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Isotopic Tracer Reveals Depth-Specific Water Use Patterns Between Two Adjacent Native and Non-native Plant Communities

Clemence P. Warren
Utah State University

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ISOTOPIC TRACER REVEALS DEPTH-SPECIFIC WATER USE PATTERNS
BETWEEN TWO ADJACENT NATIVE AND NON-NATIVE PLANT COMMUNITIES

by

Clémence Pascale Warren

A thesis submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

Ecology

Approved:

__________________________  __________________________
Karen H. Beard                 Andrew Kulmatiski
Major Professor                  Committee Member

__________________________  __________________________
Ronald Ryel                    Mark R. McLellan
Committee Member               Vice President for Research and
                                                                 Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah
2011
ABSTRACT

Isotopic Tracer Reveals Depth-Specific Water Use Patterns Between Two Adjacent Native and Non-native Plant Communities

by

Clémence Pascale Warren, Master of Science

Utah State University, 2011

Non-native plants have invaded over 100 millions of acres of western arid land in the US and dramatically altered nutrient cycling rates. Changes in water cycling caused by invasive species are of particular interest because primary production in the Western US is typically limited by water availability and aquifer recharge reflects plant demand. Large-scale invasions can, therefore, be expected to cause large-scale changes in hydrological cycles, but until recently, there have been considerable limitations in the ability to measure the timing, location, and extent of water use. Here we injected a tracer, deuterated water (D$_2$O), into five soil depths in two sampling periods (May and June) in two adjacent plant communities (native and non-native dominated). Plants were sampled at several distances from the tracer addition area to determine the horizontal and vertical extent of water use in native and non-native communities. The tracer injection was coupled with measurements of leaf level stomatal conductance, leaf area index, and volumetric soil water content to estimate plant transpiration. We found that both native
and non-native plants transpired water from primarily the top 60 cm of the soil (>75%), with a particular emphasis (≥ 50%) on shallow soil water (<10 cm) while lateral roots did not exceed 50 cm for most species. Higher leaf area index resulted in significantly more water being transpired from the native community.

Some sharp distinctions in timing and location of tracer uptake resulting from the differing phenologies of the dominant species in each community were observed and confirmed previous mechanisms thought to govern plant assemblages in these communities. In May, the non-native community dominated by annual grasses had higher tracer uptake at 10 cm than the native community but began using deep water (higher tracer uptake at 80 cm) as annual grasses senesced and tap-rooted fobs became dominant in June. The perennial native species, however, used the entire soil profile from the moment they became active until they senesced. Our approach shows promise for overcoming the lack of resolution associated with natural abundance isotopes and other enrichment approaches, and for providing detailed measurements of plant water-use space.
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Pascale Warren
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>PURPOSE OF STUDY</td>
<td>19</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>21</td>
</tr>
<tr>
<td>RESULTS</td>
<td>32</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>54</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>62</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>68</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>88</td>
</tr>
<tr>
<td>Appendix A: Tracer uptake ANOVA tables</td>
<td>89</td>
</tr>
<tr>
<td>Appendix B: Leaf area, stomatal conductance</td>
<td>92</td>
</tr>
<tr>
<td>and transpiration ANOVA tables</td>
<td></td>
</tr>
<tr>
<td>Appendix C: Pulsing summary tables and figures</td>
<td>95</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table | Page | Description
--- | --- | ---
I | 43 | Leaf area index ($m^2/m^2$) of dominant species in native and non-native communities

II | 53 | Summary of soil water loss and community-level transpiration (standardized by LAI) in native and non-native communities in May and June 2009

A-I | 90 | ANOVA results for vertical tracer uptake in May 2009

A-II | 90 | ANOVA results for vertical tracer uptake in June 2009

A-III | 90 | ANOVA results for lateral tracer uptake in May 2009

A-IV | 91 | ANOVA results for lateral tracer uptake in June 2009

A-V | 91 | ANOVA results for lateral tracer uptake in June 2009 with 5 cm pulse excluded

A-VI | 91 | ANOVA results for lateral tracer uptake in June 2000 with 5 cm pulse excluded

B-I | 93 | ANOVA results for community level patterns in leaf area index

B-II | 93 | ANOVA results for community and species level patterns in stomatal conductance

B-III | 93 | ANOVA results for community and species level patterns in plant transpiration

C-I | 96 | Summary of pulsed samples by depth
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean monthly and total monthly precipitation (mm) in Winthrop, Washington</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>Mean deuterium concentration in soil profiles of pulsed and control plots in May 2009</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Mean deuterium concentration in soil profiles of pulsed and control plots in June 2009</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>Proportion of tracer uptake by depth in native and non-native communities in May 2009</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>Proportion of native species tracer uptake by depth in May 2009</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Proportion of non-native species tracer uptake by depth in May 2009</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>Proportion of native species tracer uptake by depth in June 2009</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>Proportion of non-native species tracer uptake by depth in June 2009</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>Proportion of tracer uptake by depth in native and non-native communities in June 2009 (5 cm pulse excluded)</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>Proportion of lateral tracer uptake in May 2009</td>
<td>38</td>
</tr>
<tr>
<td>11</td>
<td>Proportion of lateral tracer uptake in June 2009</td>
<td>39</td>
</tr>
</tbody>
</table>
12 Soil water potential in native and non-native communities soil profiles in May 2009 .......................................................... 40
13 Volumetric soil water content in native and non-native communities soil profile in May 2009 .......................................................... 40
14 Soil water potential in native and non-native communities soil profiles in June 2009 .......................................................... 42
15 Volumetric soil water content in native and non-native Communities soil profile in June 2009 .......................................................... 42
16 Leaf area index of native and non-native communities at four collection dates .......................................................... 43
17 Differences in stomatal conductance within dominant native and non-native communities across months .......................................................... 44
18 Differences in stomatal conductance within dominant native species across months .......................................................... 45
19 Differences in stomatal conductance within dominant non-native species across months .......................................................... 46
20 Total monthly transpiration in native and non-native communities in May, June, and July 2009 .......................................................... 47
21 Total monthly transpiration of native species in May, June, and July 2009 .......................................................... 47
22 Total monthly transpiration of non-native species in May, June, and July 2009 .......................................................... 48
Total monthly transpiration by pulsed soil layers in native and non-native communities in May 2009 ........................................49
Total monthly transpiration by pulsed soil layers for native species in May 2009 ..........................................................50
Total monthly transpiration by pulsed soil layers for non-native species in May 2009 ..........................................................51
Total monthly transpiration by pulsed soil layers in native and non-native communities in June 2009 ........................................51
Total monthly transpiration by pulsed soil layers for native species in June 2009 ..........................................................52
Total monthly transpiration by pulsed soil layers in native and non-native communities in June 2009 ........................................52
Differences in stomatal conductance across dominant native species within months .................................................................94
Differences in stomatal conductance across dominant non-native species within months ..........................................................94
Mean deuterium concentration in treatment and control plant samples .................................................................................97
Mean deuterium concentration in native control plant samples .............................................................................................97
Mean deuterium concentration in non-native control plant samples ........................................................................................98
INTRODUCTION

Large-scale and persistent plant invasions have extensively altered the shrub lands of the Western United States. Current shrub-steppe ecosystems are drastically different from before European settlement. A long history of anthropogenic disturbances ranging from grazing, tilling, and development has removed most of the native vegetation and facilitated the establishment of invasive non-native species (Mack, 1981; D'Antonio and Vitousek, 1992; Sheley and Petroff, 1999). These invasions have had far reaching economic (Pimentel et al., 2005) and ecological impacts (Chapin et al., 1997; Dukes and Mooney, 2004). Restoration of these degraded landscapes is impeded by the lack of specific knowledge on the mechanisms through which invaders alter community properties (Levine et al., 2003). Thus, identifying the factors that contribute to the dominance of invaders is restoration priority.

In semi-arid systems, water availability plays a key role in shaping plant community dynamics (Noy-Meir, 1974; Chesson et al., 2004). Temporal and spatial partitioning of available water is one of the bases of plant coexistence (Nippert and Knapp, 2007a; Araya et al., 2011). Disturbances, however, often disrupt these biotic and abiotic interactions through biomass removal or a net increase in available resources; for example, disturbances can open windows of water-availability for non-natives to successfully establish (Davis and Pelsor, 2001; Chambers et al., 2007; Blumenthal et al., 2008). Established non-native plants often become ecosystem engineers through the introduction of novel traits that may alter regional hydrology and suppress the recolonization of native plants. Indeed, some non-native plants have become an important
component of their invaded habitat water budgets (Cleverly et al., 1997; Zavaleta, 2000; Huxman et al., 2005; Prater et al., 2006). Where this occurs, the shift in vegetation composition has caused changes in albedo (Chapin et al., 1997), litter depth (Evans et al., 2001), rooting patterns, soil moisture depletion (Obrist et al., 2004; Young et al., 2010), and evapotranspiration (Prater and DeLucia, 2006).

Water-use patterns have changed since the replacement of the perennial sagebrush and grass community by non-native annual grasses and tap-rooted forbs (Germino et al., 2004; Kulmatiski, 2006; Kulmatiski et al., 2006a; Prevéy et al., 2010). The persistence or dominance of non-native plants on the landscape has been attributed to various strategies of water use. Winter active annual grasses like cheatgrass germinate early in the spring and end their life cycle before the onset of the water shortage that longer active species experience (Cline et al., 1977; Dyer and Rice, 1999; Sheley and Petroff, 1999). The rapid near surface soil water depletion in the early spring can further impede the establishment of native perennial grass seedlings (Holmes and Rice, 1996; Enloe et al., 2004). Tap-rooted forbs that actively grow late in the summer in contrast to senescing native species or non-native annuals effectively limit soil moisture and nutrient availability in the following growing season (Hill et al., 2006; Prevéy et al., 2010). Overall, it has been suggested that the combination of early and extended phenology as well as rooting patterns might be the primary factors that determine water use patterns in invaded semi-arid environments of the Intermountain West (Kulmatiski, 2006).

While there is evidence that water acquisition patterns greatly contribute to non-native plant establishment, the difficulties in belowground research have limited measurements of the timing, location and extent of water use. Until recently, a specific
array of techniques has been used to assess root activity and plant water uptake (Boyer, 1969; Ehleringer and Dawson, 1992). Measurements of soil moisture and soil water potential have been used to indicate how much water moves in and out of specific soil depths and whether plants can physiologically access soil moisture, but this type of data cannot attribute water use to any particular plant species. Although commonly used, root abundance data or root mapping does not always correlate with root activity in resource uptake (Thorburn and Ehleringer, 1995; Casper and Jackson, 1997), not to mention the difficulty in isolating fine root materials from the soil matrix and in documenting the extent of an individual plant’s contribution to belowground biomass (Jackson et al., 1999).

Commonly used natural abundance isotopes (\(^{18}\)O and \(^{2}\)H) can distinguish between surface water, ground water, and precipitation (Dawson et al., 2002) but typically do not allow the distinction of differences in rooting depth of less than 30 cm (Kulmatiski et al., 2006a). A modification of the pulse-chase technique uses a depth-controlled tracer (Bishop and Dambrine, 1995; Plamboeck et al., 1999; Kulmatiski et al., 2010) to achieve greater resolution in the distribution of water uptake from different soil layers by injecting deuterated water into the soil to assign a known isotopic signature to a specific soil layer. The addition of a tracer not only improves the uptake and allows greater capacity to detect between sources, but also offers the promise of developing species specific descriptions of water use by depth and time.

Eddy-Correlation and Bowen ratio techniques can measure water vapor fluxes and canopy transpiration (Wullschleger et al., 1998; Baldocchi, 2003), but cannot measure the differential use of water between plant species nor identify the source pool from the
soil profile. While the pulse-chase approach can pinpoint location of water uptake in the soil profile, previous studies have not combined these measurements with estimates of transpiration to detail the contribution of specific soil layers to site water budget (Plamboeck et al., 1999; Moreira et al., 2000; Sternberg et al., 2002). Here, species-specific transpiration estimated with the Penman-Monteith model highlights the spatial heterogeneity in soil moisture depletion powered by evapotranspiration and rooting depth patterns, as illustrated by tracer uptake from pulse-chase. Such fine-scale measurements of hydrological niche partitioning, at both the soil-root and shoot-atmosphere levels, can be used to parametrize hydrological models to further our understanding of niche segregation and the potential impacts of a changing climate on regional hydrology.

