Root niche partitioning among grasses, saplings, and trees measured using a tracer technique

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Abstract

Understanding the location and timing of soil resource use by plants is a fundamental aspect of plant coexistence and elemental cycling that remains poorly understood due to the difficulties of belowground research. To measure vertical niche partitioning by grasses, planted saplings, and trees in a mesic savanna, Kruger National Park, South Africa, deuterium oxide was injected into 102,000 points in 15, 154 m^2 plots that were randomly assigned to one of five depths (0-120 cm) and one of three time periods (November, February, and May) during the 2008/2009 growing season. Grasses demonstrated a consistent exponential decline in tracer uptake from 5 to 120 cm. Saplings and trees also relied on the shallowest soils when soil water was abundant, but switched to using deeper (30–60 cm) soil depths when shallow soil water was less available at the end of the season. Saplings established roots to at least 1 m depth by February and relied on deep roots to a greater extent than grasses or trees. Niche overlap varied through the growing season and was least when resources were scarce and greatest when resources were abundant. Results highlight the problems with inferring root activity from root biomass and provide a quantitative resolution for conflicting conclusions regarding the importance of deep root activity in the study system.

Keywords Deuterium, Injection, Root, Savanna, Tracer, Water-use.

Introduction

How trees and grasses coexist in savannas remains an important but unresolved question in ecology. There is some consensus that demographic, deterministic (i.e., niche partitioning), and stochastic (e.g., fire and herbivory) processes are each involved (Sankaran et al. 2005; Sankaran et al. 2004; Scholes and Archer 1997). While demographic and aboveground stochastic processes are fairly easily measured and modeled (Adler et al. 2010; Riginos 2009), deterministic belowground processes remain poorly understood because of the difficulties inherent in belowground research (Adler et al. 2010; McKane et al. 2002; Schenk 2008). Overcoming these difficulties can be expected to help not only resolve the role of niche partitioning in savanna structure and function, but can also be expected to have the added benefit of providing a critical link between community and ecosystem ecology (Casper et al. 2003; Newman et al. 2006; Scanlon and Albertson 2003).

Woody plants have long been observed to produce deeper roots than grasses, especially in temperate savannas (Albertson 1937; Schenk and Jackson 2002; Sperry 1935). In some systems, woody plants have also been observed to tap into aquifer water (Doody and Benyon 2010; Goldstein et al. 2008; Jackson et al. 1999; Penuelas and Filella 2003). Walter (1971) used similar observations to develop the two-layer hypothesis, which suggests that deep roots allow woody plants to escape competition from dense, shallow grass root mats (Scholes and Archer 1997; Walter 1971). This intuitively attractive hypothesis has been considered an important deterministic factor in tree and grass coexistence for over 40 years (Daly et al. 2000; Foley et al. 1996; Newman et al. 2006; Sankaran et al. 2005; Scholes and Archer 1997; Seyfried et al. 2005; Walter 1971; Weng and Luo 2008).

The two-layer hypothesis, however, remains poorly constrained (Brown and Archer 1999; Brown et al. 1998). Most estimates of root activity are inferred from measures of root biomass. The strength of this inference is limited because, for example, the large roots that dominate measurements of root biomass can be largely inactive (Chen 2004; Kulmatiski et al. 2010; Peek et al. 2005). Similarly, root biomass measurements are rarely precise enough to assess changes in root growth over time and temporal resource partitioning may also be important for plants (Kulmatiski et al. 2010). Where root activity has been measured, it has produced sometimes surprising results such as, streamside trees that do not use stream water, wide lateral foraging, and patterns of root activity that do not reflect patterns of root mass (Dawson and Ehleringer 1991; Kulmatiski et al. 2010; Peek et al. 2005).

While there is a clear need for direct measurements of resource partitioning by trees and grasses, there is perhaps an even greater need for direct measurements of resource use by saplings because tree establishment has been identified as a critical 'demographic bottleneck' in savannas (Archibald and Bond 2003; Moe et al. 2009; Sankaran et al. 2004; Staver et al. 2009). Even less is known about resource partitioning by saplings than by trees and grasses (Jurena and Archer 2003; Weltzin and McPherson 1997). Sapling growth is often assumed to be limited by competition with shallow grass root mats even though saplings with deeper roots may realize limited growth due to competition with adult trees (Dickie et al. 2007; Riginos 2009). Without direct measurements of resource use, it is difficult to know the role of intra- and interspecific competition for saplings.

Tracer techniques provide an important means for measuring root activity (Ogle et al. 2004; Rodriguez et al. 2007; Schwinning et al. 2002). Here we provide the second example of the use of a recently developed tracer technique, which allows depth-specific, species-specific

69 measurements of water uptake (Kulmatiski et al. 2010). The objective of this study was to
70 measure the timing and location of vertical soil water use of grasses, saplings, and trees in a
71 savanna to test if vertical or temporal partitioning provides a potential explanation for plant
72 coexistence. In contrast to a previous study that injected tracer into 1 m^2 plots (Kulmatiski et al.
73 2010), here we inject tracer into 154 m^2 plots with planted tree saplings to better sample tree root
74 systems and to measure water uptake in tree saplings of known age.
75
76 **Materials and Methods**
77 Study site
78 Research was conducted 4 km south of Pretoriuskop, Kruger National Park, South Africa (-25°
79 12' 27" N, 31° 16' 60" E; elevation 655 m). The site is a deciduous mesic subtropical savanna
80 (Archibald and Scholes 2007; Sankaran et al. 2005). Mean annual precipitation is 746 mm,
81 occurring primarily during the summer, November to May. Mean summer and winter
82 temperatures are 24 and 18° C, respectively (Anonymous 2010). Nearby watering holes dug in
83 low-laying areas demonstrate water table depths of 6 to 13 m.
84 The study site is dominated by the C4 grasses *Hyperthelia dissoluta* (Steud.) Hutch. and
85 *Setaria sphacelata* (Schumach.) Stapf and C.E. Hubb with *Hyparrhenia filipendula* (Hochst.)
86 Stapf, *Cenchrus ciliaris* (L.) Link and *Panicum maximum* (Jacq.) present. Dominant woody
87 plants include the trees *Terminalia sericea* (Burch. ex DC.) and *Sclerocarya birrea* (A.Rich.)
88 Hochst.; and the erect shrub *Dichrostachys cineria* subsp. *africana* (Brenan and Brummitt).
89 Leaf-out by trees is rather consistent each year, while grass green-up is more variable and
90 appears to be triggered by day length and soil moisture (Archibald and Scholes 2007).
91 *Sclerocarya birrea*, has a short, thick taproot that can grow to 2 m and has a mean leaf-out date

of 15 October (Archibold and Scholes 2007). *Terminalia sericea* holds dead leaves through most of the dormant season and leaf-out typically occurs one to three weeks after *S. birrea*, often before the first rains (Childes 1988). Root mass declines exponentially with less than 0.3 g root mass kg^{-1} soil below 1 m depth (Kulmatiski et al. 2010). Mid-season ground cover was 57 ± 12% grasses, 19 ± 10% trees, 8 ± 5% shrubs, and 6 ± 2% forbs [mean ± standard deviation (SD)].

