0, 0.11, 0.22, and 0.33 mg N·mL^{-1}). When fertigated, additional plain water was added as needed but the substrate was rehydrated slightly less than container capacity to minimize leachate. Abscised leaves were collected using an open-topped white netting bag surrounding each seedling. Greenhouse temperatures averaged 15:5°C day: night.

Sampling

We randomly sampled seedlings at onset (0 week) and 5 and 9 weeks after onset of fall N fertilization; 300 seedlings total (4 N rates × 3 sample dates × 5 seedlings·replication^{-1} × 5 replications). We measured root-collar diameter and stem length (root collar to stem apex), washed seedlings free of growing media, and segregated their roots, stems, and leaves (either green or brown; brown leaves were a combination of those remaining on the stem and those that abscised). Aliquots were dried (65°C) to constant mass (~72 h). For each tissue fraction, the five seedlings within each N rate × sample date × replication combination were bulked and ground. The ratio of ^{15}N to ^{14}N ($\delta^{15}N$ %) in each tissue was expressed relative to that of air. Tissues were flash-combusted in an elemental analyzer (NC2500, CE Instruments, Milan, Italy) and analyzed using the Finnigan-MAT Delta Plus isotope mass spectrometer (ThermoFisher Scientific, Waltham, MA, U.S.A.) at the University of Idaho Stable Isotope Laboratory (Moscow, ID, U.S.A.).

Calculations of N Dynamics

For each tissue fraction, we measured the total change in N content (net N translocation amount, NTA; mg; Eq. 1), the percentage change in N content (net N translocation ratio [NTR], %; Eq. 2), and the ability of bitterbrush to uptake N (N use efficiency [NUE], %; Eq. 3) 5 and 9 weeks after onset of fall N fertilization.

$$\mathrm{NTA} = A - B + (D + \mathrm{Le}), \quad (1)$$

$$\mathrm{NTR} = \left(\frac{A-B}{B}\right) \times 100, \quad (2)$$

$$\mathrm{NUE} = \left(\frac{T-C}{F}\right) \times 100, \quad (3)$$

where A is N content (mg) after fall N fertilization; B is the N content at onset of fall fertilization (0 week); Le is the amount of leached N, assumed to be negligible in this short-term trial as noted by Miller et al. (1976) and Helmisaari (1992); D is the N content of brown leaves (attached and abscised); T is the total ^{15}N content (mg) of treated seedlings (i.e. N5, N10, N15); C is the average ^{15}N content (mg) in control seedlings (i.e. N0); and F *is* the total amount of fall-applied ^{15}N fertilizer.

Statistical Analyses

Morphological (stem length; root-collar diameter; green leaf, stem, root, and total biomass) and N (N and ^{15}N concentration and content, NTA, NTR) attributes were analyzed as repeated measures (Kuehl 2001), modeling the variance and covariance structure with a 95% level of confidence. Statistical differences between means were performed with a Tukey's honestly

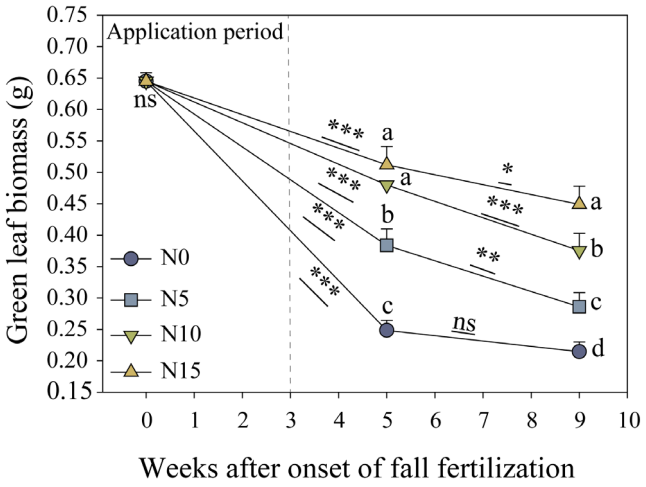

Figure 1. Mean biomass (+ SD) of bitterbrush green leaves (g) sampled at onset of, and 5 and 9 weeks after onset of, three consecutive, weekly applications of fall nitrogen (N) fertilization that delivered a total of 0 (N0), 5 (N5), 10 (N10), and 15 (N15) mg N·seedling^{-1}. Vertical dashed line indicates the cessation of fall fertilization application. Different letters indicate differences among N rates within the same week according to Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate significant differences among sample times within the same N rate according to Tukey's HSD test (ns = non-significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

significant difference (HSD) test for multiple comparison, using the PROC MIXED procedure (SAS 9.0, SAS Institute Inc., Cary, NC, U.S.A.). The effect of the different factors was assessed using the following general linear model (GLM):

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijkl}, \quad (4)$$

where y_{ijkl} is the value of the dependent variable in plant l measure in wk. ($i = 0$, 5, 9 for morphological attributes; $i = 5$, 9 for N attributes), in the j N application rates ($j = 0$, 5, 10, 15), and in the k tissue ($k = 1$, 2, 3); μ is the overall mean; α, β, and γ are the fixed effects for week, N application rate, and tissue type respectively; the double and triple terms represent interactions; and e is the error term for the hypothesis $e_{ijkl} \sim N(0, \sigma_e^2)$.