The objective of this study was to measure the location, timing, and extent of water use by dominant native and non-native plant species during the height of the growing season in a shrub-steppe ecosystem. It was hypothesized that changes in phenology, rooting depths and in plant performance traits (higher leaf area and stomatal conductance expected from non-native plants) would result in different patterns of soil drying and greater evapotranspiration rates in the non-native community. Estimates of species-specific and depth-specific water use were validated against measurements of soil water content and soil water potential.
LITERATURE REVIEW

Impacts of non-native plants on semi-arid environments

Biological invasions are a rising threat to the integrity of native ecosystems (Vitousek, 1990; Sanders et al., 2003; Simberloff, 2005). Second to land use changes, they have caused more native extinctions than any other human-induced changes (Wilcove et al., 1998). Non-native plants are a growing ecological and economic problem in the US (DiTomaso, 2000; Duncan et al., 2004; Pimentel et al., 2005). Billions of dollars are spent each year through costs associated with eradication programs and loss of ecosystem functions and services (Dukes and Mooney, 1999; Zavaleta, 2000).

Semi-arid shrub-steppe ecosystems of the western US have been particularly susceptible to biological invasions (D'Antonio and Vitousek, 1992; Pyke, 1999). Since European settlement in the 19th century, these ecosystems have been declining, with less than 20% of these ecosystems remaining unaltered (West, 1999). Originally a bunchgrass dominated ecosystem (DiTomaso, 2000), exotic annual grasses and tap-rooted forbs have successfully spread across over 100 million hectares of western shrub lands, establishing persistent stands that resist efforts to suppress them (Sheley and Petroff, 1999) and impeding native plant establishment (Lejeune and Seastedt, 2001) through increased competition for limiting resources (Suding et al., 2004). Decline in habitat quality has negatively affected wildlife dependent on native vegetation (Raphael et al., 2001).

Exotic annual grasses have disturbed the fire regime of western shrub lands (Mack and D'Antonio, 1998; Brooks et al., 2004) while resource supply and uptake rates have been significantly altered through changes in nutrient cycling and hydrology (Calder and Dye,
Changes in leaf litter quantity and quality have altered nitrogen mineralization rates in invaded ecosystems and decreased nutrient availability to native plants and soil biota (Evans et al., 2001; Booth et al., 2003; Belnap et al., 2005; Sperry et al., 2006).

Study species

The majority of noxious weeds in western rangelands are of Eurasian origin (Sheley and Petroff, 1999) whose expansion has been attributed to recurrent disturbances produced by fire, excessive grazing, agricultural practices, and construction of railroads, roads and towns that removed the native vegetation (Yensen, 1980; Mack and Pyke, 1983; Sheley and Petroff, 1999). The most ubiquitous of western rangeland plant invaders, *Bromus tectorum*, is an exotic annual grass introduced in the late 1800’s through grain contamination that rapidly spread across the Intermountain West region of the US (Mack, 1981). Its success has been partly attributed to the absence of a dominant native annual grass in shrub-steppe ecosystems (D'Antonio and Vitousek, 1992) and positive feedback with fires through the accumulation of fine fuel loads (Whisenant, 1990). The low tolerance of the already overgrazed native steppe species to fire made it easier for *Bromus tectorum* to occupy an empty niche after its introduction (Mack and D'Antonio, 1998). An early fall germinator, *B. tectorum* overwinters as small seedlings before resuming growth early in the spring and completing its life cycle before the onset of summer drought (West, 1983; Sheley et al., 1998).

Tap-rooted exotic forbs have also increasingly become a major threat to semi-arid grasslands of the West (Pyke, 1999). Since its introduction in 1907 (Roche and Talbot,
1986), *Centaurea diffusa* has been particularly successful in the interior Pacific Northwest in the bitterbrush/bunchgrass plant communities (Sheley et al., 1998). Though *C. diffusa* may also behave as a short-lived perennial or annual, it is considered a biennial that germinates in the fall and spends winter as a tap-rooted rosette before bolting in the spring (Roche and Talbot, 1986; Sheley et al., 1998). Another member of the Asteraceae family, *Tragopogon dubius*, also a tap-rooted biennial, has become established over continental America. Similarly to *C. diffusa*, *T. dubius* develops an extensive root system during its rosette phase (Upadhyaya et al., 1993) and has been shown to reduce the leaf area and shoot to root ratio of bluebunch wheatgrass in the interior Pacific Northwest (Clements et al., 1999). Numerous mustard species have also successfully invaded disturbed western rangelands, among others *Sisymbrium* spp (Yensen, 1980). *S. loeselii* is a biennial tap-rooted forb that also germinates in the winter. Many of these non-native tap-rooted forbs are non-palatable to livestock whose selective grazing of native vegetation only increase their cover (Sheley and Petroff, 1999).

The flora of intact shrub-steppe is overall depauperate (Daubenmire, 1970; West, 1983) and is usually dominated by sagebrush (or other shrub species) and bunchgrasses. *Pseudoroegneria spicatum* is by far the most dominant grass of the Intermountain West. As a perennial bunchgrass, *P. spicata* rebuilds all aboveground material starting in late spring before senescing in the early summer when soil moisture dries out (West, 1983). In eastern Washington, the forb component is usually represented by *Balsmorrhiza sagittata* and *Lupinus* spp (Daubenmire, 1970), perennial tap-rooted forbs that exhibit summer dormancy (Kitchen, 1994).
Invasibility, disturbance, and resource availability

Past research has attributed invaders success to different aspects of their biology and ecology, such as fast growth rates and high reproductive output (Rejmanek and Richardson, 1996; Williamson and Fitter, 1996b; Sutherland, 2004), but not all species that display traits characteristic of invasiveness actually become invaders (Williamson and Fitter, 1996a). Performance tends to differ across ecosystems with some ecosystems being more susceptible to invasion than others (Rejmanek, 1999). Leading theories in invasion ecology have now acknowledged that invasibility is not an inherent characteristic of any particular ecosystem but results from complex interactions between the resident species, the environmental variables of the community and the specific biological attributes of the invaders (Davis et al., 2000; Davis and Pelsor, 2001; Davis et al., 2005; Pysek and Hulme, 2005; Sharma et al., 2005). Resource availability is considered a key environmental variable that influences niches opportunities, species distribution, and survival (Tilman, 1982; Shea and Chesson, 2002). It has been argued that these same patterns that influence native communities are also relevant to the invasion process (Levine, 2000; Stohlgren et al., 2003). Successful establishment of an introduced species, just like any native species, depends on whether there are enough resources to meet their growth requirements (Shea and Chesson, 2002). There is compelling evidence that differential acquisition and utilization of resources or their net increase over space and time can result in some non-native plants excluding native plants (Burke and Grime, 1996). Resource pool dynamics, therefore, represent an important tool to understand how invasive species succeed.
Disturbances have also been shown to facilitate invasions (Hobbs and Huenneke, 1992; Lonsdale, 1999; Sher and Hyatt, 1999). They act as destabilizing forces that disrupt the biotic and abiotic interactions within a community and modify the environment in ways that creates windows of opportunity or empty niches (Elton, 1959) that are unexplored by native species (Davis et al., 2000). In countless instances, disturbances have set the stage to many plant invasions by removing or reducing native biomass (Herron et al., 2001; Cushman et al., 2004; Seastedt and Suding, 2007). Many invasive plants can quickly colonize recently disturbed areas where resources are readily available and competition from native resident species has been greatly reduced (Sheley and Petroff, 1999; Beckstead and Augspurger, 2004). While the success of B. tectorum has been attributed to the absence of a dominant native annual grass in shrub-steppe ecosystem (D'Antonio and Vitousek, 1992), its introduction coincided with high intensity grazing by domestic livestock that removed native bunchgrasses and forbs and increased resource availability (Mack and Pyke, 1983; Pyke, 1999). In an experiment manipulating snowfall, Blumenthal et al. (2008) reported increased Centaurea diffusa recruitment into established native plots that received more snowfall. The tap-rooted forb benefited from an increased availability of deep soil water during the growing season. Under increased CO2 levels, Centaurea solstitialis also increased in biomass when grown in microcosms on nutrient poor serpentine soil that usually repel its establishment (Dukes, 2002a). Similarly, Gelbard and Harrison (2005) found that exotic plants cover and richness were higher than native plants along roadsides than in adjacent interior communities. Moreover, roadsides subjected to the most disturbances also supported more exotic plants.
Resource partitioning and water as a limiting resource

Resource partitioning has been used to explain how plant species that essentially have the same requirements for light, nutrients, and water are able to coexist (Silvertown, 2004); diversifying strategies of resource acquisition can however reduce direct competition and facilitate coexistence (Barot and Gignoux, 2004). Soil moisture is often cited as being one of the most limiting factors that restrict plant growth in semi-arid environments (Lauenroth et al., 1978; Lejeune and Seastedt, 2001). In arid lands, water drives important ecosystem processes (Reynolds et al., 2004; Schwinning et al., 2004), and precipitation is considered to be the primary determinant of primary productivity (Sala et al., 1988; Churkina and Running, 1998; Adler and Levine, 2007; Heisler-White et al., 2008). The temporal and spatial variations of water availability greatly affect vegetation composition and structure (Chesson et al., 2004; Ryel et al., 2008). As such, access to soil moisture represents an important axis of niche differentiation, allowing plants of different life histories to partition resources within the soil profile in response to precipitation patterns (Chesson et al., 2004; Nippert and Knapp, 2007a).

Various studies have examined the role of resource partitioning in ecosystem invasibility (Dukes, 2002b; Maron and Marler, 2008). Intact native plant communities have a long history of common evolution through which they commensurately use available resources (Parrish and Bazzaz, 1976). Temporal or spatial partitioning of limiting resources can not only promote coexistence but also reduce resources to a level low enough that plant communities effectively resist invasions (Davis et al., 2000). Similarly, invaders that achieve site dominance can use enough of the critical resources
that re-establishment of native plants cannot occur. More diverse native communities can use resources more thoroughly and fill up the available niches, thus reducing resource availability to invaders (Tilman et al., 1996; 1997; Hooper et al., 2005; Maron and Marler, 2008) or one dominant species might be able to sequester the most resources, keeping resource levels low (McKane et al., 2002; James et al., 2008). Dominant species have been found to be able to sequester the most resources through their high biomass demand. In an arctic tundra, McKane et al. (2002) found that resource partitioning promoted coexistence between one dominant species that used the most nitrogen, over a large gradient in the soil, through time and under various chemical forms than less abundant species who had a narrower niche and survived on specific chemical forms and only used it at specific depths and time. The role of overall diversity as opposed to one dominant species in controlling resource availability needs to be resolved as management actions to combat invasive plants will depend on which factor is responsible for sequestering resources.

Rooting depth patterns of the focal native and non-native species

In shrub-steppe ecosystems, precipitation events differentially affect plants through their timing, frequency and intensity (Noy-Meir, 1974; Loik et al., 2004; Reynolds et al., 2004). Winter precipitation falling primarily as snowfall is the primary mode of soil water recharge (West, 1988; Donovan and Ehleringer, 1994). Soil moisture in the deeper soil profile is especially dependent on this winter moisture input (Donovan and Ehleringer, 1994). While deeper soil moisture does not fluctuate to a great extent
(Doborowlsky et al., 1990), high temperatures and evaporation tend to dry up the moisture in the shallow soil layers and soil surface rely mainly on summer rainfalls for moisture during the summer (Loik et al., 2004; Schwinning and Sala, 2004). Plant growth and distribution in semi-arid lands are not just a function of water availability; other factors can also be limiting and interact with each other to influence productivity (Cui and Caldwell, 1997). Limiting nutrients are strongly associated with the topsoil (Jobbagy and Jackson, 2001) and their availability coincides with moisture pulses from spring snowmelt and summer rainfalls (Bilbrough and Caldwell, 1997; Austin et al., 2004). Soil water dynamics and plant growth are also closely linked to nutrient availability and the nutrient rich shallow soil water is strongly associated with plant growth (Ryel et al., 2008). The seasonality of precipitation resulting in soil water recharge following fall and winter combined with high nutrient availability in shallow soil layers have shaped root distribution to maximize uptake of limiting resources (Seyfried and Wilcox, 2006; Schenk, 2008).