Plot and sapling establishment

In October 2007, 15 experimental plots (7-m radius) were established in a 60 m x 60 m grid of a 4-ha area in the northwest corner of the Hlangwine animal exclosure. Plots were randomly assigned to one of five depth treatment levels and one of three sampling period treatment levels. To be clear, each depth x time treatment combination (e.g., 5 cm depth in November) was represented in one experimental plot. There was also one control plot assigned to each treated plot. In October 2007, *Acacia nigrescens* seeds were germinated in 1-L potting bags filled with field soil and six-month old *T sericea* saplings were purchased from a local nursery. In February 2008, in each treatment plot, six *A. nigrescens* and two *T. sericea* saplings were transplanted into 20-cm deep holes at the center of the plots and watered resulting in 120 saplings in 15 experimental plots. Saplings were not planted in control plots. Saplings were caged to prevent herbivory and trampling. Saplings grew for eight weeks in the field before beginning to senesce in April 2008. Saplings began leaf-out just prior to the November 2008 sampling period.

D_2O tracer experiment: the timing, location and relative extent of water use

Injections were performed during three sampling periods during the 2008/2009 growing season: 10-24 November, 9-16 February, and 4-14 May. During each sampling period, 70% D_2O was

injected to each of five depths where soil water was plant available. Plant available water was assumed to be present at depths where soil water potentials were greater than observations of midday leaf water potentials (i.e., -3 MPa; Kulmatiski unpublished data). These depths were 5, 10, 20, 30 and 50 cm in November; 5, 20, 30, 50 and 100 cm in February; and 5, 30, 60, 90 and 120 cm in May. Depths were selected that emphasized shallow water use but also provided inference across the soil profile. For example, during the November 2008 sampling period, soil water was available only in the top 50 cm of soil so all five pulse depths occurred in the top 50 cm of soil. In each plot, a 15 cm x 15 cm sampling grid was laid on the 154 m^2 circular plot (7-m radius) to locate 6,838 injection points. A plank system was erected to avoid trampling in the plot. At each grid point, a 10-mm wide pilot hole was drilled to the target depth, and 150 µl of 70% D_2O followed by 2 ml of tap water were injected using custom-made needles (16 gauge, regular width hypodermic tubing; Vita Needle, Needham, MA, USA) and syringes (Kulmatiski et al. 2010). Tap water was injected to push tracer water through the long injection needles and into the soil.

The 14.7 L of tracer plus rinse water added to each 154 m^2 plot represented 0.01% of annual precipitation but this amount of water would rapidly increase the soil water content of a soil volume surrounding the injection point until soil field capacity was attained. The 2.150 mL of injected water was estimated to occupy a soil volume of 9 cm^3 and 1% of the target soil volume. So, while the injection likely created a brief pulse of water around the injection point, it was not expected to increase plant growth. Furthermore, tracer was added only to soils where soil water was plant available, thereby reducing the likelihood of inducing water uptake where plants were not previously absorbing soil water.

Three to five replicate samples from two dominant grass species and a composite of the less-common grass species were collected from each of four sampling distances (0-2, 2-6, 6-7 and 7-8 m from the center of the plot) over two days for each of 5 soil depths in each of three sampling months. This sampling design should result in 1080 grass samples (i.e., n ~ 3 replicates x 3 'species' x 4 distances x 5 depths x 2 days x 3 months = 1080 grass samples); however, not all sample combinations were always available to be sampled (e.g., grass species b in the 0-2 m distance class). Sampling was stratified by distance to control for edge effects (i.e., diluted tracer concentrations from plants at the edge of the plot). Xylem flow rates of 1-5 m day^{-1} were used to determine when samples should be taken (Fravolini et al. 2005; Meinzer et al. 2006; Kulmatiski et al. 2010), and this assumption was tested by repeating vegetation sampling over several days.

More specifically, composite grass samples from 0-2 and 2-6 m were taken over four or five days to confirm that samples were taken when peak δD values occurred. All saplings were sampled one to three days following tracer injection. All trees within the plots were sampled and most trees between 7 and 15 m of the center of the plot were sampled. Trees in the plots were sampled two and three days following tracer injection while trees outside the plots were sampled a day later for every 1-2 meters away from the plot they were located. When possible, trees within the treated area were sampled repeatedly two to seven days following tracer injection. On average 53 tree samples were removed from each plot. For all plant types, samples removed from the 100 and 120 cm depth-pulse plots were removed one day later than samples from plots pulsed at shallower depths to allow time for xylem flow to move the tracer to the sampled plant materials.

For all plant samples, non-transpiring tissues were taken so that samples represented the mean water uptake by plant roots (Dawson and Ehleringer 1993). Grasses were sampled from the

root crown. Tree stems were sampled from below the height of first leaves. All sampling tools (hands, trowels, clippers, and steel rod for filling samples into vials) were moved 20 m outside the plot and triple rinsed with tap water before taking the next sample.

At least three replicate samples of each species sampled in treated plots were also sampled from three control plots in each sampling period. Control plots were located 30 m from any treated plot and at least three were randomly selected during each sampling month for the collection of control samples. Control samples were collected following the methods used for treated plots except that naturally-occurring (i.e., not planted) saplings were sampled in control plots.

Soil cores were drilled in each treatment plot and one control plot during each sampling period to identify the location of the injected tracer in the soil. Cores were drilled in a randomly selected location one to three days following tracer injection. Soil samples were taken at eight depths to confirm the location of the pulse.

For each collected sample, as much plant or soil material as could fit in the bottom 10 cm of the prepared borosilicate (19-mm outer diameter) tubes was collected. Then, the tubes were sealed with parafilm, placed on ice, transported to a freezer within six hours, and extracted within two weeks. Water samples were extracted from plant tissues and soils using a batch cryogenic distillation procedure (Vendramini and Sternberg 2007) in Skukuza, Kruger National Park. Samples were analyzed at the University of Cape Town Stable Light Isotope Laboratory 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, $x1000\ \partial = \frac{R_{sa}-R_{std}}{R_{std}}$ X 1000 expressed as "parts per mil" or "‰", where R = the

ratio of heavy to light isotope, *sa* = sample, and *std* = standard, and *Rstd* ≈ 1/6412. Analytical precision (2σ) was 0.9 ‰ for δD.

Soil water availability

Soil water potentials were determined using an array of calibrated Campbell Scientific (Logan, UT, USA) 229L heat dissipation sensors placed at 5, 10, 20, 30, 50, 75, 120, and 150 cm in the soil profile of three soil pits (Flint et al. 2002; Kulmatiski et al. 2010). The three soil pits were located in the study area and within 50 m of each other. One pit was located in an area dominated by grasses, one in an area dominated by trees, and one in a mix of trees and grasses. Soil water potentials in the three pits were averaged and, therefore, expected to be representative of a broad range of soil moisture conditions at the study site. Additionally, soil water volumetric content was determined at the same depths in the mixed tree and grass pit using capacitance/frequency domain measurements (Decagon Devices EC-5 sensors; Decagon Devices, Pullman, WA, USA). Following sensor installation in October 2007, the soil pits were filled and compacted, and grass root mats were replaced. Measurements were recorded every two hours on a multiplexed Campbell Scientific CR1000 datalogger.