The NUE was assessed with a one-way analysis of variance (ANOVA) for a completely randomized design using a PROC GLM procedure (SAS Institute Inc., Cary, NC, U.S.A.). We verified assumptions of normality and homoscedasticity using Shapiro–Wilk and Levene tests, respectively. Differences among means were determined with a Tukey's HSD test for multiple comparisons.

Nitrogen concentration by tissue type (leaves, stems, and roots) was used to calculate a weighted mean concentration at the whole plant level (Tang et al. 2018) and this whole plant concentration along with whole plant biomass was used to create a nutrient vector nomogram (Haase & Rose 1995). Resulting vector direction and magnitude revealed relative changes in biomass, N content, and N concentration of entire bitterbrush seedlings. Brown leaf biomass and N concentration were analyzed using Equation 4 with $wk = 5$ and 9, and $k = 1$.

Results

Plant Growth Response

The interaction of N rate and weeks after onset of fall N application was significant for green leaf biomass ($p < 0.0001$; Fig. 1) but none of the other morphological traits ($p \geq 0.1569$; Table S1). Five weeks after onset of fall N application (i.e. 2 weeks after the final N application), biomass of green leaves was significantly greater in the highest N rates (N10 and N15), followed by N5, and lastly by the control (N0) treatment. Nine weeks after onset, this pattern remained, despite a reduction in green leaf biomass across all treatments (Fig. 1).

As independent variables, N rate was not significant for any morphological variable ($p \geq 0.2649$) except green leaf biomass ($p < 0.0001$), whereas weeks after onset of fall fertilization was significant for all variables ($p \leq 0.0009$) except stem length ($p = 0.5720$; Tables 1 & S1). In general, significant increases in most biomass amounts because of N rate were observed 5 weeks after onset of fall fertilization, and no additional gains were noted 9 weeks after onset. Brown leaf biomass was unaffected by weeks ($p = 0.6603$; 0.24 vs. 0.25 g), N rate ($p = 0.0994$; 0.28, 0.26, 0.23, and 0.21 g for N0, N5, N10, and N15, respectively), or their interaction ($p = 0.1420$).

N and ^{15}N Concentration and Content and Nutritional Status

The three-way interaction of N rate × tissue type × weeks after onset of fall N fertilization was significant for N concentration ($p = 0.0335$) and N ($p = 0.0176$) and ^{15}N ($p = 0.0002$)

Table 1. Mean stem length, root-collar diameter, and biomass (± standard errors) and p-values of bitterbrush seedlings sampled at onset of, and 5 and 9 weeks after onset of, three consecutive, weekly applications of fall fertilization ($n = 100$ for each sample time). Bold p-values indicate significant differences for each variable at $p < 0.05$. According to Tukey's HSD test ($\alpha = 0.05$), different lowercase letters within a column indicate statistically significant differences.

			Biomass (g)		
Weeks after onset of fertilization	*Stem length (cm)*	*Root-collar diameter (mm)*	*Stem*	*Root*	*Total*
0	20.3 ± 0.8	2.22 ± 0.02 a	0.29 ± 0.02 a	0.50 ± 0.01 a	1.42 ± 0.03 a
5	21.6 ± 1.0	2.43 ± 0.04 b	0.41 ± 0.03 b	0.63 ± 0.02 b	1.68 ± 0.05 b
9	20.5 ± 1.0	2.38 ± 0.04 b	0.38 ± 0.03 b	0.67 ± 0.03 b	1.63 ± 0.07 b
p-values	0.5720	**<0.0001**	**<0.0001**	**<0.0001**	**0.0009**