Sun et al. (1997) found that 65% of temperate grassland species are shallow rooted and have the greatest root concentration in the top 20 cm of the soil profile. Around 80% of temperate grassland and temperate desert species reach a maximum depth of one meter (Doborowlsky et al., 1990; Jackson et al., 1996). Weaver (1919) extensively mapped shrub-steppe root systems from the Intermountain West and provided a rich source of rooting depth patterns for the dominant native grasses and forbs included in this study. Some species of Lupinus, for example, were found to have a rooting range between 1.5 and 3 m while B. sagittata’s woody tap root could reach down to as much as 1.9 m. Roots of P. spicata were by contrast coarse and fibrous with short laterals, which
is characteristic of bunchgrasses and could reach up to 1.5 m, although on average they only went down to 0.75 m.

Among the non-native species, \textit{B. tectorum} has well documented root systems with its fibrous roots concentrated in the upper 30 cm of soil (Klemmedson and Smith, 1964). Harris (1967) reported that \textit{B. tectorum} roots can attain up to 1.5 m while Peek \textit{et al.} (2005) found that they only grew to 1.2 m but had 75\% of their root biomass above 0.5 m and less than 5\% of total root length below one meter. Another study by McKenzie \textit{et al.} (1986) found that \textit{B. tectorum} maximum rooting depth ranged from 76 cm to 120 cm. Little is known about the root systems of the other non-native species in the present study. McKenzie \textit{et al.} (1986) reported that \textit{Sysimbrium altissimum}, a species that often co-occur with \textit{S. loeselii} reached a maximum depth of 97 cm while \textit{T. dubius} had roots that were found as deep as 137 cm (Waugh \textit{et al.}, 1994). \textit{C. maculosa}, a congener of \textit{C. diffusa}, had close to 40\% of its roots below 30 cm and as much as 4 times the amount of roots of \textit{P. spicata} in the 45-60 cm portion of the soil profile (Marler \textit{et al.}, 1999).

Very little is known about the lateral root growth of plant species in the Intermountain West. It is expected however that plant species growing in a semi-arid climate, where low precipitation limits infiltration depth, would spread out long lateral roots at relatively shallow depths in order to maximize water uptake (Jobbagy and Jackson, 2001). For example, Weaver (1919) described \textit{B. sagittata} as having profuse lateral roots that spread out 60-90 cm on average. In contrast, the lateral roots of \textit{B. tectorum} only attained 20-30 cm (Hulbert, 1955).

While root mapping offers valuable insight into root distribution and resource partitioning between different life forms, it lacks accuracy in measuring belowground
resource uptake. This approach disproportionately exposes large suberized roots at the expense of fine roots that contribute the most to water and nutrient uptake (Chen et al., 2004; Peek et al., 2005). Further research is needed to fully understand how precipitation and its effects on the spatial and temporal soil water dynamics influence rooting patterns in semi-arid environments (Young et al., 2010).

*Previous research on plant water use and evapotranspiration in semi-arid environments*

Changes in plant community composition associated with plant invasions have resulted in significant changes to ecosystem soil moisture dynamics in the semi-arid west (Ryel et al., 2010). Woody plant encroachment into shrub lands has been found to increase ecosystem water use through changes in community rooting depth, canopy cover and litter depth (Hester et al., 1997; Calder and Dye, 2001; Huxman et al., 2005; Potts et al., 2006). Replacement of native plants by non-native species of similar life form can also affect community water use as reported by Cavalieri and Sack (2010) who found that invasive plants had higher stomatal conductance than native plants. When combined with other traits such as low tissue construction cost and higher leaf area (Daehler, 2003), fast growing non-native plants have the potential to change the water balance in their invaded region.

Plant communities now dominated by exotic annual grasses like *B. tectorum* become active much earlier in the spring than native communities. Annual grass seedlings are able to start root elongation at colder temperatures than the native bunchgrasses (West, 1983; Sheley et al., 1998). Early phenology allows *B. tectorum* a
head start by using available water from snowmelt and spring precipitation to rapidly complete its life cycle before water gets scarce, effectively decreasing available soil water to other plants especially in surface soils critical for germination and early establishment (Mack and Pyke, 1983). Obrist et al. (2004) found that herbaceous exotic annuals used more water than intact sagebrush communities, had lower soil water recharge following winter snowfall and lower soil water content from the shallow soil layers early in the growing season. Similar patterns were observed in California grasslands where shallow rooted exotic annual grasses allocated more biomass to belowground structures, had high growth rates, became active early and ended their life cycle early (Holmes and Rice, 1996).

Similarly, many tap-rooted exotic forbs remain physiologically active in late summer when native species senesce (Sheley et al., 1998). Their deeper root distribution in the soil profile allows them access to deeper soil moisture that herbaceous native species cannot reach. Scarce summer precipitation in the Intermountain West does not usually result in deep infiltration and is typically offset by evaporative losses (West, 1983; Ehleringer et al., 1998). As a result, native bunchgrasses and forbs start senescence in the early summer to avoid prolonged drought conditions. In contrast, tap-rooted exotic forbs have been shown to stay photosynthetically active longer, setting seeds well into late summer and increasing summer water use in invaded habitats (Sperber, 2001; Gerlach, 2004; Germino et al., 2004). Soil water content has been reported to be lower underneath stands of C. maculosa as well as C. solstitialis compared to similar sites that had not been invaded by these species (Gerlach, 2004; Young et al., 2010).
Hill *et al.* (2006) reported greater leaf area index, higher rates of transpiration and carbon assimilation at sites dominated by *C. maculosa* compared to native grasses in Montana grasslands; this was attributed to *C. maculosa* being able to access a more constant water supply from deeper soil layers. In addition, alterations in fire regime that accompany the conversion from shrub-steppe to annual grasslands has also accelerated soil moisture depletion and resulted in greater evapotranspiration rates (Prater and DeLucia, 2006). Previous research in Washington suggested that early season shallow water use from annual grasses combined with late summer deep water use by tap-rooted invasive forbs might be the mechanism explaining how adjacent native and non-native plant communities are unable to invade each other over decadal time, instead forming alternative stable state plant communities (Kulmatiski, 2006; Kulmatiski *et al.*, 2006a). Limitations associated with available tools and techniques have prevented the resolution of this assumption; newly developed techniques in plant ecophysiology make possible a clear test of this hypothesis.

*Stable isotopes in plant ecology*

Recent progresses in plant physiology have made available tools, such as stable isotopes, that have proven to be extremely valuable in improving our knowledge of resource use and belowground resource partitioning by finally making it possible to measure root activity (Dawson *et al.*, 2002). Due to inherent properties of isotopes (Lajtha and Marshall, 1994), many biogeochemical processes have a specific isotopic signature measured as the ratio of heavy to light isotopes (Dawson, 1993; Lajtha and Marshall, 1994). The field of plant ecophysiology has benefitted enormously from the use
of isotopes that provide a non-destructive alternative for measuring water acquisition by plants. Within the soil profile, there are naturally occurring gradients of isotopic abundance in soil water (Dawson, 1993). Shallow soil layers submitted to higher evaporative demand tend to be enriched in heavy isotopes relative to deeper soil water layers (Dawson, 1993). As plants do not discriminate against heavy isotopes during water uptake, xylem water in non transpiring plant tissues retain the isotopic signature of the water source from which it was derived (Flanagan and Ehleringer, 1991). Isotopic abundance in xylem sap corresponds to the range of isotopic composition of all water taken up by the plant roots, and can determine the average depth of a plant’s soil water uptake (Dawson, 1993; Dawson et al., 2002).

Water utilization between plants of different life forms was commonly inferred through root presence in the soil using root distribution maps (Weaver, 1919; Walter, 1971; Weltzin, 1997) until the advent of stable isotopes. Measurements of root activity with stable isotopes have since demonstrated that presence of roots does not always equate to water uptake (Ehleringer et al., 1991; Nippert and Knapp, 2007b). The natural abundance technique, however, does not provide very detailed estimate as it can only distinguish between surface water, ground water, and precipitation (Dawson et al., 2002). In these instances, the enrichment approach (or pulse chase) can be used where small amounts of a labeled substance are added at a concentration higher than the natural background concentration to act as a tracer. Adding a tracer improves the uptake and allows greater capacity to detect between sources. To date, pulse-chase techniques have involved irrigating the surface of the soil with deuterated water (Schwinning et al., 2002). By measuring the isotopic content of plant tissues after pulse label addition, it is possible
to determine the extent to which different plants accessed the added label (Moreira et al., 2000). While the pulse chase technique offers the advantage of measuring root activity, it has not provided high resolution measurements of water use by different plant types; this is because irrigation results in a wide pulse front in the soil so it is difficult to distinguish differences in water use over vertical distances of less than 40 cm. Furthermore, it is not possible to compare concentrations in herbaceous and woody plants unless the dilution effect of water present in woody plant tissues can be determined.

Fortunately, it has become possible to achieve greater resolution in the distribution of water uptake from different soil layers with a modification of the pulse-chase technique that uses a depth-controlled tracer (Bishop and Dambrine, 1995; Plamboeck et al., 1999; Kulmatiski et al., 2010). This approach finally overcomes the unresolved issue of overlapping root zones and the lack of resolution in the isotopic signatures between soil water source pools by assigning a known isotopic signature to specific soil layers that can be retained by plant tissues. This method is described below.
PURPOSE OF STUDY

Several invasive non-native species of Eurasian origin have become established in the shrub-steppe ecosystems of north-central Washington (Sheley et al., 1998; Sheley and Petroff, 1999) and are now the dominant vegetation of abandoned agricultural lands and other disturbance-prone sites (Kulmatiski, 2006). Once established, these species become extremely hard to eradicate as they often employ various mechanisms that promote their self growth (Kulmatiski et al., 2006b; Sperry et al., 2006). Some invaded sites have been found to persist for several decades in the same successional state where ruderal non-native replace other ruderal non-native species without ever progressing to a later successional stage (Kulmatiski, 2006). These invaded communities become unpalatable to livestock, reduce forage and habitat to wildlife, can contaminate croplands, and lose their recreational value (Sheley and Petroff, 1999). Restoration efforts aimed at reducing the associated economical and ecological losses are often not successful largely in part because of the lack of knowledge of the mechanistic processes through which invaders transform their landscapes (Levine et al., 2003).

The goal of the study is to use the modified pulse chase technique to obtain the most detailed measurements so far of plant water use space in an invaded shrub-steppe ecosystem. Past research stopped short of identifying specific mechanisms that could explain the patterns of community assemblages observed in the Methow Valley due to the inaccuracy of the then available tools to measure water use (Kulmatiski et al., 2006a). We propose using deuterium as a tracer to inject a known isotopic signature into five soil depths in May and July to determine the location and timing of water uptake in two
adjacent plant communities of different land use history (native and non-native). Paired with measurement of leaf area index, and stomatal conductance, our approach will allow estimation of the extent of species transpiration at particular soil depths. We hypothesize that differences in water use patterns are consistent with the development of alternative stable state communities that exist side by side without ever invading each other.

The results of the study will be presented in the next chapter and will be submitted for publication to the journal *Ecohydrology* with Drs. Karen H. Beard and Andrew Kulmatiski as co-authors.
MATERIALS AND METHODS

Study site

Research was conducted in the shrub-steppe ecosystem of the Methow Valley, near Winthrop, Washington, USA (48°37’N, 107°10’W). Mean annual precipitation at the study site was 35.4 cm from 1906 to 2009 (WRCC, 2009; Figure 1). Snowfall from October to April contributes 70% of annual precipitation and is the primary mechanism of soil water recharge as much of the precipitation received during the growing season satisfies evaporative demand before reaching soils (Kulmatiski et al., 2006a). The growing season usually begins during freeze-thaw cycles in late April and lasts until snowfall around November. By early July most native grasses and forbs have senesced and stay dormant until late April, though some species germinate in the fall or early spring.