Plant available water (PAW) was calculated as the sum of positive increments in soil volumetric water content when soil water potentials were > -3MPa (i.e., plant available). These water potentials were similar to mean mid-day leaf water potentials measured at the site using the chilled mirror technique on grab samples (WP4T, Decagon Devices, Pullman WA, USA; data not shown). This approach provided good estimates of PAW at a specific depth, but overestimated the total amount of PAW throughout the soil profile because water that moves from one depth to another is counted twice. To estimate the total amount of PAW that moves

through the soil profile, the positive increments in PAW measured at 5 cm were added. To account for water that may have passed to deeper soils between the bihourly readings, any increases in soil volumetric content that occurred in the 10 and 15 cm depths at the same time-step as increases in the 5 cm were included in PAW measurements.

Data analyses

Tracer uptake by plant type and depth was analyzed in three ways: as a standardized proportion of the number of samples with δD values > 2 SD above mean control values, as mean δD values, and as a proportion of tracer uptake. First, to assess the number of samples demonstrating tracer by a plant from a specific depth, a count of the number of samples of each plant type receiving tracer [i.e., δD values greater than two standard deviations (SDs) above mean δD values in control samples] was determined. This count was then standardized to control for species-specific traits. More specifically, for a plant type by depth and time period, the standardized proportion of the number of samples receiving tracer = $P_{obs}\left(\frac{1}{P_{max}}\right)$, where P_{obs} is the proportion of samples within a depth (e.g., 5 cm depth in February) that received tracer, and P_{max} is the maximum proportion of samples from a plant type that received tracer at any depth. For example, if the greatest proportion of grass samples in February to receive tracer was 0.75 and this occurred at the 5 cm depth, then the proportion of samples receiving tracer at all depths was scaled to a maximum value of 0.75. This calculation controlled for the fact that different numbers of samples were taken from grasses, saplings, and trees and for the fact that species-specific traits, such as rooting area (i.e., tracer was added to a larger proportion of the rooting zone of a plant with a small rooting area relative to a plant with a large rooting area) and plant volume (i.e., tracer absorbed by trees will be diluted in a larger storage pool) are likely to affect

the number of samples receiving tracer (Bishop and Dambrine 1995; McKane et al. 2002; Schwinning et al. 2002). Because this measure is based on a count of samples, there was no associated error and differences among plant types were not tested statistically.

Second, mean δD values for each plant type by depth and time were calculated (i.e., the mean δD value of trees in the 20 cm injection plot in February). Differences in δD values for each plant type by depth were tested using analysis of variance (ANOVA) computed using the MIXED procedure in SAS/STAT for Windows, Release 9.1.3 (SAS Institute Inc., Cary, North Carolina). To compare δD values across depths by plant type, the fixed effect was plant type and replicate samples from each plot were used as random effects. Analyses, therefore, provide inference at the plot level, not the landscape level because replicates within plots were used as the source of error. To compare δD values across 'days since injection' by plant type, the fixed effect was 'days' and replicate samples from each depth were used as random effects. Data were transformed to meet assumptions of normality and homogeneity of variance when necessary. Tukey post-hoc pairwise comparisons were used to determine mean differences at the alpha < 0.05 level.

Third, to account for species-specific differences in tracer concentrations, the proportion of tracer uptake at each depth and each distance was calculated as follows: $\frac{S_n - C}{\sum_{n=i}^{j}(S_n - C)}$ where S_n is the mean δD value of samples from a treatment level *n* (e.g., 5 cm depth), and *C* is the mean δD value of control samples for that functional type (e.g., trees; Kulmatiski et al. 2010). The error associated with the proportional uptake by each plant type from each depth was propagated assuming that total uptake was a fixed value so that error was proportional to the error associated with tracer concentrations at a particular depth (Goodman 1960). Differences within plant types among depths and differences among plant types within depths in proportional uptake were

calculated for each soil depth using a standard equivalency test: two proportional values were considered different when $|Pa - Pb| \leq 2\sqrt{\sigma_a^2 + \sigma_b^2}$, where *Pa* is the proportion of tracer uptake for plant a, and σ_a^2 is the variance associated with plant a. Tests of the proportion of tracer uptake were the only analyses for which we compared differences in uptake among plant types within a depth. Tests among plant types were not possible for the standardized proportion data (because there is no error associated with those values) and were not appropriate for the δD values (due to inherent species differences).

Finally, the proportion of tracer uptake and measures of plant available water (PAW) were used to calculate niche overlap. Niche overlap was calculated using Pianka's standardized overlap value (i.e., $O_{jk} = \frac{\sum e_{ij} e_{ik}}{\sqrt{\sum e_{ij}^2 e_{ik}^2}}$, where O_{jk} is a measure of overlap between species *j* and *k*, e_{ij} or the electivity index = p_{ij}/R_j, where p_{ij} is the proportion that resource *i* is of the total resource used by species *j*, p_{ik} is the proportion that resource *i* is of the total resources used by species *k*, and *Rj* is a measure of the availability of resource state *j*) (Pianka 1986). This unitless measure ranges from 0 to 1, where 0 indicates that no resources are used in common (i.e., complete niche partitioning). To determine if observed overlap values were likely to result by chance, the species utilization matrices were compared to predictions from a randomized null model. Null model predictions were developed using EcoSim ver. 7 (Gotelli and Entsminger 2011). Randomization algorithm three, in which niche breadth is retained and zero states are reshuffled, was used (Gotelli and Entsminger 2011). This algorithm was used because niche breadth did appear to differ by species, zero uptake did not appear to be a fixed species trait for any depth (i.e., all plants accessed some tracer from every depth sampled during one time period or another), and this approach is usually superior in detecting non-random overlap (Winemiller andPianka 1990). To estimate *Rj,* we used our measurements of PAW by depth (Gotelli and Entsminger 2011).

More specifically, mean PAW values by depth for the three weeks prior to sampling were used to calculate resource availability by depth (i.e., the electivity matrix). For example, if the greatest water availability occurred at the 10-20 cm depth and one half as much water was available at the 60-70 cm depth, then the electivity value would be 1 for the 10-20 cm depth and 0.5 for the 60-70 cm depth. Random niche overlap values were derived from 1000 Monte Carlo permutations derived from the data matrix. Observed overlap values were then compared to the distributions of the randomized values. A P value of < 0.05 indicates that observed niche overlap values were greater than or less than niche overlap values produced by the randomized model.

Results

Soil water availability

During the 2008/2009 growing season, there was 871 mm of precipitation and 225 mm of this was plant available in the top 15 cm of soil (Fig. 1). This reflected 0.7, 1.1 and 0.7 mm of PAW becoming available each day in the October to January, January to April, and April to June time periods. A difference between precipitation and plant available water presumably reflected: 1) the loss of water to evaporation on plant surfaces, 2) plant uptake that occurred between the bihourly soil measurements, and 3) differences in precipitation events between the study site and the precipitation collection 4 km away. Most PAW occurred in the middle of the growing season and near the soil surface (Fig. 2). Soil water was not plant available between 0-150 cm at the start of the growing season (not shown) and had infiltrated to 50 cm by the first sampling tracer addition. Soil water was abundant throughout the profile during the second tracer addition. Small amounts of soil water were plant available throughout the soil profile during the final tracer addition (Fig. 2).

