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#### RAPID SAVANNA RESPONSE TO CHANGING PRECIPITATION INTENSITY

by

### Ryan S. Berry

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

of

#### MASTER OF SCIENCE

in

Ecology

Approved:

Andrew Kulmatiski Major Professor Karen H. Beard Committee Member

Scott Jones Committee Member Mark R. McLellan Vice President for Research and Dean of the school of Graduate Studies

UTAH STATE UNIVERSITY Logan, Utah

2016

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#### ABSTRACT

#### Rapid Savanna Response to Changing Precipitation Intensity

by

Ryan S. Berry, Master of Science

Utah State University, 2016

Major Professor: Dr. Andrew Kulmatiski Program: Ecology

As the atmosphere warms, precipitation events are likely to become less frequent but more intense. While extensive efforts have been made to understand how changes in mean annual precipitation will affect plant growth, particularly in semi-arid systems, relatively little is known about how increasing precipitation intensity will affect plant growth and hydrologic cycles. A recent study by Kulmatiski and Beard (2013) found that small increases in precipitation intensity increased woody plant growth and decreased grass growth in a three-year experiment in a savanna system, Kruger National Park. Here we report results from the following two years of that experiment. Due to naturally large precipitation events, plant available water was similar between treatment and control plots in the last two years of the study allowing us to test woody plant and grass responses to treatment removal (*i.e.*, legacy effects). Treatment effects on grass and tree growth disappeared within months of treatment removal. However, due to a legacy effect of treatments, tree mass was greater in treatment than control plots at the end of the experiment. Measurements of root recruitment and hydrological tracer uptake, but not root volume helped explain plant growth responses to treatments. Results suggest that savanna plants respond rapidly to changes in precipitation intensity, but because of legacy effects, occasional increases in precipitation intensity can result in long-term shrub encroachment.

(65 pages)

#### PUBLIC ABSTRACT

## Rapid Savanna Responses to Changing Precipitation Intensity Ryan S. Berry

Climate change has the potential to cause large-scale changes in plant growth, biodiversity, and biosphere-climate feedbacks. A pervasive aspect of climate change is that as the atmosphere warms, precipitation events are likely to become less frequent but more intense, because warmer air can hold more water. Larger precipitation events can be expected to change plant productivity and community composition, particularly in semiarid ecosystems such as savannas. Savannas are of particular interest because they are spatially expansive at the global scale, they are important to humans for food production, and they are known to be sensitive to changes in soil water availability. Extensive efforts have been made to understand how increases or decreases in total precipitation will affect plant growth, but relatively little is known about how increasing precipitation intensity will affect grass and tree growth in savannas.

Here we use precipitation manipulation shelters in a semiarid savanna system, Kruger National Park, South Africa to examine grass and tree response to changes in precipitation intensity. The shelters collected and stored 50% of ambient precipitation, then redeposited collected water as relatively large precipitation events. Grass and tree growth, and root growth and activity were monitored in treated plots and untreated control plots from 2008-2013.

Small changes in precipitation intensity resulted in large increases in plant available water, particularly at 30-60 cm depths during the first three years of the study. Due to naturally large precipitation events, plant available water was similar between treatment and control plots in the last two years of the study. In the first three years, grass growth decreased and tree growth increased in treatment relative to control plots. Treatment effects disappeared for both grasses and trees in the last two years of the study when treatment effects were small. Our study revealed rapid savanna responses to changes in precipitation intensity.

Our results suggest increased precipitation intensity plays a part in shrub encroachment alongside fire suppression, grazing, and rising atmospheric CO<sub>2</sub> concentrations. Increasing precipitation intensity could decrease grass litter buildup, affect fire regimes, and could mean better habitat for browsers, worse habitat for grazers. While small increases in precipitation, like those in this study, are likely to increase plant productivity or aquifer recharge, larger increases may increase runoff and erosion and decrease productivity. Moreover, our data could be used to help develop new crop strains more compatible with climate change in this part of the world.