Research was conducted during the 2009 growing season (May to July) and total precipitation from May to August was 6.25 cm or 24% of annual precipitation (Figure 1; WRCC 2009). Mean annual rates of pan evaporation derived from the closest meteorological stations with similar climate, Wenatchee (155 km) and Spokane (300 km), were 104 and 123 cm year\(^{-1}\), respectively (WRCC 2009). The water table is between 30 and 130 m below the soil surface, as suggested by active wells near the study site.

Research was conducted on the Newbon soil series (coarse-loamy, mixed mesic Typic Haploxerolls).

In the Methow Valley, non-native species commonly invade abandoned agricultural (ex-arable) fields while native plants dominate undisturbed areas (Kulmatiski
Research focused on the dominant grasses and forbs species in each community: *Baslamorhiza sagitatta* Pursh., *Lupinus* spp., and *Pseudoregnia spicatum* Pursh. in the native community and *Bromus tectorum* L., *Centaurea diffusa* Lam., *Sysimbrium loeselii* L., and *Tragopogon dubius* Scop in the non-native community.

Figure 1. Mean monthly (1906-2009, WRCC) and observed monthly precipitation in 2009 (collected from rain gauge), Winthrop, Washington.

*Deuterium tracer experiment*

Treatments consisted of five pulse depth plots plus a control plot for each pulsing time period (May and June) in each plant community for a total of 24, 1.5-m radius (7.07 m$^2$) plots (six treatments x two time periods x two plant communities). These 24 plots were placed 20 to 30 m apart in four parallel, 180-m long transects. Two of the four transects with six plots were located in a native field and two transects were located in an adjacent non-native field. Each plot within a plant community was randomly assigned
one of the 12 treatments. Exact plot location was determined by the presence of representative samples of all focal species.

Early-season injections (May) emphasized the shallow depths 10, 20, 30, 50 and 90 cm. Late-season injections (June) emphasized greater depths: 5, 40, 60, 80, and 120 cm. Pulsing in June at 5 cm was used to take advantage of a 7-mm rainstorm that re-wetted surface soils. Each pulse treatment (e.g., pulsing at 10 cm depth) was conducted within one day of each other in each plant community to minimize climatic differences among treatment levels. All treatments were applied within one week of each other. In each pulsed plot, 413, 1.25 cm-diameter holes spaced 13 cm apart were drilled, either by hand or with an electric hammerdrill fitted with a steel rod, to the assigned treatment depth resulting in an evenly spaced injection grid covering the entire 7m² plot and representing 4.6% of total plot surface. In each hole (pulse point), 1 ml 70% D₂O followed by 2 ml tap water were injected using a custom-made needle (16 gauge, regular width hypodermic tubing; Vita Needle, Needham, MA, USA) with an attached 2-ml syringe. The tap water injected after the tracer was intended to 1) expel all tracer from syringe and needle, 2) push tracer into the soil surrounding the base of the hole, and 3) prevent tracer contamination as the needle was removed from the hole. Holes were filled with soil when needles were removed. The resulting 3 ml of combined water and tracer injected into each point added up to 1242 ml of water per plot (0.05% of total annual precipitation) and was not expected to affect plant growth (Kulmatiski et al., 2010).

With the exception of samples from the plots receiving tracer at 120 cm depths, non-transpiring, suberized plant tissues that retain mean isotopic signature of source water (Flanagan and Ehleringer, 1991) were collected for isotopic composition analysis
one day following tracer injection. Plants from the 120 cm depth plots were collected two
days following injection to allow time for sapflow to bring the tracer to the surface
(Kulmatiski et al., 2010). Paired plant samples were collected from control plots that
received no deuterium or tap water.

To estimate lateral root activity, sampling was stratified by distance from the
center of the plot (0-100, 100-150, 150-200, 200-250 cm). Because the pulse area was 3
m in diameter, only the first two sampling distances were within the pulse area. One to
six samples (depending on available biomass) composited by species were collected at all
four distances in and around each plot. All sampling tools (hands, trowels, clippers, and
steel rod for filling samples into vials) were moved 20 m away from the transect and
rinsed with tap water between samples. For each sample, enough plant biomass was
collected to fill the bottom 12 cm of borosilicate test tubes (19-mm outer diameter). Test
tubes were immediately sealed with Parafilm and placed on ice in the field to prevent
sample fractionation. Leaf-level stomatal conductance indicated that by mid-June, B.
tectorum had stopped transpiring; therefore, no plant tissue was collected for that species
during the second pulsing period. Samples were transported to a freezer within four
hours, and kept frozen until extraction. Within one week after pulsing, soil pits were dug
within pulsed and control plots, and soil samples were collected at 10 cm increments to
120 cm. Additional samples were removed at 5 cm increments near the injection depth to
confirm the location of the pulse. In control plots, soil samples were taken at 20 cm
increments.

Plant and soil water was extracted using cryogenic vacuum distillation using
custom-made heating and cooling blocks that accommodated our 19 mm sample tubes (as
in Vendramini and Sternberg, 2007) at Utah State University. Extracted plant and soil water samples were shipped to the University of Alaska Anchorage where they were stored at 4º C until analyzed on a Picarro water isotope analyzer L2120-i for the determination of deuterium to hydrogen ratios. All isotope values are expressed in delta notation (δ) as the D / H ratio relative to a standard (Vienna standard mean ocean water).

For clarification, \[ \delta = \frac{R_{sa} - R_{std}}{R_{std}} \times 1000 \] expressed as “parts per mil” or “‰”, where R = the ratio of heavy to light isotope, sa = sample, and std = standard and \( R_{std} \approx 1/6420 \).

Tracer absorption was measured as deuterium excess as follows \( \delta_e = 8 \times \delta^{18}O + 10 \). Deuterium excess was used to account for any enriched δD values that reflected natural (i.e., evaporative) and not tracer enrichment.

To account for tracer dilution differences associated with differently sized plants and differently sized rooting zones, tracer concentrations were converted to proportional tracer uptake as a function of soil depth. This allows a direct comparison of root activity among different species (Kulmatiski et al., 2010). Tracer uptake for each species was normalized by depth or distance, as appropriate, for the analyses using \[ \frac{S_n - C}{\Sigma_{n=1}^{l}(S_n - C)} \], where \( S_n \) is the mean δD value of samples from treatment level \( n \) (e.g. 5 cm depth), and \( C \) is the mean δD value of control samples for that species (e.g. B. tectorum) (Bishop and Dambrine, 1995; Kulmatiski et al., 2010).

**Soil water availability**

On 23 May 2009, 3 m-deep soil pits were dug in the non-native and native fields with an excavator. Pits were located 10 m from the plot transects and between the parallel
transects in each field closest to the field boundary. Bulk soil samples were collected at 15 cm interval from the soil surface to 120 cm, every 30 cm from 120-210 cm, and every 50 cm from 210-300 cm. On June 12 2009, 100 cm soil pits were dug in the native and non-native fields, and bulk soil samples were collected at 15 cm intervals from the soil surface down. Bulk soil samples were sealed in plastic bags, weighed in the laboratory, then dried at 105°C until constant weight. Oven-dried soils were re-weighed and passed through a 2-mm mesh sieve. Rock and root (> 2 mm) mass was subtracted from wet and dry soil mass and percent moisture was determined as total water mass loss divided by dry mass of fine-soil material. Gravimetric soil water content was converted to volumetric soil water content using previously estimated bulk density (Kulmatiski et al., 2006a). Volumetric water content results indicated that considerable soil water moisture had been lost from the top 100 cm of the soil surface by the time the two communities were sampled. It was assumed that the soil profile was at field capacity at snowmelt. Volumetric water content at field capacity was then set to the maximum value of water content recorded in each community in May, or 22% and 23% in the non-native and the native community, respectively. Estimates of soil water storage and soil water loss presented below were calculated using these numbers as initial soil water content at the beginning of the growing season.

Fine-root biomass concentration was determined as the mass of oven-dried fine roots divided by the oven-dried mass of fine-soil material. The remaining sieved soil was kept to derive soil moisture characteristic curves using a Decagon Devices WP4T water potential meter (Decagon Devices, Pullman, WA, USA) and obtain a gradient of soil water potential for the soil profile to be correlated with measurements of volumetric
water content in the field. Soil water was considered to be plant available above permanent wilting point (i.e., water potential \((\psi) > -3\) MPa (Sauer et al., 1984; Kulmatiski et al., 2010)

**Water budget and evapotranspiration**

Species-specific transpiration was estimated using a modified Penman-Monteith approach calculated on a daily basis. More specifically, the Penman-Monteith approach as described by Allen et al. (1998) describes the energy and water balance as follows:

\[
\lambda ET = \frac{\Delta (R_n - G) + \rho_a c_p (e_s - e_a) r_s}{\Delta + \gamma (1 + \frac{r_s}{r_a})}
\]

where \(\lambda ET\) is evapotranspiration expressed as mm day\(^{-1}\), \(R_n\) is net radiation at the vegetation surface (MJ m\(^{-2}\) day\(^{-1}\)), \(G\) is soil heat flux density (MJ m\(^{-2}\) day\(^{-1}\)), \((e_s - e_a)\) is the vapor pressure deficit of the air (kPa), \(\rho_a\) is the mean air density at constant pressure (1.293 kg m\(^{-3}\)), \(c_p\) is the specific heat of the air at constant pressure (1.013 \(10^3\) MJ kg\(^{-1}\)C\(^{-1}\)), \(\Delta\) is the slope of saturation vapor pressure versus temperature curve (kPa °C\(^{-1}\)), \(\gamma\) is psychrometric constant (0.067 kPa °C\(^{-1}\)), \(r_s\) is bulk surface resistance (s m\(^{-1}\)), and \(r_a\) is bulk aerodynamic resistance (s m\(^{-1}\)). Hourly weather data including temperature, dew point temperature, wind speed, and solar radiation were collected from a nearby weather station in Malott, WA (48° 17’N, 119° 42’W; Washington Agricultural Weather Network).

Plant heights were needed to estimate \(r_a\), and were estimated from measurements of 10 individuals in each of nine different fields from across the study site. Stomatal
conductance measurements were needed to estimate \( r_s \) and were measured on the focal species throughout the day from May to July with a solid-state leaf porometer (SC-1; Decagon Devices, Pullman, WA, USA). All species were measured three times within 30 minute intervals (to control for variability in the environmental conditions driving conductance), resulting in 106 conductance datasets with three to six replicate measurements per species (3310 total measurements). Preliminary plotting of the data suggested that stomatal conductance followed a Gaussian distribution over the course of the day, where conductance is at a minimum during hours ranging from sunset to sunrise and reaching a maximum during mid-day (when field measurements were taken). Recent data suggested that significant night-time stomatal conductance occurred in a broad range of annual and perennial grasses, shrubs and trees species (Snyder et al., 2003; Caird et al., 2007; Dawson et al., 2007; Novick et al., 2009). Conductance measurements were aggregated over consecutive days when measurements were taken at similar time to produce larger datasets. A quadratic curve was then fitted to the larger datasets with the intercept set the minimum observed conductance value. A weighed daily value of stomatal conductance \((g_s)\) was obtained from the average of function over the course of the day to account for the decrease in conductance for the period of time when stomatal conductance was not measured.

Leaf area index (LAI) was estimated from the surface area sum of 10, 1 m x 1 m plots of photosynthetically active vegetation collected in each field before and after each pulse event for a total of 80 plots (10 quadrats x 4 time periods x 2 plant communities). Plots were located every 5 m along a 200-m transect line parallel and between the plot transects in each field. Each plot within a plant community was randomly assigned a
collection period. Bare ground and plant height were first estimated and then all
aboveground vegetation within the quadrat was clipped and collected by species.
Collected vegetation was kept frozen until it could be separated into green only material
and scanned using a LI-COR leaf area meter LI-3000A (LI-COR Biosciences, Lincoln,
NE, USA). LAI was calculated as the surface sum of all green vegetation collected in
each quadrat divided by one m². The single-sided leaf area of grasses was doubled to
account for the presence of both adaxial and abaxial stomates, which was not observed on
forbs and shrubs.