297

298 Tracer detection

299 Of the 2,216 plant and 144 soil samples taken, 1,464 plant and 109 soil samples were
300 successfully extracted and analyzed for δD values. The remaining samples including all soil
301 samples from the November 30 cm soil core and all sapling samples from the November 50 cm
302 plot were lost during shipping or due to glass failure during the extraction process. From treated
303 plots, a total of 1,370 vegetation samples were extracted and analyzed: 758 grass, 87 sapling, and
304 525 tree. Of these 57, 85, and 44%, respectively, demonstrated δD values that were two SDs or
305 more above controls (i.e., received tracer). Of 94 control samples, five (5%) demonstrated δD
306 values that were two SDs above the mean. These samples were either contaminated with tracer
307 or had realized significant enrichment due to evaporation. Control grass samples demonstrated
308 δD values (mean ± SD) of -3.2 ± 14.4, -5.6 ± 15.0 and -10.2 ± 21.9 ‰ in November, February,
309 and May, respectively. Control tree samples demonstrated δD values of 25.9 ± 4.1, -19.0 ± 5.6,
310 and -4.9 ± 5.6 ‰ in November, February and May, respectively.

311 Soil core samples showed large δD values at or near the target depths for the November
312 5, 10, 20, and 50 cm plots (Fig. 3a), the February 30 and 50 cm plots (Fig. 3b), and the May 5
313 and 30 cm plots (Fig. 3c), but failed to capture the location of other tracer injections. Also, the
314 May 60 cm sample showed a wide tracer distribution from 40 to 80 cm (Fig. 3c). Missing tracer
315 distributions were likely to have resulted because the 5 cm wide soil core did not intersect with
316 injection points, which were separated by 15 cm. Control soil δD values of soils across the
317 profile were 15.8 ± 11.2, -14.0 ± 2.4, and 13.3 ± 6.3 ‰ in November, February, and May,
318 respectively. Repeated vegetation sampling indicated that, in general, grasses and trees realized
319 peaks in δD values two and three days after tracer injection, respectively (Fig. 4).

320

321 Plant uptake

322 In all months in the 5 cm plots, the standardized proportion of the number of samples with δD
323 values > 2 SD above controls indicated that tracer was commonly absorbed by all plant types. In
324 November, the greatest standardized proportion of grass, sapling, and tree samples receiving
325 tracer occurred at 20, 10, and 30 cm, respectively (Fig. 5a). In February, the greatest
326 standardized proportion of grass, sapling, and tree samples receiving tracer occurred at 20, 5-30,
327 and 30 cm, respectively (Fig. 5b). In May, the greatest standardized proportion of grass, sapling,
328 and tree samples receiving tracer occurred at 5, 120, and 120 cm, respectively (Fig. 5c).

329 Mean δD values indicated that in November, values were largest for all plants in the 5
330 cm plot (though only one sapling replicate sample was available for some depths; Table 1). In
331 the middle of the growing season (February), δD values were again largest for all plants in the 5
332 cm plot. At the end of the growing season (May), grass δD values again decreased with depth,
333 but sapling δD values were fairly even across depths, and tree δD values were greatest at 30 and
334 60 cm depths.

335 Mean δD values and the proportion of tracer uptake values by plant type and depth were
336 very similar (Online Resource 1; Fig 6). In November for grasses, the proportion of tracer uptake
337 was greater at 5 cm than all other depths. For trees, similarly, the proportion of tracer uptake was
338 greater at 5 cm than 10, 20, or 30 cm. We could not reliably test differences for saplings and
339 other plants because only one sample was available for some depths in November. For all plant
340 types in February, the proportion uptake was greater at 5 cm than at 50 or 100 cm. In May,
341 grasses continued to obtain the greatest proportion of tracer uptake from the shallowest depth.

342 Saplings, however, demonstrated no difference in proportion uptake among depths. Trees
343 demonstrated the greatest proportion uptake at 30 and 60 cm.
344 We also observed differences in the proportion uptake by depth among plant types. In
345 November, the proportion of grass uptake at 20 cm was greater than that of trees (Fig. 6a). In
346 February, the proportion of sapling uptake was greater than grass or tree uptake at 30 and 50 cm
347 and greater than grass uptake at 100 cm (Fig 6b). In May, the proportion of grass uptake at 5 cm
348 was greater than that for saplings or trees (Fig. 6c). The proportion of tree uptake from 30 cm
349 was greater than that for grasses or saplings. The proportion of tree uptake was also greater than
350 grass uptake at 60 and 120 cm. Finally, sapling uptake was greater than grass uptake at 120 cm.
351
352 Niche overlap
353 Niche overlap values did not differ from the null model in November, but in February observed
354 niche overlap was greater than predicted from the null model for grasses and saplings, grasses
355 and trees, and trees and saplings, respectively (Table 2). In May, sapling and tree overlap was
356 greater than null model predictions but overlap between grasses and saplings and grasses and
357 trees were not (Table 2).
358
359 **Discussion**
360 Constraining the two-layer hypothesis
361 Consistent with the two-layer hypothesis, grasses relied most heavily on the shallowest soils and
362 least heavily on the deepest soils. However, results also differed from predictions of the two-
363 layer hypothesis in several ways. First, all plants relied heavily on shallow soil water (Figs. 5 and
364 6). More specifically, across the growing season grasses, saplings, and trees absorbed 61, 42, and

35% of their tracer from the shallowest 5 cm depth, respectively. Second, all plants accessed some tracer from all soil depths sampled. So, use of water resources was not exclusive at any depth. Third, trees did not heavily rely on exclusive access to deep soil water. Across the growing season grasses, saplings, and trees absorbed 2, 16, and 8% of their tracer from the deepest depths sampled, respectively.

The two-layer hypothesis is often viewed as a fixed plant trait (Arora and Boer 2003; Scanlon and Albertson 2003). This assumption appears appropriate for grasses, which always relied most heavily on the shallowest soils (Fig. 6; Kulmatiski et al. 2010). Sapling and tree root activity, however, changed within the growing season and did not always rely on shallow or deep soil water (Ashton et al. 2010; Kulmatiski et al. 2010; Nippert and Knapp 2007). As a result, and in contrast to a fixed-trait perspective of plant root activity, niche partitioning was greatest when resources were scarce and least when resources were abundant (Figs. 2 and 6 and Table 2). When combined with results from a previous study at the same site, which showed that grasses and trees partition soil resources between 5 and 20 cm depths (Kulmatiski et al. 2010), it is clear that niche partitioning is not a fixed-trait and can occur over short temporal and spatial scales (McKane et al. 2002).