#### ACKNOWLEDGMENTS

This study would not have been possible without the cooperation of South African National Parks and Kruger National Park (project registration number 213896412). I owe thanks for funding to the Andrew Mellon Foundation, Utah State University for a research catalyst grant, and the Utah Agriculture Experimental Station, and to dozens of researchers and technicians including E. February, W. Bond, and the University of Capetown for the rainout shelters. Thanks also to my major professor: Andrew Kulmatiski, my thesis committee: Karen Beard and Scott Jones, and S. Durham for assistance with statistical analysis. Thank you to L. Forrero for assistance analyzing deuterium isotope samples, R. Tobin for assistance with data analysis, the field managers: M. Cooper, M. Mazzacavallo, M. Keretetsi, S. Heath and L. Hierl, and the field/laboratory assistants: W. Sibuye, R. Mashele, V. Sibuye and M. Rogers.

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#### **INTRODUCTION**

As the atmosphere warms, precipitation events are predicted to become fewer, but larger [1–8]. This is because the water-holding capacity of air increases 7-10 % per degree Celsius [9,10]. This is referred to as the Clausius-Clapeyron rate, and recent evidence suggests the percent increase may be even larger than previously thought [11]. With global climate models predicting increasing temperatures of several degrees Celsius over the next century, changes in air water-holding capacity and consequently precipitation event sizes on the order of 23-33% appear likely [1, 3, 7]. Recent observational evidence supports these predictions with increased precipitation intensity observed around the world [10, 12–17].

Extensive efforts have been made to understand how increases or decreases in total precipitation amount will affect ecosystem dynamics and water cycling [18, 19], but relatively little is known about how increasing precipitation intensity will affect ecosystem dynamics and water cycling [1, 7, 20–26]. Among studies that have been performed, increased precipitation intensity has been found to decrease shallow soil water content [21]. This is likely to decrease the growth of shallow-rooted plants, such as grasses [27–29]. However, inference from these studies is limited because only about half were multi-year studies and about a third were in the same North American grassland [1, 7, 20]. Longer-term studies are needed to determine if increased precipitation intensity effects increase or decrease over time, or if treatments produce lag or legacy effects on plant growth [1, 24].

Ecohydrological models can be used to help understand ecosystem responses to increased precipitation intensity [7]. These well-developed models suggest that

ecosystem responses are very sensitive to increased precipitation intensity [7]. For example, plant productivity can be expected to increase, decrease, or have no response to increased precipitation intensity depending on parameters such as event size, slope, and soil characteristics [7, 24, 30, 31]. It is easy to imagine, for example, that a small increase in precipitation intensity may increase plant productivity by decreasing water loss to interception and evaporation. Alternatively, a large increase in precipitation intensity may decrease plant productivity due to overland flow or deep soil infiltration. Further, root distributions are an important but poorly understood component of ecohydrological models [7, 30, 32–35]. There remains, therefore, a need for experiments to test the belowground effects of increased precipitation intensity in multi-year experiments, particularly in non-North American study sites [1, 7, 24, 36].

Semiarid systems cover 40% of earth's surface [37, 38], support a large percentage of the human population [38], and are particularly vulnerable to changes in mean precipitation intensity and the timing of precipitation events [7, 30, 39]. These systems have exhibited a large increase in tree and shrub encroachment over the last 50 years that may be due, in part, to increasing precipitation intensity [40–44].

Our objective was to measure plant growth responses to increased precipitation intensity in a semi-arid system. To do this, we measured grass and woody plant responses to increased precipitation intensity treatments applied by a shelter experiment, Kruger National Park, South Africa. Results from the first three years of treatment indicated that increased precipitation intensity led to increased woody plant growth and decreased grass growth [40]. Here we report aboveground and belowground responses of grasses and woody plants over an additional two years of treatment. Soil moisture measurements were used to assess treatment effects on water availability. Measurements of aboveground growth were used to assess treatment effects on grasses and woody plants. Two approaches were used to estimate root growth responses to treatments. First, root image analyses were used to assess total plant root recruitment and biomass in treated and control plots. Second, a hydrologic tracer experiment was performed to estimate vertical patterns of root water uptake in treatment and control plots.