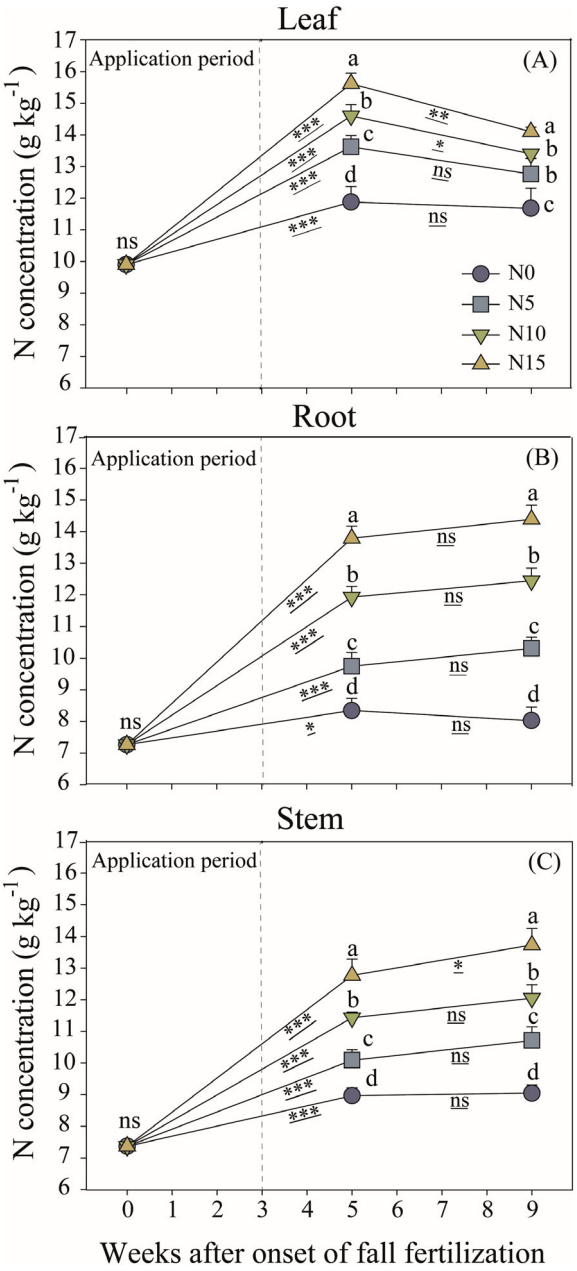

Figure 2. Mean nitrogen (N) concentration ($g \cdot kg^{-1}$ + SD) of bitterbrush green leaves (A), roots (B), and stems (C) sampled at onset of, and 5 and 9 weeks after onset of, three consecutive, weekly applications of fall N fertilization that delivered a total of 0 (N0), 5 (N5), 10 (N10), and 15 (N15) mg $N \cdot seedling^{-1}$. Vertical dashed line indicates the cessation of fall fertilization application. Different letters indicate differences among N rates within the same week according to Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate significant differences between weeks within the same N rate according to Tukey's HSD test (ns = non-significant; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

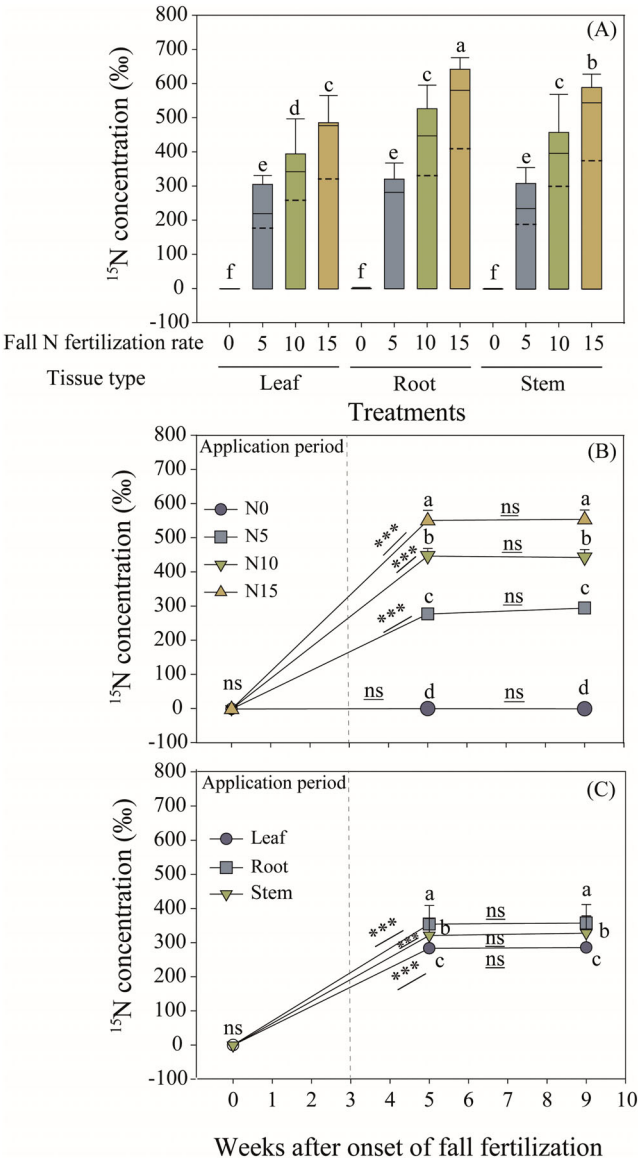

Figure 3. Status of nitrogen (N) isotope concentration (‰ + SD) in bitterbrush seedlings. ^{15}N concentration (‰) of fall-fertilized bitterbrush tissue types at different N rates (A). ^{15}N concentration of bitterbrush seedlings sampled at 5 and 9 weeks after onset of, three consecutive, weekly applications of fall N fertilization that delivered a total of 0 (N0), 5 (N5), 10 (N10), and 15 (N15) mg $N \cdot seedling^{-1}$ (B) and in green leaves, roots, and stems (C). Vertical dashed line indicates cessation of fall fertilization application in (B) and (C). Within each bar (A), the solid line indicates the median whereas the dashed line indicates the mean. Different letters indicate significant differences among tissue types and N rates according to Tukey's HSD test ($\alpha = 0.05$). Different letters indicate significant differences among N rates within the same sample time (B) and between tissue type during the same sample time (C) according to Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate significant differences among sample times at the same N rate (A) and between weeks of the same tissue type (B) according to Tukey's HSD test (ns = non-significant; $***p < 0.001$).