While it seems unlikely that these species are groundwater dependent because they are deciduous or otherwise inactive during the dry season, it is crucial not to discount groundwater as a source when confining a study to the top 150 cm of soil. Groundwater at the study site (δD value of -24 ‰; Leyland and Witthüser 2008) is more depleted than soil water at the site (10, -14, and 13 ‰ in November, February, and May, respectively). Plants from control plots with δD values lower than those of the soil, therefore, may be using groundwater. Alternatively, low δD values may reflect the use of rainwater, for which we do not have data, but which should have

388 δD values similar to those of the groundwater. We did not, however, observe a consistent pattern
389 of control tree samples being depleted relative to soils or grasses in this study or in a related
390 study conducted in the previous year (A. Kulmatiski unpubl. data). Because δD values of trees in
391 control plots were rarely more depleted than either grasses or soils, there was little evidence to
392 suggest that trees use groundwater and if they do, they do not appear to use more groundwater
393 than grasses.
394
395 Sapling root activity
396 Contrary to our expectations, saplings rapidly established deep roots. By the middle of their
397 second growing season saplings absorbed tracer from at least 1 m depths and saplings relied on
398 deep soil water more than grasses or trees (Figs. 5b, 6b, 6c). The rapid establishment of deep root
399 activity by saplings is impressive but is not surprising given that annual and biennial plants in
400 other systems have been observed to develop roots to nearly 1 m depths (Peek et al. 2005;
401 Kulmatiski et al. 2006). Our results suggest that it may not be difficult for plants to establish
402 deep roots. Rather there is likely to be a selective pressure to maintain shallow roots. Shallow
403 roots, for example, provide access to the greatest amount of precipitation in nutrient-rich soils.
404 That saplings relied more heavily on deep soil water than grasses or trees in February, suggests
405 that saplings were dedicated to the development of deep roots despite shallow soil water
406 availability. We did not assess the effects of grasses on tree germination and first-year
407 establishment where competition could be expected be intense, but our results suggest that by
408 their second year, saplings can avoid competition with grasses by relying on deep soil resources.
409
410 Root biomass, active roots, and root activity

411 Root activity is typically inferred from measures of root biomass (February and Higgins 2010;
412 Hipondoka et al. 2003; Schenk and Jackson 2002) though the nature of this relationship is poorly
413 understood because direct measurements of root activity are rare. Root biomass at the study site
414 and in most systems demonstrates an exponential decrease with depth (February and Higgins
415 2010; Kulmatiski et al. 2010; Schenk and Jackson 2002). Our results show that this pattern is
416 consistent with grass root activity, which declined exponentially with depth in each sampling
417 period. Sapling and tree root activity, however, varied over time and did not demonstrate an
418 exponential decline with depth. Root biomass, therefore, may be appropriate for estimating root
419 activity for grasses or when soil resources exceed biotic demand (Figs. 2 and 6b) but not for
420 assessing niche partitioning or root activity when resources are limiting (Figs. 2 and 6c).
421 Our results provide a unique perspective on the difference between the presence of active
422 roots and root activity. We suggest the standardized proportion of samples demonstrating tracer
423 indicates the presence of active roots and the δD values indicate root activity. For example, the
424 greatest standardized proportion of tree samples demonstrating tracer uptake in February
425 occurred in the 30 cm plot (Fig. 5b), yet the greatest δD values in trees in February occurred in
426 the 5 cm plot (Fig. 6b). The large standardized proportion of samples demonstrating tracer
427 uptake at 30 cm suggests that trees have the greatest number of active roots at this depth. The
428 large δD values in trees at 5 cm, however, suggests that roots at 5 cm, while fewer in number,
429 absorbed more soil water. This pattern is assumed to reflect greater water availability at 5 cm
430 (Fig. 2). Thus, water uptake was a function of both active root abundance and water availability.
431 Though this seems like an obvious conclusion, it has not previously been possible to observe
432 using root biomass, minirhizotron, or natural abundance stable isotope approaches (February and
433 Higgins 2010; Kulmatiski et al. 2006; Kulmatiski et al. 2010; Peek et al. 2005).

434 The first test of the depth-controlled tracer technique only examined grass and tree, and
435 not sapling, water use at the study site in the previous growing season (Kulmatiski et al. 2010).
436 That study was performed during a year with a mid-season drought that resulted in a loss of
437 PAW from throughout the soil profile. Despite differences in approaches (1 m^2 vs. 154 m^2 plots)
438 and climate, results were qualitatively similar in both studies. Kulmatiski et al. (2010) found that
439 grasses consistently relied on 5 cm soils and trees showed a more temporally variable reliance on
440 soil water at 20 cm. The current study found greater water use by all plants below 20 cm, which
441 is consistent with greater deep soil water infiltration in a wet year.
442
443 Conclusion
444 By quantifying plant water use by depth, our measurements help resolve conflicting conclusions
445 of previous researchers that have alternately suggested that grasses and trees rely on different soil
446 depths (Goldstein et al. 2008; Schenk and Jackson 2002; Walter 1971; Weltzin and McPherson
447 1997) and that grasses and trees both rely on shallow soil depths (February and Higgins 2010;
448 Hipondoka et al. 2003; Leroux et al. 1995). Our results are from one mesic, sandy site and so
449 will require further testing in a wider range of sites, but support a weak version of the two-layer
450 hypothesis: one in which all plants access soil water throughout the soil profile but grasses rely
451 more heavily on shallow soils and trees rely on a dynamic range of soil depths. Perhaps more
452 importantly, we found that niche partitioning occurs over small spatial and temporal scales that
453 are not necessarily well predicted by the two-layer hypothesis. Precise techniques are, therefore,
454 likely needed to assess niche partitioning in plant systems and parameterize niche partitioning
455 and ecohydrological models (Scanlon and Albertson 2003; Williams and Albertson 2004).
456

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614

615

Table 1. Mean δD values (‰) ± 1 SE in plant materials sampled from plots that had received tracer at the indicated soil depths (e.g., 5 cm) and time of year (e.g., November). Values in a row followed by a different lower case letter are different at the 0.05 level.

Plant type	Soil depth				
	November 2008				
	5 cm	10 cm	20 cm	30 cm	50 cm
Grass	76 ± 18a	-18 ± 12b	31 ± 3ab	-45 ± 45b	-1 ± 28ab
Sapling	289 ± 151a	105	73	42	Na*
Tree	188 ± 56a	13 ± 14b	-1 ± 22b	70 ± 40ab	70 ± 85ab
	February 2009				
	5 cm	20 cm	30 cm	50 cm	100 cm
Grass	96 ± 18a	60 ± 13b	19 ± 5c	-3 ± 6d	3 ± 9cd
Sapling	105 ± 41a	41 ± 8b	64 ± 11ab	11 ± 8c	30 ± 10bc
Tree	54 ± 21a	10 ± 5b	6 ± 3ab	-9 ± 2ab	2 ± 5ab
	May 2009				
	5 cm	30 cm	60 cm	90 cm	120 cm
Grass	944 ± 76a	229 ± 25b	90 ± 15c	107 ± 19c	76 ± 28c
Sapling	189 ± 127a	148 ± 25a	646 ± 598a	72 ± 18a	93 ± 17a
Tree	65 ± 24b	161 ± 24a	226 ± 79a	36 ± 10b	84 ± 11ab

*Na = data not available

620 **Table 2.** Observed and predicted niche overlap ± variance of water use by depth for grasses and
621 saplings, trees and saplings, and trees and grasses throughout a growing season. Niche overlap is
622 a unitless value that ranges from 0 to 1, with 0 reflecting no overlapping resource use and 1
623 reflecting complete overlap. A null (randomized) model was used to develop predicted niche
624 overlap values (see text for details). *P* = probability that the observed value is different than
625 predicted value.