Transpiration was only estimated for the dominant species on which the pulse
experiment was focused and for which stomatal conductance was measured. The focal
species measured represented 56 % and 92 % of the total LAI in the non-native and the
native community, respectively (presented below). Total species transpiration estimates
were extrapolated to community-scale estimates by multiplying total transpiration by 1
over the proportion of leaf area sampled in each community or 1.78 (1/0.56) for the non-
native and 1.08 (1/0.92) for the native community. Estimated community-level
transpiration was used to calculate each community’s water budget.

Statistical Analysis

The effects of community type (native or non-native) and species on proportional
tracer uptake were assessed using a split-split-plot design and generalized linear mixed
model with a beta distribution and logit link. A beta distribution was used to
accommodate distributional properties of proportion data, notably skewness,
heterogeneity of variance, and range constraints. Community- and species-level patterns
of tracer uptake by depth (i.e. 10, 20, 30, 50, and 90 cm for May) were determined using community as a whole plot factor, distance as sub-plot factor, and species within community as sub-sub-plot factor. Random effects included distance(community) and depth*distance(community). Community level patterns of tracer uptake by distance (i.e. 100, 150, 200, and 250 cm) were determined using community as a whole plot factor, depth as a sub-plot factor, and species within community as a sub-sub-plot factor. Random effects included depth(community) and distance*depth(community). Only distances of 100 cm and 150 cm were used as replicates for the depth analysis because uptake in pulsed areas was different than uptake outside pulsed areas (200 cm and 250 cm; presented below). All depths (i.e. 10, 20, 30, 50, and 90 cm) were used as replicates for the distance analysis. Due to different pulsing depths, May and June data were analyzed as separate datasets. For June, statistical analyses were conducted with and without the 5 cm depth as soil moisture at that depth in June is not typically plant available. To make post-hoc pairwise mean comparisons, the MULTTEST procedure using the false discovery rate method was used to adjust P-values for type I error.

Differences in leaf area between community types and collection date were assessed with a two-way factorial analysis of variance (ANOVA) in a completely randomized design. To meet assumptions of homogeneity and variance, leaf area was log transformed. Differences in community and species level patterns of stomatal conductance and transpiration by month were assessed with a two-way factorial analysis of variance (ANOVA) in a completely randomized design with stomatal conductance and transpiration log transformed as well.
All analyses were computed using the GLIMMIX procedures in SAS/STAT for Windows, Release 9.2 (SAS Institute Inc., Cary, North Carolina). Fixed effects are only discussed in text when interactions are not significant. Statistical significance was considered at \( p < 0.05 \). Actual \( p \) values are reported; means ± 1 SE are presented unless otherwise stated.

A standard equivalency test was performed, when applicable, to determine differences in amount of water transpired by soil layer. Errors were propagated using standard errors associated with tracer uptake proportion and transpiration estimates. Results were declared different from each other if they differed by more than two times the calculated error.
RESULTS

*Tracer detection and uptake*

In total, 767 plant samples from experimental plots and 72 from control plots were extracted and analyzed for isotope ratios. From the experimental plots, 376 samples (49%) demonstrated isotope ratios that were two SDs or more above the control mean of -195.05 (± 50.78 SD) [i.e., received tracer as in Kulmatiski et al. (2010); Table C-I, Table C-II]. Of the 72 control plant samples, only one (0.01%) demonstrated an isotope ratio that was two SDs above the species control mean (Figure C-1, Figure C-2, Figure C-3).

A total of 134 soil samples from experimental plots and 40 from control plots were taken one week following tracer injection and these samples showed clear differences in tracer concentration with depth among the treatments though there was some redistribution of tracer 10 cm in either direction (Figure 2 and Figure 3). Mean deuterium concentration of soil samples collected from control plots was 139.56 ppt ± 37.74 SD (range -233.67 to -47.84). Mean deuterium concentration of soil samples collected within 10 cm of target pulse depth in treatment plots was 4607.36 ppt ± 7486.04 SD (range -163.13 ppt to 32963.43).

In May, tracer uptake by community type (native and non-native) differed with depth ($F_{4,10} = 7.69, p = 0.0042$) as a result of higher native than non-native uptake at 50 cm and higher non-native than native uptake at 10 cm (Figure 4). For native plants, the proportion of tracer uptake was greatest at 10 cm (Figure 4) followed by at uptake at 50 cm. The proportion of tracer uptake was smallest at 20 cm, 30 cm, and 90 cm (Figure 4).
Figure 2. Deuterium concentration in pulsed and control plots in native and non-native communities in May 2009, Winthrop, Washington.

Figure 3. Deuterium concentration in pulsed and control plots in native and non-native communities in June 2009, Winthrop, Washington.
For non-native plants, the proportion of tracer uptake was also greatest at 10 cm (Figure 4). There were no differences in the proportion of tracer uptake at 20 cm, 30 cm, and 50 cm; uptake was lowest at 90 cm (Figure 4).

In May, tracer uptake by species also differed with depth ($F_{20, 25} = 3.82, p < 0.001$). In the native community, *P. spicata* and *Lupinus* had more than 50% of their tracer uptake at 10 cm, more than at any other depth (Figure 5). *B. sagittata* had only 18% of its tracer uptake at 10 cm and its uptake at 10 cm was not different from uptake at the other depths (Figure 5). The proportion of tracer uptake for tap-tooted *B. sagittata* was higher than uptake for *P. spicata* at 50 cm (Figure 5).

![Graph showing proportion of tracer uptake by depth in May 2009 in native and non-native communities.](image)

**Figure 4.** Proportion of tracer uptake by depth in May 2009 in native and non-native communities, (upper row) showing differences across depths within each community and (lower row) showing differences between communities within each depth ($n = 2$). Different letters indicate significant differences at $p < 0.05$. 
In May, in the non-native community, all non-natives, except *S. loeselii*, had more than 50% of their tracer uptake at 10 cm, more than at any other depth (Figure 6). *S. loeselii* had only 38% of its tracer uptake at 10 cm and uptake at 90 cm was the lowest (Figure 6). Tracer uptake for *B. tectorum* was similar at 20 cm, 30 cm, and 50 cm, but uptake at 90 cm was lower than at 20 cm and 30 cm (Figure 6). Tracer uptake for *C. diffusa* was similar at 30 cm, 50 cm, and 90 cm, but uptake at 90 cm was lower than at 20 cm (Figure 6). Tracer uptake for *T. dubius* was similar at 20 cm, 30 cm, and 50 cm, but uptake at 90 cm was lower than at 20 cm (Figure 6).

In June, with the 5 cm pulse included, there was a difference in tracer uptake among species by depth ($F_{15, 19} = 4.44$, $p = 0.0014$). In the native community, both *Lupinus* and *P. spicata* had 77% and 47% of their tracer uptake at 5 cm, respectively,
while *B. sagittata* had only 15% of tracer uptake at 5 cm (Figure 7). Comparing species, *B. sagittata* had lower tracer uptake than *Lupinus* at 5 cm (Figure 7). In the non-native community, *S. loeselii* had 67% of its tracer uptake at 5 cm while *C. diffusa* and *T. dubius* had only 7% and 43% of their tracer uptake at 5 cm, respectively (Figure 8).

In June, when the 5 cm pulse was not included, community types differed in tracer uptake by depth (*F*₃,₆ = 5.53, *p* = 0.0306) due to higher tracer uptake at 80 cm in the non-native community than the native community (Figure 9), but there were no species differences by depth (*F*₁₁,₁₅ = 1.99, *p* = 0.11).

Lateral tracer uptake in both communities was higher in the tracer addition area than outside the addition area in both May (*F*₃,₂₄ = 39.91, *p* < 0.0001) and June (*F*₃,₂₄ = 33.45, *p* < 0.0001) (Figure 10 and Figure 11).

![Bar chart](image_url)

**Figure 6.** Proportion of non-native species tracer uptake by depth in May 2009, (upper row) showing differences across depths for each species and (lower row) showing differences across species within each depth (*n* = 2). Different letters indicate significant differences at *p* < 0.05.
Figure 7. Proportion of native species tracer uptake by depth in June 2009 when 5 cm pulse is included, (upper row) showing differences across depths for each species and (lower row) showing differences across species within each depth ($n=2$). Different letters indicate significant differences at $p < 0.05$.

Figure 8. Proportion of non-native species tracer uptake by depth in June 2009 when 5 cm pulse is included, (upper row) showing differences across depths for each species and (lower row) showing differences across species within each depth ($n=2$). Different letters indicate significant differences at $p < 0.05$. 
Figure 9. Proportion of tracer uptake by depth in June 2009 in native and non-native communities when 5 cm pulse is excluded, (upper row) showing differences across depths in each community and (lower row) showing differences across communities within each depth ($n =2$). Different letters indicate significant differences at $p < 0.05$.

Figure 10. Proportion of tracer uptake by distance in May 2009, showing differences across distance ($n =5$). Different letters indicate significant differences at $p < 0.05$. 
Figure 11. Proportion of tracer uptake by distance in June 2009, showing differences across distance (n = 5). Different letters indicate significant differences at $p < 0.05$.

Soil water availability

In May, based on a pre-defined cutoff, soil water at the pulsed depths in both native and non-native communities was plant available (e.g., $\Psi > -3$ MPa) (Figure 12). Volumetric water content in the native community was higher than in the non-native community from the surface to 100 cm in May but the non-native community had higher soil moisture content from 180 cm down (Figure 13). The native soil profile had more moisture stored in the first 105 cm than the non-native soil profile in May (121.62 mm and 96.37 mm, respectively). In June, soil water at the pulsed depths in both native and non-native communities was plant available (Figure 14) except at 5 cm. The bulk soil samples used to assess volumetric water content and soil water potential collection were collected before the precipitation event that rewetted the soil surface before the 5 cm pulse. By June, the native community had less moisture stored in the first 105 cm than
Figure 12. Soil water potential determined from bulk soil samples collected in native and non-native soil profiles in May 2009; available water is at $\Psi > -3$ MPa.

Figure 13. Volumetric water content in native and non-native soil profiles from soil pits dug in May 2009, Winthrop, Washington.
the non-native community (39.01 mm and 49.17 mm, respectively; Figure 15). Overall, the native soil profile lost more moisture than the non-native soil profile (109.51 mm and 99.38 mm, respectively).

Leaf area

The six species with the most leaf area in the native community in descending order were *P. spicata*, *B. sagittata*, *Lupinus*, *P. tridentata*, *Calochortus macrocorpus* Douglas, and *Achillea millefolium* L. (Table I). The six species with the most leaf area in the non-native community were *B. tectorum*, *Hesperostipa comata* Trin. & Rupr (a common native bunch grass that readily colonizes invaded communities), *S. loeselii*, *T. dubius*, *Poa bulbosa* L. and *C. diffusa* (Table I). Leaf area index varied between communities by collection time ($F_{3,72} = 3.83$, $p = 0.01$). The native community had higher LAI than the non-native community except on 25 May, when they had comparable LAI (Figure 15). In the native community, there were no differences in LAI between collection times (Figure 16). In the non-native community, however, LAI on 15 May and 25 May was higher than LAI on 15 June (Figure 16). Together, the species on which the pulse was focused accounted for 92% of the total LAI in the native community and 56% of the total LAI in the non-native community.
Figure 14. Soil water potential determined from bulk soil collected in native and non-native soil profiles in June 2009; available water is at $\Psi > -3$ MPa.

Figure 15. Volumetric water content in native and non-native soil profiles from soil pits dug in June 2009, Winthrop, Washington.
Table I. Leaf area index (± SE) of the dominant native and non-native species in the native and non-native communities measured four times throughout the growing season, 2009, Winthrop Washington (n =4).