content (Table S2). Progressive increases in N rate from N0 to N15 yielded progressive increases in N concentration of all tissues (i.e., green leaves, stems, and roots) observed 5 weeks after onset of fall N fertilization (Fig. 2). Nine weeks after onset, however, seedlings receiving the most N (N10 and N15) presented significant decreases (8.2 and 9.7%, respectively) in leaf N

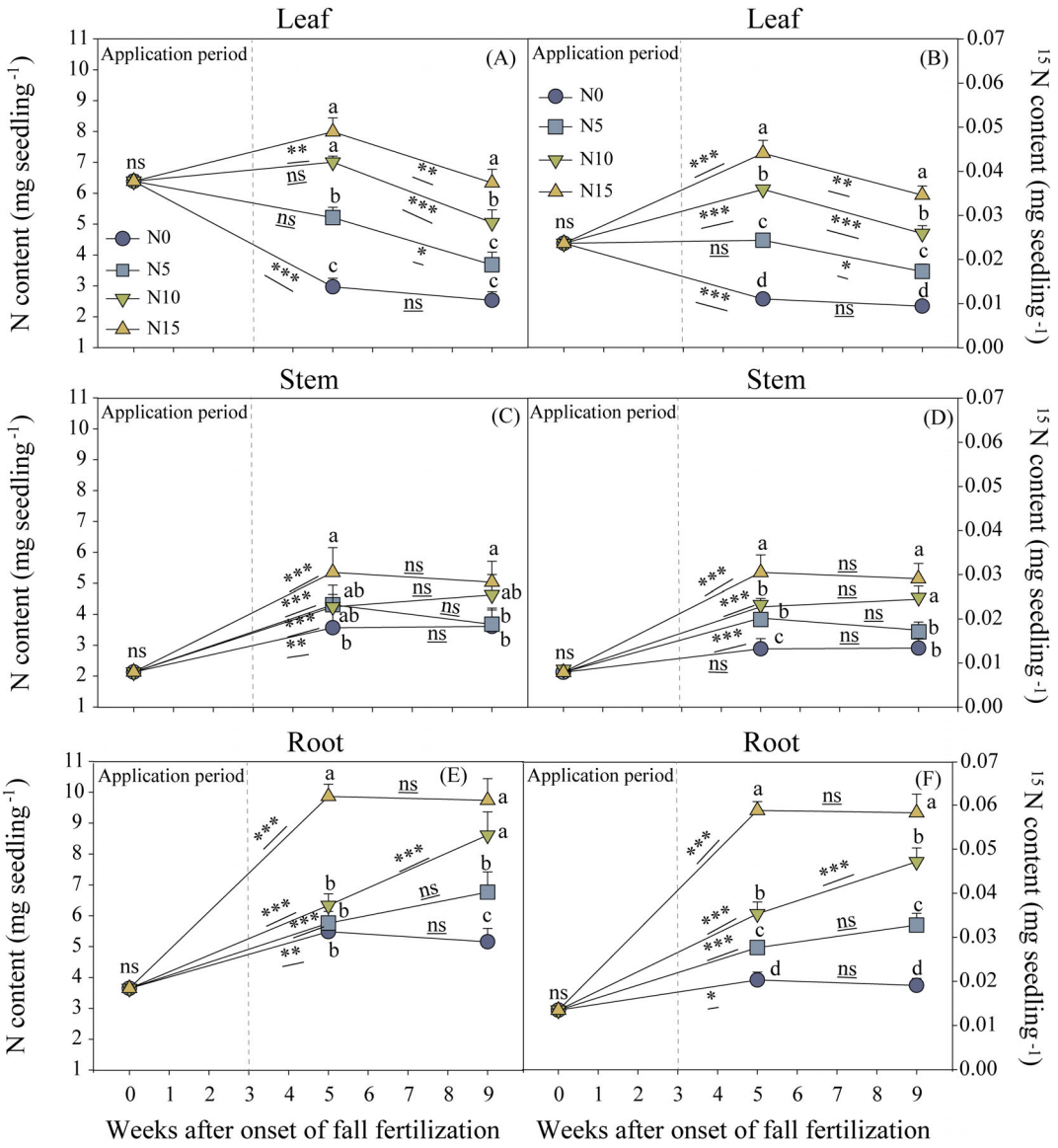

Figure 4. Status of nitrogen (N) and N isotope content (mg·seedling^{-1} + SD) in bitterbrush seedlings. Nitrogen (A, C, E) and ^{15}N (B, D, F) contents of green leaves (A, B), stems (C, D), and roots (E, F) sampled at 5 and 9 weeks after onset of, three consecutive, weekly applications of fall N fertilization that delivered a total of 0 (N0), 5 (N5), 10 (N10), and 15 (N15) mg N·seedling^{-1}. Vertical dashed line indicates cessation of fall fertilization application. Different letters indicate differences among N rates within the same sample time according to Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate significant differences among sample times of the same N rate according to Tukey's HSD test (ns = non-significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