Comparison	November 2008			February 2009			May 2009		
	Observed	Predicted	*P*	Observed	Predicted	*P*	Observed	Predicted	*P*
Grass vs. Sapling	0.69	0.67± 0.01	0.22	0.94	0.62 ± 0.03	0.03*	0.44	0.54± 0.05	0.62
Tree vs. Sapling	0.76	0.74 ± 0.00	0.71	0.91	0.76 ± 0.02	0.03*	0.93	0.51± 0.05	0.01*
Tree vs. Grass	0.43	0.31 ± 0.11	0.53	0.89	0.61 ± 0.03	0.01*	0.57	0.63 ± 0.03	0.48

626

627 **Figure Legends**

628 **Fig. 1.** Observed precipitation and plant available water (PAW; mm mo^{-1}) that entered the soil
629 from January 2007 through December 2009, Pretoriuskop, Kruger National Park, South Africa.
630 PAW defined as positive increments of soil volumetric water content when soil water potentials
631 were greater than -3 MPa.

632 **Fig. 2.** Plant available water (PAW; mm day^{-1}) by depth for the three weeks prior to tracer
633 addition in (a) November 2008, (b) February 2009, and (c) May 2009.

634 **Fig. 3.** δD values (‰) in soils during the (a) November 2008, (b) February 2009, and (c) May
635 2009 sampling periods. Each point represents a soil sample from a single soil core from each
636 plot. There is no data for 30 cm in November (see text).

637 **Fig. 4.** δD values (‰) in (a, c, e) grass and (b, d, f) tree samples taken by day following
638 deuterium oxide tracer injection during the (a,b) November 2008, (c, d) February 2009, and (e, f)
639 May 2009 sampling periods.

640 **Fig. 5.** The standardized proportion of the number of grass, sapling, and tree samples
641 demonstrating δD values greater than two standard deviations above those measured in paired
642 control samples in (a) November 2008, (b) February 2009, and (c) May 2009. The proportion of
643 the number of samples receiving tracer was standardized to the greatest value observed for a
644 plant type at any depth during a sampling period. This was done to allow a comparison of
645 different plant types.

646 **Fig. 6.** Proportion of tracer uptake by grasses, planted saplings, and trees in November (a),
647 February (b) and May (c) 2008/2009, Pretorioskop, Kruger National Park, South Africa. Error

was propagated assuming that total tracer uptake was a fixed value. Plant types by depth with different lower case letters were different using a standard equivalency test. Letters represent grasses, trees and tree saplings, respectively. Lines between data from different depths are shown for ease of interpretation but cannot be used to estimate the proportion of tracer uptake at depths other than those sampled.

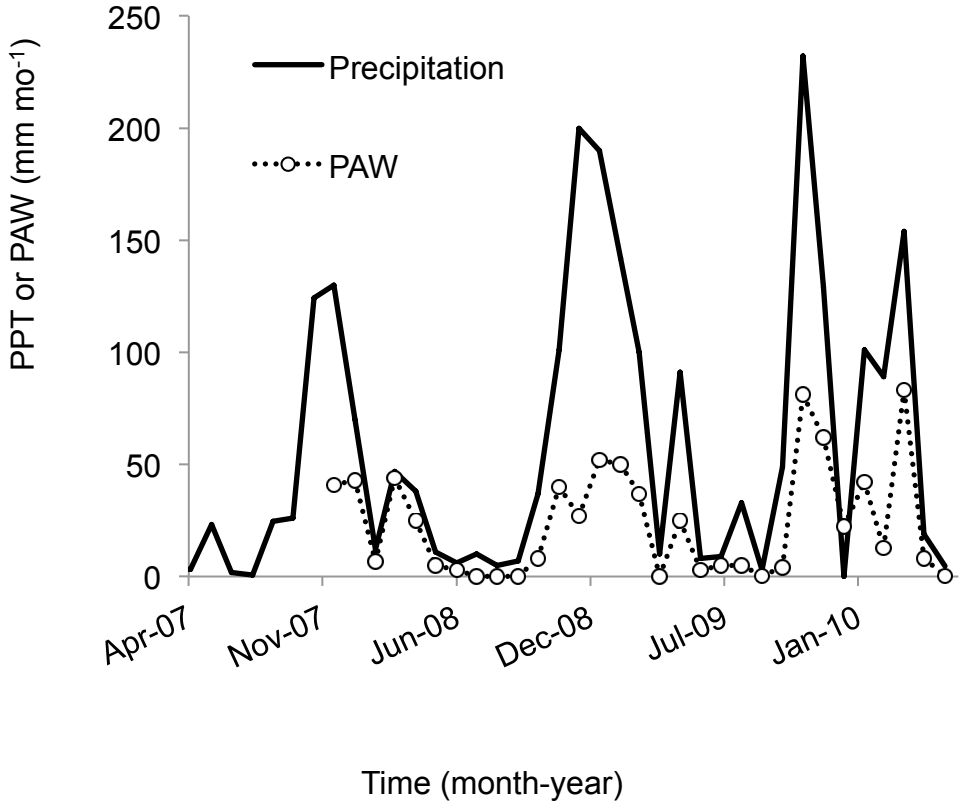
250
200
150
100
50
0
PPT or PAW (mm mo-1)
Precipitation
PAW
Apr-07
Nov-07
Jun-08
Dec-08
Jul-09
Jan-10
Time (month-year)

Fig. 1.

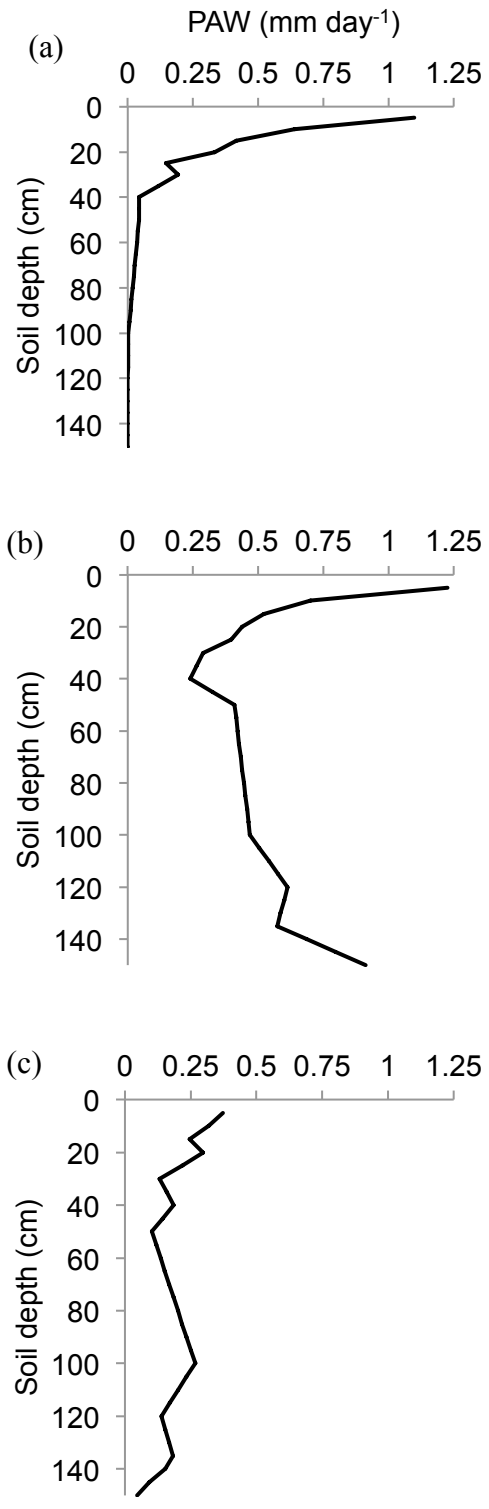
(a)
PAW (mm day-1)
0 0.25 0.5 0.75 1 1.25
Soil depth (cm)
0 20 40 60 80 100 120 140
(b)
0 0.25 0.5 0.75 1 1.25
Soil depth (cm)
0 20 40 60 80 100 120 140
(c)
0 0.25 0.5 0.75 1 1.25
Soil depth (cm)
0 20 40 60 80 100 120 140

Fig. 2.