<table>
<thead>
<tr>
<th>Native species</th>
<th>Non-native species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudoroegneria spicata</em></td>
<td><em>Bromus tectorum</em></td>
</tr>
<tr>
<td>0.34 ± 0.03</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td><em>Balsamorhiza sagittata</em></td>
<td><em>Hesperostipa comata</em></td>
</tr>
<tr>
<td>0.30 ± 0.04</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td><em>Lupinus</em> spp</td>
<td><em>Sisymbrium loeselii</em></td>
</tr>
<tr>
<td>0.06 ± 0.01</td>
<td>0.03 ± 0.004</td>
</tr>
<tr>
<td><em>Purshia tridentata</em></td>
<td><em>Tragopogon dubius</em></td>
</tr>
<tr>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.004</td>
</tr>
<tr>
<td><em>Calochortus macrocarpus</em></td>
<td><em>Poa bulbosa</em></td>
</tr>
<tr>
<td>0.004 ± 0.004</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td><em>Centaurea diffusa</em></td>
</tr>
<tr>
<td>0.003 ± 0.003</td>
<td>0.01 ± 0.006</td>
</tr>
</tbody>
</table>

Figure 16. Leaf area index (± SE) in native and non-native communities measured four times throughout the growing season, 2009, Winthrop, Washington (n =7-12). Letters indicate significant differences (top row) between communities at each collection time (lower row) within community across collection times.
Stomatal conductance

Stomatal conductance did not differ between the native and non-native communities \((F_{1,3290} = 0.13, p = 0.71)\), but did differ between months for each community. Conductance differed in the two communities across month \((F_{2,3290} = 15.68, p < 0.001)\). In both communities, conductance was highest in May, followed by June and lowest in July (Figure 17); the drop in July was greatest in the native community (Figure 17). Stomatal conductance for species also differed by month \((F_{9,3290} = 10.70, p < 0.0001)\). For all species in the native community, conductance declined each month, across the season (Figure B.1). In May and June, conductance for \textit{B. sagittata} was higher than the other species, but by July all species were equal (Figure 18).

![Figure 17](image-url).

Figure 17. Stomatal conductance (mmol m\(^{-2}\)s\(^{-1}\) ± SE) of focal species measured throughout the 2009 growing season. Letters indicate significant differences within a community across months; \((p < 0.05 \text{ (n=260-959)})\).
Figure 18. Stomatal conductance (mmol.m\(^{-2}\).s\(^{-1}\) ± SE) of focal native species measured throughout the 2009 growing season. Letters indicate significant differences within a month across species (\(p < 0.05; n = 80-240\)).

In the non-native community overall, conductance declined for each species across months (Figure B.2). Conductance was highest for \(S.\) \(loeselii\) and lowest for \(B.\) \(tectorum\) in May and June (Figure 19). By July, there were no differences in species stomatal conductance (Figure 19).

**Transpiration**

Transpiration was higher in the native than in the non-native community in June and July, but not in May (\(F_{2, 593} = 93.51, p < 0.0001\)) (Figure 20). In the native community, transpiration was lowest in May and highest in June (Figure 20). In the non-native community, transpiration was highest in May, followed by June, and lowest in July (Figure 20). There were also some species differences in transpiration across months (\(F_{10, 593} = 55.87, p < 0.0001\)). In the native community, transpiration for \(B.\) \(sagittata\) was
highest in May, followed by June and lowest in July (Figure 21). *P. spicata* maintained steady rates of transpiration in May, June, and July while *Lupinus* transpiration rates were lower in May than in June and July (Figure 21). Moreover, *B. sagittata* and *P. spicata* had similar transpiration rates, but were both higher than *Lupinus* in May, June, and July (Figure 21).

In May, *B. tectorum* and *S. loeselii* transpired the most water while *C. diffusa* and *T. dubius* had similar transpiration rates (Figure 22). In June, *S. loeselii* had the highest transpiration rates, followed by *C. diffusa*; *T. dubius* had the lowest transpiration rates (Figure 22). By July, there were no species differences (Figure 22).

![Figure 19](image_url)

**Figure 19.** Stomatal conductance (mmol.m$^{-2}$.s$^{-1}$ ± SE) of focal non-native species measured throughout the 2009 growing season. Letters indicate significant differences within a month across species (($p < 0.05$; $n=80-240$).
Figure 20. Mean daily transpiration in May, June, and July 2009 in native and non-native communities, showing (upper row) differences across months within each community and (lower row) differences between communities within each month ($n = 30-31$). Different letters indicate significant differences at $p < 0.05$

Figure 21. Mean daily transpiration in May, June, and July 2009 for native species, showing (upper row) differences across months for each species and (lower row) differences between species within each month ($n = 30-31$). Different letters indicate significant differences at $p < 0.05$. 
Figure 22. Mean daily transpiration in May, June, and July 2009 for non-native species, showing (upper row) differences across months for each species and (lower row) differences between species within each month ($n = 30-31$). Different letters indicate significant differences at $p < 0.05$.

In May, transpiration differed between communities by soil layers, as a result of higher transpiration in the native community than in the non-native community from the 50 cm soil layer (Figure 23). The native and non-native communities otherwise moved similar amount of soil moisture out of the other pulsed depths (Figure 23). The native community transpired more water from the first 10 cm of the soil profile than from any other pulsed depths, except at 50 cm (Figure 23). Transpiration from the 50 cm soil layer was also comparable to transpiration from the 30 cm soil layer but higher than at 20 cm and 90 cm (Figure 23). The non-native community transpired the most water from the first 10 cm of the soil and relied the least on soil moisture at 50 cm and 90 cm (Figure 23). There were also some differences in species transpiration by soil layers in May. In the native community, $B. sagittata$ and $P. spicata$ transpired more water than $Lupinus$ did at 10 cm and 20 cm, but all three moved the same amount of moisture from the 90 cm
soil layer (Figure 24). *P. spicata* transpired more water from 30 cm than *Lupinus* while *B. sagittata* transpired more water than *Lupinus* and *P. spicata* at 50 cm (Figure 24). In the non-native community, *B. tectorum* transpired more soil water from 10 cm and 20 cm than all the other non-native species, but as much as *S. loeselii* from the 30 cm soil layer (Figure 25). *S. loeselii* transpired more water from 50 cm than *C. diffusa* and *T. dubius* while *C. diffusa* transpired the most water from the 90 cm soil layer (Figure 25).

In June, transpiration was higher in the native than in the non-native community at every soil layer pulsed except at 80 cm where both communities transpired similar amount of soil water (Figure 26). In the native community, *B. sagittata* transpired more soil water than *Lupinus* and *P. spicata* from 60 and 120 cm, but all three species

![Transpiration graph](image)

**Figure 23.** Total monthly transpiration by depth in May 2009 in native and non-native communities, showing differences (lower row) within depths between communities and (upper row) differences across depths in each community (n = 3-4). Different letters indicate significant differences based on standard equivalency test.
transpired similar amount of soil water from 80 cm (Figure 27). At 40 cm, *B. sagittata* and *P. spicata* transpired more soil water than Lupinus but at 5 cm, *P. spicata* transpired more soil water than *B. sagittata* (Figure 27). In the non-native community, *S. loeselii* transpired the most soil water from 5 cm and 60 cm, but similar amount of soil water than *C. diffusa* at 80 cm (Figure 28). There were no species differences in transpiration at 40 cm and 120 cm (Figure 28).

Over the course of the summer, the native and non-native community each transpired 132 mm and 113 mm of soil water, respectively. Total soil water loss starting from snowmelt until July amounted to 180 mm and 172 mm of water in the native and non-native community, respectively (Table II).

![Figure 24. Total monthly transpiration by depth in May 2009 for native species, showing differences within depths between species (*n* =2). Different letters indicate significant differences based on standard equivalency test.](image)
Figure 25. Total monthly transpiration by depth in May 2009 for non-native species, showing differences within depths between species (n =2). Different letters indicate significant differences based on standard equivalency test.

Figure 26. Total monthly transpiration by depth in June 2009 in native and non-native communities, showing differences (lower row) within depths between communities and (upper row) differences across depths in each community (n =3-4). Different letters indicate significant differences based on standard equivalency test.
Figure 27. Total monthly transpiration by depth in June 2009 for native species, showing differences within depths between species \((n = 2)\). Different letters indicate significant differences based on standard equivalency test.

Figure 28. Total monthly transpiration by depth in June 2009 for non-native species, showing differences within depths between species \((n = 2)\). Different letters indicate significant differences based on standard equivalency test.
Table II. Summary of soil water loss and community-level transpiration (standardized by LAI) in native and non-native communities in May and June 2009 in Winthrop, WA

<table>
<thead>
<tr>
<th></th>
<th>Soil water loss (mm)</th>
<th>Transpiration (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>Native</td>
<td>26.90</td>
<td>82.61</td>
</tr>
<tr>
<td>Non-native</td>
<td>52.18</td>
<td>47.20</td>
</tr>
</tbody>
</table>
DISCUSSION

Native and non-native communities differed in the location and timing of water use in a way that is consistent with the presence of two alternative state plant communities. Non-native plants used shallow soil water early in the growing season and deep soil water late in the growing season. Native plant communities rapidly extracted water from throughout the soil profile in the middle of the growing season. These patterns may explain the presence of alternative state communities because early-season shallow water use in non-native communities is likely to inhibit native plant establishment. At the same time rapid mid-season water use by native communities is likely to prevent early-season non-native plants from completing their life cycles in native communities. Finally, late-season deep soil water use by non-native is likely to further inhibit establishment by native plants in non-native communities. Results supported findings suggested in a previous study, but provided quantitative support for not only vertical patterns of uptake but also horizontal patterns of uptake as well as quantified estimates of transpiration. More specifically, all plants relied on soil water beneath their crowns but all plants also accessed water at least 1m away from the stem – a distance greater than crown diameter for any species. Horizontal foraging did not differ between native and non-native plants.

Vertical tracer uptake

The switch from shallow tracer uptake in May to deep tracer uptake in June in the non-native community was the result of changes in community composition and
phenological differences between species. All non-native species with the exception of *S. loeselii* had high tracer uptake at 10 cm. *B. tectorum* was the dominant species in the non-native community in May and was responsible for most of the uptake at 10 cm during that month. The higher tracer uptake observed in the non-native community early on is supported by previous findings of higher fine root biomass in the non-native communities in the top 15 cm of the soil profile (Kulmatiski *et al.*, 2006a). Moreover, non-native communities with high annual grass cover are expected to rapidly pull out soil moisture in shallow soil layers early on because of early phenology; these species become physiologically active at lower temperatures in early spring than native plants (Sheley and Petroff, 1999). We expected and found an increase in tracer uptake at deeper depths in the non-native community in June; this was driven by high tracer uptake of *C. diffusa* at 80 cm whose tap-root, confers an advantage that allows access to deep soil water late in the season (Enloe *et al.*, 2004; Kulmatiski *et al.*, 2006a).

In the native community, species relied on both shallow and deeper soil water from early on, resulting in the complete use of soil water (from both shallow and deep layers) driven again by differences between species in root distribution. While both tap-rooted *Lupinus* and *B. sagittata* had high tracer uptake at 50 cm in May, *P. spicata* mostly took up tracer at shallow depths resulting in the complete use of the soil profile to supply water. This strategy is common for species in plant communities that evolved together to fully and efficiently partition a limiting resource to allow coexistence (Parrish and Bazzaz, 1976; Carpinelli, 2000). Further evidence of this comes from higher root mass in the native community (Kulmatiski *et al.*, 2006a), suggesting that species in this community allocate biomass to capture water in the soil profile and invest in perennial
belowground structures that acquire resources as plants become active in the spring rather than build completely new structures each year (West, 1983; Jackson and Roy, 1986).

Although an important rain event allowed re-wetting of the soil surface in June, not all species were able to take advantage of that extra moisture. Tap-rooted non-native *C. diffusa* switched from using shallow to deep soil water in June and did not utilize the additional input of soil moisture from summer precipitation. Tap-rooted native *B. sagittata* also started using deep soil water early on and maintained this strategy throughout. Short term water availability such as summer precipitation can provide a respite from drought conditions to plants in semi-arid environments, but the efficiency of use of that short term moisture varies widely (Schwinning et al., 2004). Our results are similar to previous research indicating that deep-rooted plants may experience less water stress during the gap between precipitation events or might be slower than annual species to respond to precipitation pulses (Schwinning and Ehleringer, 2001; Ogle and Reynolds, 2004; Huxman et al., 2005).