concentration whereas low N rates (N0 and N5) remained constant. From 5 to 9 weeks after onset of fall N fertilization, N concentrations for roots and stems also remained stable (Fig. 2). Within each tissue type, ^{15}N concentration increased with increasing N rate, with concentrations within roots > stems > leaves (Fig. 3A & 3C). As with N concentration, ^{15}N concentration peaked 5 weeks after onset of fall N fertilization and remained static through the 9-week measurement (Fig. 3B). Nitrogen content results were more variable. Five weeks after onset of fall N fertilization, N content in leaves of seedlings receiving the most N (N15) had increased significantly, whereas the moderate rates (N10 and N5) did not, and the control (N0) showed a significant decline (Fig. 4A). In roots, N content significantly increased regardless of N rate, but the highest rate (N15) had more N 5 weeks after onset of fall N fertilization (Fig. 4E). Stem N content was affected less by N rate, with little change in content between observations at 5 and 9 weeks after onset of fall N fertilization (Fig. 4C).

Tissue content of ^{15}N was significantly ($p < 0.0001$) affected by all possible two-way interactions (i.e. tissue type × weeks after onset of fall fertilization; tissue type × N rate; N rate × weeks after onset of fall fertilization; Table S2), with content patterns similar to N content except that roots appeared more sensitive to ^{15}N changes based on N application rate

(Fig. 4). Vector analysis revealed that all rates of N fertilization increased N uptake relative to the control and were generally associated with increases in biomass at 5 and 9 weeks after onset of fall fertilization (Fig. 5).

In brown leaves, the two-way interaction of N rate × weeks after onset of fall N fertilization was not significant ($p = 0.8390$) for N concentration but N and week were ($p = 0.0003$ and 0.0155, respectively). Week 9 concentrations (5.94 ± 0.14 g·kg^{-1}) were greater than week 5 concentrations (5.56 ± 0.12 g·kg^{-1}) for all N rates combined. The control N concentration (5.18 ± 0.14 g·kg^{-1}) was significantly less than treatments receiving N; N5 (5.63 ± 0.18) was significantly less than N15 (6.14 ± 0.15), but N10 (6.03 ± 0.15) was not different from N0 or N15.

Nitrogen Translocation and Utilization

The interactions of tissue type × weeks after onset of fall N fertilization and of tissue type × N rate were significant for net ^{15}N translocation amount (^{15}NTA; $p = 0.0008$ and <0.0001, respectively) and ratio (^{15}NTR; $p = 0.0455$ and 0.0003, respectively; Table S3). The role of weeks as an independent factor was, however, much less pronounced ($p \geq 0.6482$) when compared with the roles of tissue type and N rate ($p \leq 0.0001$; Table S3). Overall, 5 weeks after onset of N fertilization, ^{15}NTA was highest in roots, followed by stems and green leaves (Fig. 6A). From 5 to 9 weeks after onset of fall N fertilization, ^{15}NTA significantly increased in roots, remained constant in stems, and decreased in green leaves (Fig. 6A), although translocation ratio within each tissue type was not significant (Fig. 6C). In all tissue types, ^{15}NTA and ^{15}NTR increased with increasing N rate, with roots and stems having similar ^{15}NTR (Fig. 6B & 6D). Green leaves at the lowest N rates (N0 and N5) presented negative values for ^{15}NTA and ^{15}NTR (Fig. 6B & 6D). Results for NTA were similar to those observed in ^{15}NTA.

For NTR, the independent variables tissue type and N rate were significant. NTR was higher in stems ($103 \pm 64\%$) and roots ($98 \pm 59\%$) than in green leaves ($-20 \pm 32\%$), and accumulation ratio increased in concert with increasing N rate (N0 = $20 \pm 70\%$, N5 = $45 \pm 70\%$, N10 = $69 \pm 67\%$, and N15 = $108 \pm 79\%$). Fall N fertilization use efficiency (NUE) was unaffected by N rates ($p = 0.6502$) and averaged $\sim 26 \pm 4\%$ (data not shown).

Discussion

Effect of Fall N Fertilization on Seedling Growth

Fall fertilization has proven to be an effective technique to avoid nutrient dilution during autumn in several species, ranging from conifer (Birchler et al. 2001; Boivin et al. 2002, 2004) to broadleaved (Oliet et al. 2009, 2013) species, by increasing internal nutrient concentration in plants without affecting total biomass (Timmer 1997). Accordingly, our results with bitterbrush seedlings showed that fall N fertilization does not significantly affect total biomass, stem height, or root-collar diameter. Similar results have been reported in genera such as larch (*Larix*; Wei et al. 2014), oak (*Quercus*; Oliet et al. 2011; Wang et al. 2016), pine (*Pinus*; Li et al. 2016), and spruce (*Picea*; Boivin et al. 2004) and satisfies the objective of adding N during fall fertilization without affecting seedling biomass. Lower temperatures accompanied by the decrease in day length during fall