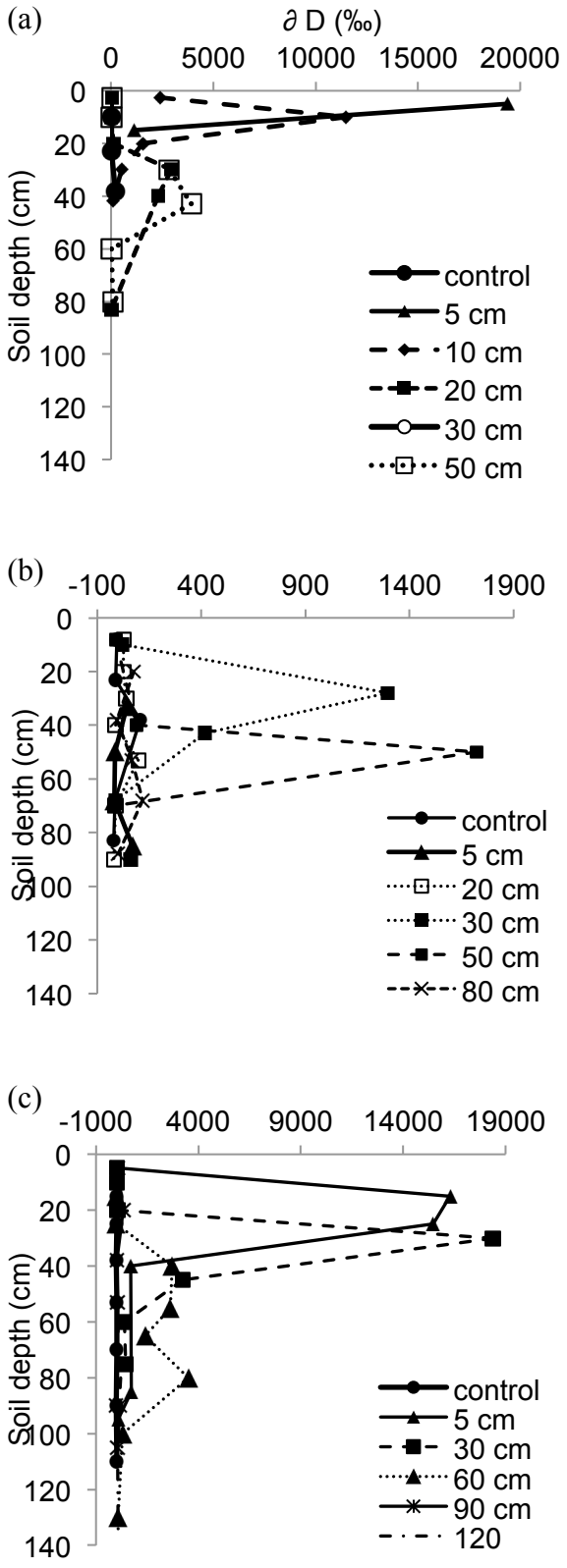
(a)
∂ D (‰)
0
5000
10000
15000
20000
Soil depth (cm)
0
20
40
60
80
100
120
140
control
5 cm
10 cm
20 cm
30 cm
50 cm
(b)
-100
400
900
1400
1900
Soil depth (cm)
control
5 cm
20 cm
30 cm
50 cm
80 cm
(c)
-1000
4000
9000
14000
19000
Soil depth (cm)
control
5 cm
30 cm
60 cm
90 cm
120

Fig. 3.

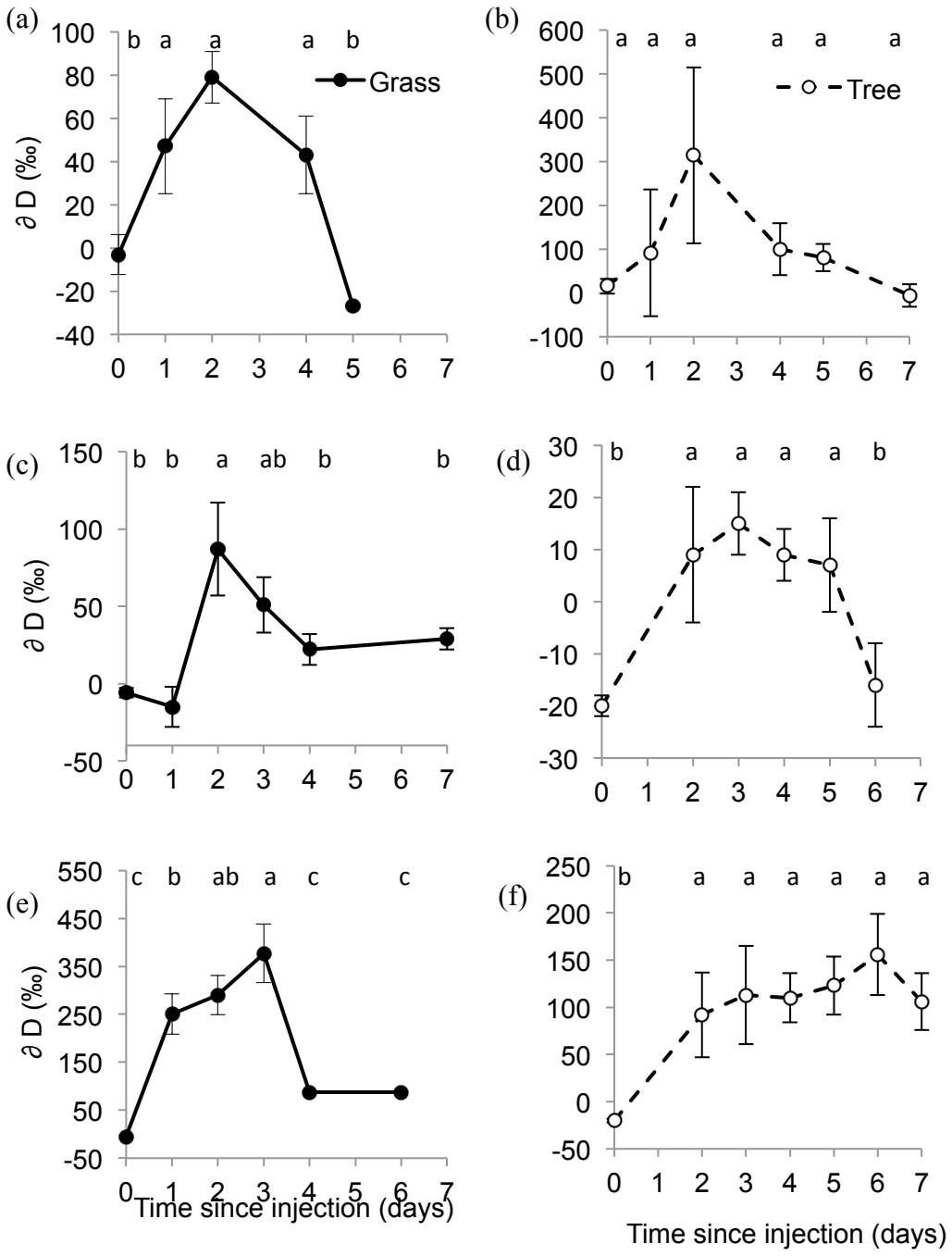
(a)
b a a a b
Grass
∂ D (‰)
(b)
a a a a a a
Tree
(c)
b b a ab b b
(d)
b a a a a b
(e)
c b ab a c c
(f)
b a a a a a a
Time since injection (days)
Time since injection (days)

Fig. 4.