*Lateral tracer uptake*

The bulk of lateral foraging for water was restricted to within 50 cm in both communities in May and June. Although we expected to see some species differences, mainly for annuals to have shorter lateral roots than perennials (Schenk, 2006), water uptake was restricted to the first 50 cm regardless of life history (annuals or perennials) or community. The annual grass, *B. tectorum*, had a comparable breadth of lateral water use space to the longer lived specie. Though data is scarce on lateral roots in the literature, *B. sagittata* was expected to have lateral roots longer than what we found (≈ 1m
long). This discrepancy might be from differences in techniques used; excavations and root mapping show presence of roots but do not correlate well to resource uptake. Overall, both native and non-native plants had a central core of roots that spread out to 50 cm, but they also occasionally sent out long runners over one meter in length.

Soil moisture dynamics

Overall, there is good corroboration between the volumetric soil water measurements and the tracer uptake from the two pulsing events. The two adjacent communities were chosen to minimize differences in soil texture, aspect, slope, elevation, drainage and heat loading. Land use history was the only variable that varied between the two plant communities (Kulmatiski et al., 2006a), and differences in community composition best explained the dynamics in soil moisture over the growing season. In May, soil water content was lower in the surface soil layers in the non-native soil profile consistent with our measurements of rapid shallow water use by winter active plants. Soil water content was also lower in the deeper soil layers in the native community suggesting that deep infiltration is less in these communities. This is also consistent with our measurements of greater water use by the native community. Changes in moisture content in the communities were most obvious in the first 15 cm and were consistent with the higher tracer uptake recorded in the non-native community in May at 10 cm, as well as supported by the greater fine root mass in the non-native soil in the first 15 cm (Kulmatiski et al., 2006a). The decrease in deep soil water content in the native soil profile in May is linked to the presence of deep-rooted shrubs (Purshia tridentata) that are photosynthetically active all year and are able to access deep moisture to support
transpiration during the summer. This drying out of deep soil moisture is still noticeable even after recharge from snowmelt. The patterns of soil water depletion and resulting lower soil moisture content in the native than in the non-native soil profile are consistent with the life histories of the dominant plants of these communities. Shallow rooted bunchgrasses, native tap-rooted forbs and woody shrubs with deep penetrating roots use water from the entire soil profile before the non-natives, in accordance with the extensive root systems from perennials evolved in a dry environment to maximize uptake of limited resources (Kulmatiski et al., 2006a). Soil water was plant-available at all pulsed depths in both communities in both May and June, except at 5 cm, but bulk soil samples were collected before the precipitation event.

Tracer injection effectiveness

Several pieces of information confirm the general effectiveness of our pulsing methods. First, there was very little redistribution even one week after pulsing; redistribution might have been even smaller had the soil coring been done the same day that the plant samples were collected. Second, little vertical movement of the tracer suggests that the injection technique is a better alternative to using natural abundance and allows for not just better resolution but also for detection of horizontal root activity. Moreover, the natural abundance approach is incapable of providing accurate data at depths greater 60 cm (due to lack of evaporative enrichment), where the pulse-chase technique still picked up many species differences in root activity. Third, there was little to no contamination observed; <1% of the control samples showed values that were 2 SD’s above the pulsed value. This, however, likely reflects a naturally occurring high
value caused by evaporative enrichment and not actual tracer contamination as the deuterium value was relatively low. In addition, the control soil samples showed distinct isotopic signatures compared to pulsed depths.

With some small improvements, the pulse chase method could provide more results. Increasing the number of pulsed plots would greatly improve statistical power and inference on the landscape. Sampling soils closer to the injection date, and repeated sampling over consecutive days would determine how labile the tracer is in the soil and allow documentation of hydraulic redistribution, an important mechanism in semi-arid soils that can alleviate drought conditions (Richards and Caldwell, 1987; Ryel et al., 2002). Lastly, a longer delay in sampling would allow enough time for the tracer to move through woody plants in plots where injection depths were deeper.

Leaf area, stomatal conductance and transpiration

Contrary to previous reports of plant invasions altering regional hydrology by drawing up large amount of moisture from the soil (Zavaleta, 2000; Glenn and Nagler, 2005; Huxman et al., 2005), here non-native plants used less water than native plants overall. Environmental drivers of ET were the same between communities; as a result ET was driven by differences in stomatal conductance, LAI, and phenology. In both communities, early season water use was primarily located in the shallow soil layers where nutrients are located (growth pool) (Ryel et al., 2008; Schenk, 2008; Ryel et al., 2010). Indeed, 95% and 99% of the soil water in the native and the non-native community, respectively, was transpired from shallow soil layers (upper 50 cm) in May.
As surface soil moisture decreased in June, plants relied more on deeper soil moisture (maintenance pool, not primarily associated with nutrients uptake). Native and non-native plants increased deep soil water use from 5% and 1% to 13% and 23%, respectively, obtained from 80 cm and deeper. Longer active and tap-rooted non-native plants, in particular, relied more on deep soil water to support their extended phenology and set seeds in late summer. In both communities, LAI proved to be a good indicator for estimating how much water will be taken up by a particular species. Partitioning of available water showed that the dominant species (B. sagittata and P. spicata in the native community and B. tectorum, and S. loeselii in the non-native community) had access to the largest water pools both in terms of absolute amount of water used and the gradient over which they used water in the soil profile. Estimates of transpiration (132 mm in native vs. 113 mm in non-native) were less than the net soil water loss estimated from measured volumetric soil water content. However, soil evaporation, deep drainage, and residual soil moisture were not accounted for but would have otherwise increased the losses registered from evapotranspiration calculations.

With LAI being the main driver of transpiration, dominant species extracted more soil moisture than the subordinate species. Tracer injection distinctly illustrated the spatial distribution and relative importance of different soil layers in supplying moisture for transpiration. Contrary to previous work, non-native plant invasion did not result in an accelerated use of a limiting resource. The emerging patterns of spatial and temporal partitioning of water provide additional support for what differences in natural abundance isotopes have suggested. Early season shallow water use combined with late season deep water use by non-native species may indeed prevent the establishment of
native species in abandoned agricultural fields and represents an important tool for land managers endeavoring to restore degraded rangelands.
CONCLUSIONS

Implications of the study

The objective of this study was to provide detailed measurements of plant water use space as previously available techniques lacked the resolution to directly measure resource uptake at specific locations in the soil profile. We also intended to investigate how the change in community composition resulting from invasions affected processes like water balance. Our results clearly demonstrate water partitioning at a finer scale than previous studies in semi-arid environments and illustrate species-specific soil profile exploitation of soil water and the proportional contribution of specific soil layers to a species water budget. Both communities relied heavily on the upper soil layers; 75% of the tracer uptake and the water transpired came from shallow soil layers rich in soil nutrients (>60 cm). Clear distinctions however emerged during the study due to differences in phenological timing and in rooting distributions of the dominant species in the two communities. Specifically, in May, tracer uptake in the non-native community was higher at 10 cm than in the native community and decreased monotonically at the remaining depths while uptake in the native community peaked again at 50 cm. In June, tracer uptake at 80 cm was higher in the non-native community than in the native community, a trend that reflects the onset of summer dormancy in the native community.

Our results echo past studies that have tried to elucidate the mechanisms of the persistence of these alternative stable state communities (Kulmatiski et al., 2006a). While disturbance frequently sets the stage for invasion (in the Methow Valley, these invaded plant communities occur on ex-arable lands), the successional trajectory of the
invaded plant communities does not follow customary trends (Kulmatiski, 2006).

Decades after initial colonization, non-native ruderal species are still being replaced by similar species. These annual-dominated plant communities preemptively decrease shallow soil moisture availability early in the growing season. Due to their delayed phenology, native seeds begin germination in a drier environment, often light limited from dense stands of early active non-native species (Coleman and Levine, 2007). Removal of litter would reduce seed bank density, improve light penetration and create a more suitable microclimate for establishment and germination of native plant seedlings (Dyer et al., 1996; Coleman and Levine, 2007). Though sowing native seeds might be more cost-effective in restoration settings, planting mature native plants with more developed root structures that are tall enough to escape light competition from a crowded plant canopy is a strategy more likely to succeed.

Farming has also resulted in the complete removal of the shrub component of many shrub steppe-ecosystems that are now solely colonized by invasive non-native herbaceous species whose phenological timing strongly differs from that of the original native vegetation. Because of their disproportionate effect on local hydrology, sagebrush and species of similar plant functional type have been referred to as a foundation species in the Intermountain West (Germino et al., 2004; Prevéy et al., 2010); their loss can have cascading effects in resource supply rates. The absence of this evergreen woody species whose yearlong photosynthetic activity translates into deep soil water use to maintain its perennating structures leaves a niche unfilled (Inouye, 2006). Longer active tap-rooted non-native forbs now have access to the untapped soil moisture late in the summer when native vegetation becomes dormant (Sperber, 2001). We saw a corresponding increase in
tracer uptake at 80 cm in the non-native community translating to deep soil water use later in the season. In the presence of shrubs, these tap-rooted forbs would be directly competing with them for that resource; bunchgrass roots typically do not reach that depth or are dormant at that time. The extended growth period of tap-rooted non-native forbs can even increase nutrient depletion in the next year (Enloe et al., 2004). Many restoration projects focus largely on creating a native herbaceous layer, but this approach risks leaving an unfilled niche and creating opportunities for late season tap-rooted forbs to establish. In the absence of external disturbance forces, the combination of mature perennial bunchgrasses and woody shrubs is one that is most likely able to resist conversion to non-native stands by completely using the available resources (Davis et al., 2000).

Non-native plants did not consistently out-perform native plants. Leaf-level stomatal conductance did not differ between communities; instead conductance in both communities decreased from May to July in response to soil moisture depletion and increased vapor pressure deficit. Earlier onset of senescence in the native community contributed to a sharper decrease in conductance in the native community than in the non-native community, as some late season tap-rooted non-native forbs were still able to access deeper soil moisture (80 cm pulse) at that time. Both transpiration estimates and volumetric soil water measurements indicated that more soil moisture was lost from the native community than from the non-native community. Collectively, the native plants had higher leaf area index as well as well-developed perennial rooting structures that were very efficient at drawing up soil moisture from the complete soil profile (Jackson and Roy, 1986). LAI was a good indicator of resource use as dominant species in both
communities used more water than subordinate species did. Our results suggest that direct competition for limiting water or the presence of “invasive traits” in non-native species may not be the causes of the maintenance of these two distinct communities. Instead, phenology and rooting distribution were most likely the main drivers responsible for incompatibility in spatial and temporal patterns of soil water depletion.

_Potential problems with study design_

In the Intermountain West, growing conditions become suitable for annual grasses like _B. tectorum_ long before native species (Sheley _et al._, 1998). The first pulsing did not start until all species were already active and missed the opportunity to measure the extent of soil profile used by these early annuals. The study could have also benefitted from multiple pulsing events as opposed to just the two done in May and June given limited time. Additional pulsing should be done at key phenologically important times of the year to capture early season water use, to document the senescence of the focal species (but continued activity of shrub) and finally, to illustrate which species benefit most from fall precipitation. Pulsing depths differed between May and June and prevented us from comparing soil profile utilization in both pulsing events. Future pulsing should be done at the same depths to allow better interpretation of the temporal partitioning of soil water.

Limited funding restricted replication in our study but increasing replication at the community level (and across different paired communities) would allow better insights into landscape trends, rather than just plot level inference. Furthermore, premature plant tissue collection from _P. tridentata_ did not allow adequate time for tracer uptake and no
pulse was registered in any of the shrub samples which were consequently dropped from further analysis. The highly variable density of *H. comata* in the non-native community made it difficult to include it in the study but this would have otherwise offered valuable insight into water partitioning between the non-native species and the only native bunchgrass that is successful in the non-native community.