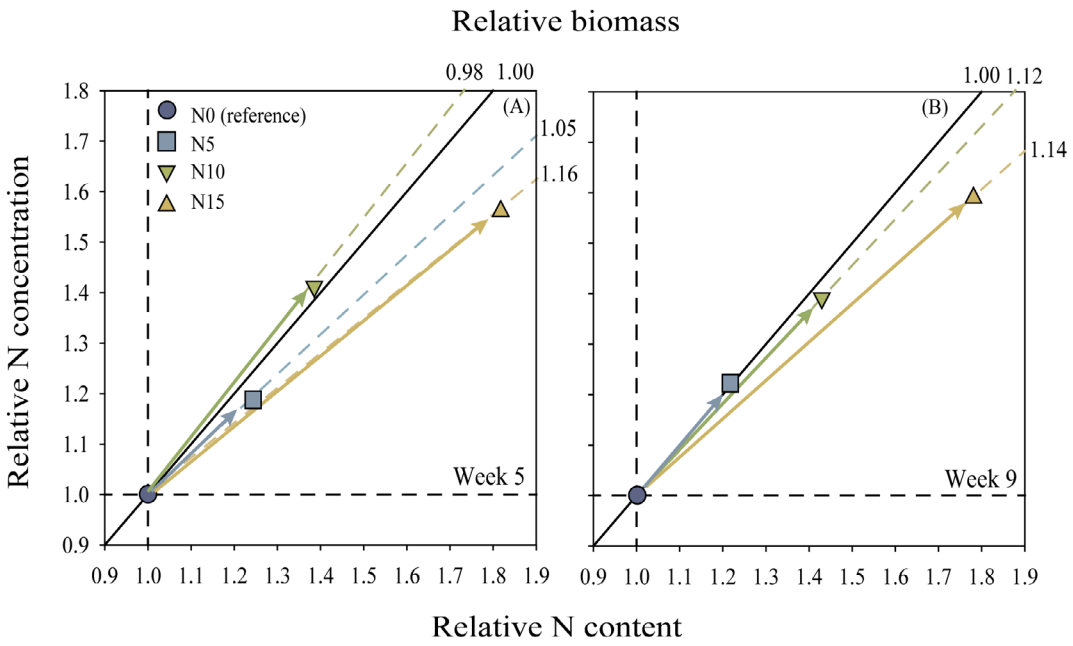

Figure 5. Vector nomogram of relative changes in biomass (solid diagonal line), nitrogen (N) content (*x*-axis), and N concentration (*y*-axis) of entire bitterbrush seedlings 5 (A) and 9 (B) weeks after onset of, three consecutive, weekly applications of fall N fertilization that delivered a total of 5 (N5), 10 (N10), or 15 (N15) mg N·seedling^{-1}. Characteristics of control seedlings supplied with 0 (N0) mg N·seedling^{-1} were used as the reference (relative) values.