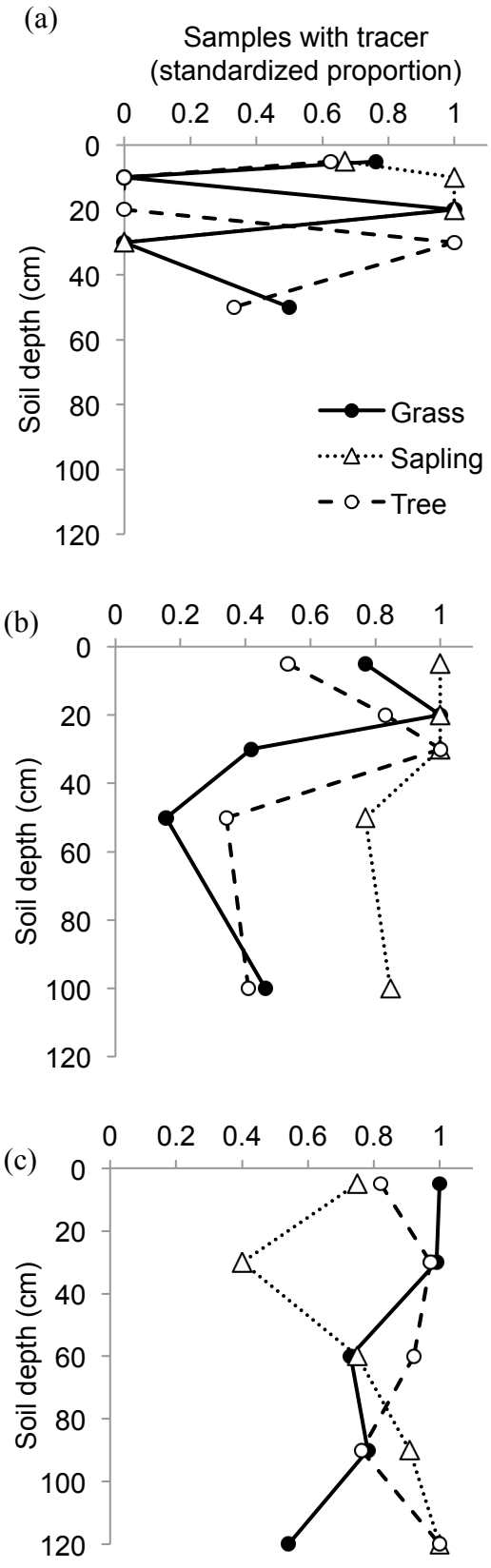
(a)
Samples with tracer
(standardized proportion)
0
0.2
0.4
0.6
0.8
1
Soil depth (cm)
0
20
40
60
80
100
120
Grass
Sapling
Tree
(b)
(c)

Fig. 5.

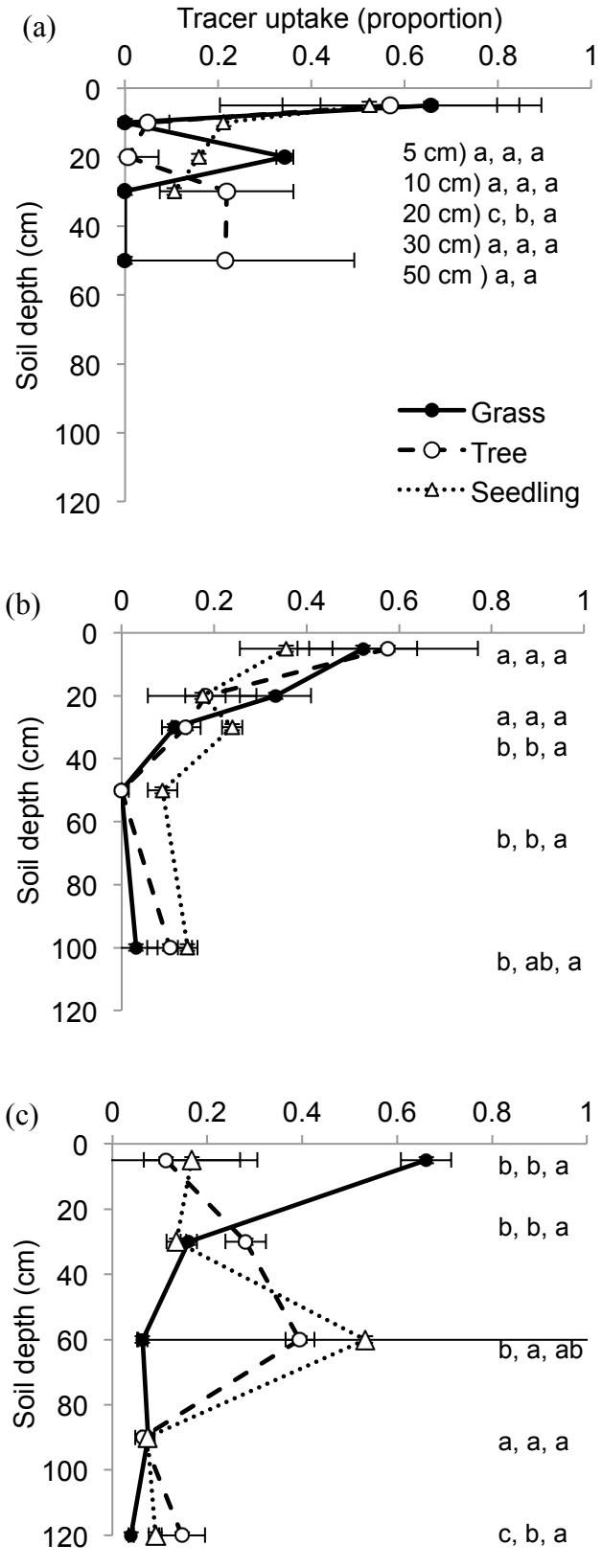
(a)
Tracer uptake (proportion)
0
0.2
0.4
0.6
0.8
1
Soil depth (cm)
0
20
40
60
80
100
120
5 cm) a, a, a
10 cm) a, a, a
20 cm) c, b, a
30 cm) a, a, a
50 cm) a, a
Grass
Tree
Seedling
(b)
a, a, a
a, a, a
b, b, a
b, b, a
b, ab, a
(c)
b, b, a
b, b, a
b, a, ab
a, a, a
c, b, a

Fig. 6.