*Future directions*

Future directions should focus on addressing some of the shortcomings of the study, mainly the inclusion of the woody vegetation layer as it is an essential component of shrub-steppe ecosystems in terms of accessing a resource that the herbaceous layers cannot. Soil samples from the pulsed plots indicated that the tracer is labile in the soil, and repeated sampling of the soil profile following injection can provide a precise mean of measuring the extent and breadth of hydraulic redistribution. The use of the tracer is able to identify changes occurring at specific locations in the soil profile and gives valuable information that can be used in building theoretical models to predict soil water dynamics and vegetation composition under expected future changes in climate. It could also be used to inform on and improve the timing and selection of materials for restoration planting. Repeated pulsing over several years can also show how root activity and plasticity might change under varying environmental conditions such as temperature, and precipitation. The tracer injection approach could be used in mixed plant communities where native and non-native species are able to coexist to ascertain the differences in root distribution and the timing of water uptake that allow such
coexistence. In general, competitive interactions are difficult to measure but tracer injection offers a quantifiable mean to measure these interactions.
REFERENCES


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Potts DL, Huxman TE, Cable JM, English NB, Ignace DD, Eilts JA, Mason MJ, Weltzin JF, Williams DG. 2006. Antecedent moisture and seasonal precipitation influence


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APPENDICES
APPENDIX A
Tracer uptake ANOVA tables
Table A-I. Community-and species-level pattern of tracer proportion uptake by depth using distances 100 and 150 cm as replicates in May 2009, Winthrop, Washington.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>community</td>
<td>1</td>
<td>10</td>
<td>0.00</td>
<td>0.9646</td>
</tr>
<tr>
<td>depth</td>
<td>4</td>
<td>10</td>
<td>59.30</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>community*depth</td>
<td>4</td>
<td>10</td>
<td>7.69</td>
<td>0.0042</td>
</tr>
<tr>
<td>species(community)</td>
<td>5</td>
<td>25</td>
<td>0.10</td>
<td>0.9903</td>
</tr>
<tr>
<td>depth*species(community)</td>
<td>20</td>
<td>25</td>
<td>3.82</td>
<td>0.0010</td>
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</table>

Table A-II. Community- and species-level pattern of tracer proportion uptake by depth using distances 100 and 150 cm as replicates in June 2009, Winthrop, Washington.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10</td>
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<tr>
<td>depth</td>
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<td>10</td>
<td>12.78</td>
<td>0.0006</td>
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<tr>
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<td>10</td>
<td>3.00</td>
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<td>4</td>
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<tr>
<td>depth*species(community)</td>
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<td>4.44</td>
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</table>

Table A-III. Community- and species-level pattern of tracer proportion uptake by distance using all depths as replicates in May 2009, Winthrop, Washington.

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<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>community</td>
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<td>8</td>
<td>1.00</td>
<td>0.3473</td>
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<tr>
<td>distance</td>
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<td>24</td>
<td>39.91</td>
<td>&lt;.0001</td>
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<tr>
<td>community*distance</td>
<td>3</td>
<td>24</td>
<td>0.57</td>
<td>0.6399</td>
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<tr>
<td>species(community)</td>
<td>4</td>
<td>64</td>
<td>0.71</td>
<td>0.5872</td>
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<tr>
<td>distance*species(community)</td>
<td>12</td>
<td>64</td>
<td>1.75</td>
<td>0.0765</td>
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</table>
Table A-IV. Community- and species-level pattern of tracer proportion uptake by distance using all depths as replicates in June 2009, Winthrop, Washington.

<table>
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<th>F Value</th>
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</thead>
<tbody>
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<td>0.26</td>
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<td>distance</td>
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<td>24</td>
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<td>24</td>
<td>0.96</td>
<td>0.4263</td>
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<td>60</td>
<td>0.11</td>
<td>0.9773</td>
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<tr>
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<td>12</td>
<td>60</td>
<td>1.73</td>
<td>0.0831</td>
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</tbody>
</table>

Table A-V. Community- and species-level pattern of tracer proportion uptake by depth (without 5 cm pulse) using distances 100 and 150 cm as replicates in May 2009, Winthrop, Washington.

<table>
<thead>
<tr>
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<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
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<tr>
<td>depth</td>
<td>3</td>
<td>6</td>
<td>12.50</td>
<td>0.0054</td>
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<td>community*depth</td>
<td>3</td>
<td>6</td>
<td>5.53</td>
<td>0.0366</td>
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<tr>
<td>species(community)</td>
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<td>15</td>
<td>0.88</td>
<td>0.4974</td>
</tr>
<tr>
<td>depth*species(community)</td>
<td>11</td>
<td>15</td>
<td>1.99</td>
<td>0.1080</td>
</tr>
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</table>

Table A-VI. Community- and species-level pattern of tracer proportion uptake by distance using all depths (except for 5 cm pulse) as replicates in June 2009, Winthrop, Washington.

<table>
<thead>
<tr>
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<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
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<td>6</td>
<td>0.09</td>
<td>0.7752</td>
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<td>distance</td>
<td>3</td>
<td>18</td>
<td>48.05</td>
<td>&lt;.0001</td>
</tr>
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<td>community*distance</td>
<td>3</td>
<td>18</td>
<td>0.85</td>
<td>0.4831</td>
</tr>
<tr>
<td>species(community)</td>
<td>4</td>
<td>44</td>
<td>0.22</td>
<td>0.9232</td>
</tr>
<tr>
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<td>12</td>
<td>44</td>
<td>3.49</td>
<td>0.0012</td>
</tr>
</tbody>
</table>
APPENDIX B
Leaf area index, stomatal conductance, and transpiration ANOVA tables
and stomatal conductance figures
Table B-I. Community level patterns for leaf area index across four collection dates in May and June 2009, Winthrop, Washington.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Den DF</th>
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</tr>
</thead>
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<td>community</td>
<td>1</td>
<td>72</td>
<td>77.92</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>time</td>
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<td>72</td>
<td>5.91</td>
<td>0.0012</td>
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<td>community*time</td>
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<td>72</td>
<td>3.83</td>
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</table>

Table B-II. Community- and species-level patterns for stomatal conductance from May to July 2009, Winthrop, Washington.

<table>
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<tbody>
<tr>
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<td>1</td>
<td>3290</td>
<td>0.13</td>
<td>0.7136</td>
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<tr>
<td>species(community)</td>
<td>5</td>
<td>3290</td>
<td>75.20</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>month</td>
<td>2</td>
<td>3290</td>
<td>235.70</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>community*month</td>
<td>2</td>
<td>3290</td>
<td>15.68</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Species(community)*month</td>
<td>9</td>
<td>3290</td>
<td>10.70</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Table B-III. Community- and species-level patterns for transpiration from May to July 2009, Winthrop, Washington.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num DF</th>
<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>community</td>
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<td>593</td>
<td>536.18</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>species(community)</td>
<td>5</td>
<td>593</td>
<td>146.87</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>month</td>
<td>2</td>
<td>593</td>
<td>30.37</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>community*month</td>
<td>2</td>
<td>593</td>
<td>93.31</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>species*month(community)</td>
<td>10</td>
<td>593</td>
<td>55.87</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Figure B.1. Stomatal conductance (mmol. m\(^{-2}\).s\(^{-1}\) ± SE) of focal native species measured throughout the 2009 growing season. Letters indicate significant differences within species across months; \((p < 0.05; n =80-240)\).

Figure B.2. Stomatal conductance (mmol.m\(^{-2}\).s\(^{-1}\) ± SE) of focal non-native species measured throughout the 2009 growing season. Letters indicate significant differences within species across months; \((p < 0.05; n =80-240)\).
APPENDIX C
Pulsing summary tables and figures, control treatment and pulsed samples figures, pulsed soil profile figures and transpiration figures
Table C-I. Summary of the samples from the experimental plots that registered a pulse (2 SD above the species control mean) in May and June 2009.

<table>
<thead>
<tr>
<th>Pulse depth</th>
<th>Native species</th>
<th>Non-native species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. sagittata</td>
<td>Lupinus spp</td>
</tr>
<tr>
<td>10 cm</td>
<td>6/12</td>
<td>7/13</td>
</tr>
<tr>
<td>20 cm</td>
<td>7/14</td>
<td>3/11</td>
</tr>
<tr>
<td>30 cm</td>
<td>5/15</td>
<td>2/15</td>
</tr>
<tr>
<td>50 cm</td>
<td>14/19</td>
<td>7/11</td>
</tr>
<tr>
<td>90 cm</td>
<td>6/14</td>
<td>3/9</td>
</tr>
<tr>
<td>5 cm</td>
<td>3/15</td>
<td>5/11</td>
</tr>
<tr>
<td>40 cm</td>
<td>4/11</td>
<td>2/9</td>
</tr>
<tr>
<td>60 cm</td>
<td>6/14</td>
<td>6/11</td>
</tr>
<tr>
<td>80 cm</td>
<td>1/15</td>
<td>1/12</td>
</tr>
<tr>
<td>120 cm</td>
<td>4/13</td>
<td>2/13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pulse depth</th>
<th>Native species</th>
<th>Non-native species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. spicata</td>
<td>B. tectorum</td>
</tr>
<tr>
<td>10 cm</td>
<td>15/20</td>
<td>15/18</td>
</tr>
<tr>
<td>20 cm</td>
<td>16/16</td>
<td>10/11</td>
</tr>
<tr>
<td>30 cm</td>
<td>11/18</td>
<td>10/13</td>
</tr>
<tr>
<td>50 cm</td>
<td>10/14</td>
<td>15/20</td>
</tr>
<tr>
<td>90 cm</td>
<td>3/15</td>
<td>1/12</td>
</tr>
<tr>
<td>5 cm</td>
<td>11/15</td>
<td>N/A</td>
</tr>
<tr>
<td>40 cm</td>
<td>14/19</td>
<td>N/A</td>
</tr>
<tr>
<td>60 cm</td>
<td>13/16</td>
<td>N/A</td>
</tr>
<tr>
<td>80 cm</td>
<td>7/18</td>
<td>N/A</td>
</tr>
<tr>
<td>120 cm</td>
<td>3/18</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Pulse depth</th>
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<th>Non-native species</th>
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</thead>
<tbody>
<tr>
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</tr>
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<td>6/9</td>
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<tr>
<td>120 cm</td>
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<td>4/10</td>
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</table>

Table C-II. Summary of the samples from the experimental plots that registered a pulse (2 SD above the species control mean) in May and June 2009.

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>Native species</th>
<th>Non-native species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. sagittata</td>
<td>Lupinus spp</td>
</tr>
<tr>
<td>0-100</td>
<td>14/16</td>
<td>8/13</td>
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<td>100-150</td>
<td>15/21</td>
<td>10/15</td>
</tr>
<tr>
<td>150-200</td>
<td>8/17</td>
<td>3/16</td>
</tr>
<tr>
<td>200-250</td>
<td>1/20</td>
<td>1/15</td>
</tr>
<tr>
<td>0-100</td>
<td>14/16</td>
<td>8/13</td>
</tr>
<tr>
<td>100-150</td>
<td>15/21</td>
<td>10/15</td>
</tr>
<tr>
<td>150-200</td>
<td>8/17</td>
<td>3/16</td>
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<tr>
<td>200-250</td>
<td>1/20</td>
<td>1/15</td>
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<td>100-150</td>
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<td>3/16</td>
<td>7/19</td>
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<td>200-250</td>
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<tr>
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<td>0/8</td>
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<table>
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<tr>
<th>Distance (cm)</th>
<th>Native species</th>
<th>Non-native species</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B. sagittata</td>
<td>Lupinus spp</td>
</tr>
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<td>100-150</td>
<td>18/20</td>
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</tr>
<tr>
<td>150-200</td>
<td>18/22</td>
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<tr>
<td>200-250</td>
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<td>N/A</td>
</tr>
<tr>
<td></td>
<td>15/22</td>
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<tr>
<td></td>
<td>0/15</td>
<td>N/A</td>
</tr>
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</table>
Figure C-1. Mean deuterium concentration ± 2SD of all plant samples from control plots (control, \( n = 72 \)) and all samples from treatment plots (treatment, \( n = 767 \)).

Figure C-2. Mean deuterium concentration ± 2 SD of native species in control plots (\( n = 7-12 \)).
Figure C-3. Mean deuterium concentration ± 2 SD of non-native species in control plots ($n = 7\text{-}12$).