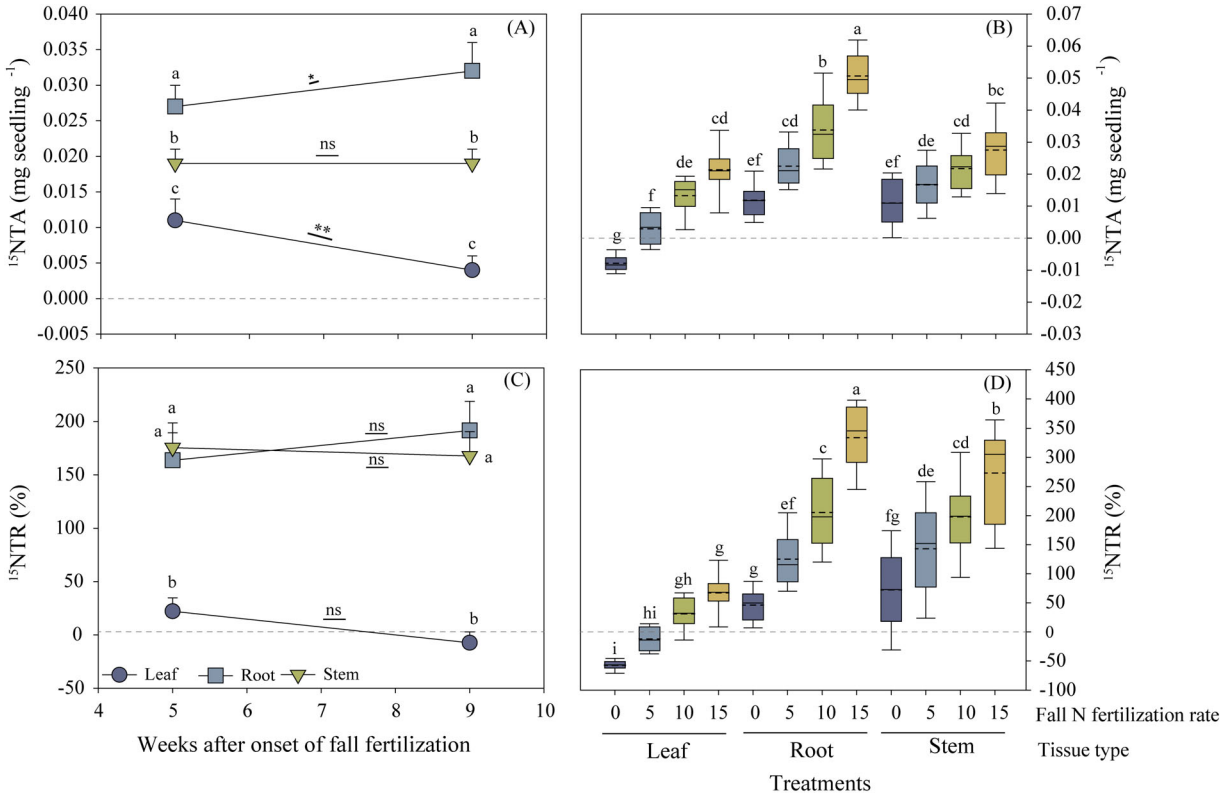

Figure 6. Net ^{15}N translocation amount (^{15}NTA, mg·seedling^{-1} + SD) (A, B) and net ^{15}N translocation ratio (^{15}NTR, % + standard deviation) (C, D) of bitterbrush green leaves, roots, and stems sampled at 5 and 9 weeks after onset of, three consecutive, weekly applications of fall N fertilization (A, C) that delivered a total of 0 (N0), 5 (N5), 10 (N10), and 15 (N15) mg N·seedling^{-1} (B, D). In (B) and (D), the solid line within each box indicates the median whereas the dashed line indicates the mean. Different letters indicate significant differences among tissue types within the same sample time (A, C) and among tissue types and N rate (B, D) according to Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate significant differences among weeks within the same tissue type (A, C) according to Tukey's HSD test (ns = non-significant; *$p < 0.05$, **$p < 0.01$).

could inhibit shoot growth and biomass accumulation of evergreen species adapted to areas that experience summer drought (Fernández et al. 2008), allowing nutrient accumulation without additional growth.

Bitterbrush is an evergreen species that produces two sets of distinct leaves during the year; large ephemeral leaves form during the spring and smaller, overwintering leaves replace the more abundant summer foliage in the fall, which then abscise with the onset of spring growth (Shaw & Monsen 1986; Bilbrough & Richards 1993). Although N fertilization increased N uptake and translocation, leaves (presumably the ephemeral spring leaves) mostly abscised between the onset of fertilization and sampling 2 weeks after the cessation of fertilization regardless of N rate and despite showing additions of ^{15}N and N concentration that increased with N rate. Thus, N additions to these leaves did not affect biomass or appear to delay or prevent normal abscission. A delay in leaf abscission with N fertilization was observed in a semi-evergreen oak (Villar-Salvador et al. 2013).

Although N fall fertilization had little effect on brown leaves, N rate and weeks after onset of fall fertilization significantly interacted to affect green leaf biomass. Because no additional stem growth was observed, this increase in biomass may have been due to a change in green leaf morphology. In a hybrid poplar (*Populus*), additions of N increased biomass in mature leaves within 2 weeks of application, along with rate of photosynthesis and amount of chlorophyll (Cooke et al. 2005).

Nutritional Status

In general, our results showed that higher fall N fertilization rates enhanced bitterbrush N status, observed as an increase in seedling N content and concentration at the whole plant level. Similar results have been reported in diverse species with distinct leaf habits (Boivin et al. 2004; Zhu et al. 2013; Li et al. 2016). Nitrogen distribution and uptake patterns were, however, dependent on tissue type and weeks after onset of fall N fertilization. N content and concentration increased through week five with little additional change through week nine; autumn-like environmental conditions could have prevented N uptake due to a decrease in plant metabolism, indicating a proper application period of fall fertilization to induce N uptake. Also, content of N and ^{15}N in green leaves significantly decreased between weeks five and nine in the N5, N10, and N15 treatments, in accordance with a decrease in green leaf biomass in

these treatments. In the control treatment, N concentration increased between the onset of the fall fertilization treatments and week five, indicating a continuous assimilation of the fertilization applied during the growing season. This agrees with Wang et al. (2016) who reported that pre-hardening fertilization and fall fertilization affected oak nutritional status. Also, the additional N may originate from the substrate we used. Peat initially contains appreciable N that may have subsequently been mineralized and available for plant uptake. In their review, Cheng and Kuzyakov (2005) report that rapid soil wetting-drying cycles tend to increase mineral soil organic matter mineralization; this condition is common in small-volume seedling containers such as the ones we used, especially as the seedlings became larger (Landis et al. 1989). Moreover, the presence of roots can lead to a threefold increase in mineralization (Cheng & Kuzyakov 2005).

Five and 9 weeks after onset of fall N fertilization, ^{15}N concentration was significantly higher in roots, followed by stems and finally leaves, indicating that N applied during fall fertilization was mainly directed toward the perennial tissues. This contrasts with other evergreen species where most fall-applied N is stored in leaves and stems (Boivin et al. 2004; Islam et al. 2009). Our results indicate bitterbrush roots are the major sink of N during fall, and aligns with research showing that levels of total nonstructural carbohydrates also increase in roots at the end of the growing season (McConnell & Garrison 1966; Menke & Trlica 1981). In bitterbrush, this dynamic of N distribution among tissues was also dependent on N rate during fall fertilization; only in the higher N fall fertilization treatments (N10 and N15) did the ^{15}N content increase in leaves, while in the N5 treatment the ^{15}N content only increased in roots. This suggests that under limited N supply during fall, N uptake is directed toward roots as the main source of N storage. Accordingly, Salifu and Timmer (2003) indicated that plants growing in N rich soils increase N uptake toward roots.

At the seedling level, vector analysis revealed that the application of fall N fertilization induced a near-luxury or luxury consumption (elevation of N status with little or no change in total biomass) compared with the control treatment (N0) despite observed significant changes in green leaf biomass. The shifts in relative total biomass by fall N fertilization are likely a result of that increase in leaf biomass, and that additional growth may be reflected in the slight dilution of N in the N10 and N15 treatments. Similar results were observed in pine where 9.75 mg N·seedling^{-1} induced luxury consumption, while higher doses caused N dilution due to an increase in seedling biomass (Timmer & Armstrong 1987; Miller & Timmer 1994), and an analogous pattern of nutritional status with N fall fertilization was reported in oak (Uscola et al. 2015).

Nitrogen Translocation During Fall Fertilization

To explain the improved N status of seedlings during fall N fertilization and how that may support new growth and seedling establishment after outplanting, an understanding of the movement of N among tissues is key (Salifu et al. 2008). Here, the use of a stable isotope (i.e. ^{15}N) allowed the discrimination of N uptake from substrate with internal remobilization within the seedlings during fall fertilization.

The ^{15}NTA and ^{15}NTR results reveal that fall N fertilizer was readily absorbed (week 5) and substantially accumulated in green leaves, but during the next month was subsequently translocated from leaves to roots; the amount of N translocated was dependent on N rate with higher N rates supporting increased translocation from leaves to roots. Thus, in seedlings from the N15 treatment, ^{15}NTR reached 400% in roots and only 50% in leaves, indicating that newly acquired ^{15}N was allocated toward roots. These results confirmed that translocation is stimulated by nursery nutrient loading (Imo & Timmer 1992; Salifu & Timmer 2003) and similar results have been reported in species across diverse evergreen (olive, *Olea*; pine and oak) and deciduous (larch and oak) genera (Li et al. 2012, 2014; Zhu et al. 2013; Uscola et al. 2015).

Before the application of fall N fertilization, N concentration in green leaves (9.9 g·kg^{-1}) was significantly greater than roots (7.2 g·kg^{-1}) and root concentration was not significantly different than stems (7.4 g·kg^{-1}). This supports the hypothesis that in evergreen species leaves are the major sink of nutrient reserves during the growing season (van den Driessche 1984; Islam et al. 2009; Millard & Grelet 2010). On the contrary, at the end of fall fertilization treatments, N translocation was directed from leaves to roots, indicating that during this season roots are the main sink of N, which is also observed through higher ^{15}N uptake toward roots, supporting the hypothesis of translocation driven by sink strength (Nambiar & Fife 1991; Salifu & Timmer 2003). Previous research has reported variable amounts of N translocated toward roots from leaves, ranging from 24 to 36% in oak and maple (*Acer*) (Millard & Proe 1991; Li et al. 2014) to 83% in larch (Zhu et al. 2020), suggesting that the amount of translocated N from leaves is species specific. The shift in sink-source relationships between the growing and fall seasons is, however, sustained across species, including bitterbrush.

Our results clearly indicate that higher N fertilization rates during fall caused an increase in concentration and content of this nutrient in bitterbrush seedlings. Considering that bitterbrush is an important browse species for wild and domestic ungulates, it is prudent to consider the effects that increased N content/concentration could have on browsing. Wild ungulates are known to increase browsing frequency on plant species identified as luxury N consumers (Tripler et al. 2002), fertilized plants (Ball et al. 2000; Månsson et al. 2009), and plants that grew larger because of fertilization regardless of foliage chemical composition (Hartley et al. 1997). Thus, whether it is an effect of increased N plant concentration or plant size promoted by higher fertilization rates, bitterbrush seedlings produced under higher N fall fertilization rates may be more palatable to browsing animals. Moreover, mule deer preferentially browsed the woody portions of younger bitterbrush plants during winter (Krannitz & Hicks 2000). This could negatively affect survival during the first year of establishment.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Mean stem length, root-collar diameter, and biomass (±SE) and *p*-values of bitterbrush seedlings.
Table S2. Summary of three-way repeated measures ANOVA *p*-values for tissue type.
Table S3. Analysis of variance *p*-values for seedling tissue type.

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