#### **METHODS**

#### Study Site Information

Research was conducted in the Cape Buffalo enclosure located near the Satara Rest Camp, Kruger National Park South Africa (24°24'18.30" S, 31°44'52.81"E [45]). Mean annual precipitation is 489 mm, with most falling during December and January (Fig 1). During the five years of the study (2008-2013), annual precipitation was 459 mm, 654 mm, 473 mm, 388 mm, and 756 mm, respectively. Annual average temperatures ranged from highs of 40° C in summer to 8° C in winter. Soils are basalt-derived dark brown to black pedocutanic clay loams [41]. Common C4 grasses at the study site include *Bothriochloa radicans* (Lehm) A. Camus, *Panicum maximum* (Jacq.), and *Themeda triandra* (Forssk.). Common woody plants include the nitrogen-fixing shrub *Dichrostachys sinerea* subsp. *africana* (Brenan & Brummitt) and shrub/tree *Flueggea virosa* (Roxb. Ex Willd.) Voigt. *T. triandra, D. sinerea,* and *F. virosa* are common, widespread species in southern Africa [46,47]. Grass cover at a similar nearby site was approximately 47 % and tree cover was approximately 20 % [29]. All measured species are native; non-native species are uncommon.

#### Experimental Design

The experimental design is described elsewhere [40]. Briefly, at the end of the 2007 to 2008 (henceforth, 2008) growing season, six 8 m by 8 m by 2.5 m shelters constructed of a steel frame and clear, polycarbonate plastic roofing [45] were erected. Roofing sheets, 13.3 cm wide, covered half the roof and collected 50 % of ambient precipitation. From 2008-2013, collected precipitation was stored in four 200 L barrels in

each shelter. When full, a self-flushing flout mechanism released the equivalent of 1 cm precipitation events through a drip irrigation system that was 60-100 cm above the ground. [40]. Approximately 40 % of natural daily precipitation events were 1 cm or larger, so treatments represented a relatively small increase in precipitation intensity (see SI). Treatment plots were paired with untreated plots without roofs [40]. Responses to treatments were measured from 2009 to 2013.



**Fig 1. Long-term average and monthly observed precipitation patterns.** Long-term average (dashed lines) and observed (solid lines) monthly precipitation for Satara Rest Camp, Kruger National Park, South Africa. Treatments were installed March 2008 and ended June 2013. Dates are shown in month-year format.

*Soil moisture:* Soil moisture was measured at eight depths between 5 and 140 cm in one treatment and one control plot. Measurements were taken hourly during the growing season and every three hours during the winter. Soil water content was measured using capacitance/frequency domain sensors (Decagon Devices EC-5 sensors, Pullman, WA, USA) and soil water potential was measured using heat-dissipation sensors (Campbell Scientific 229 sensors, Logan, UT, USA [40]). Heat dissipation sensors were

individually calibrated for specific depths using soil from the site, and were assumed to measure water potential because salinity was low and osmotic potential was assumed negligible [48]. Soil water potential measurements were made to estimate plant available water (PAW [49]). Water was assumed plant available at or above water potentials of -3 MPa. A common midday leaf water potential at our study site was -2.5 MPa, so -3 MPa was used as a more conservative estimate of PAW [29]. PAW calculations were not sensitive to the specific water potential value used because there is only a small difference in water content (*i.e.*, 0.008 g g<sup>-1</sup>) between -3.0 MPa and -2.5 MPa [41].

*Aboveground Growth:* A disc pasture meter (DPM) measured bulk grass height in treatment and control plots. A DPM measures the height aboveground that a grass canopy supports a metal plate, and measurements are correlated with biomass harvest measurements [50]. DPM measurements were taken approximately four times annually on 20 or 40 subplots per plot.

Sixty trees and shrubs (five per plot) were outfitted with small-diameter dendrometer bands (Agricultural Electronics, Tucson, AZ, USA), which measured woody plant circumference increment in treatment and control plots. Circumference increment is a measure of change in trunk circumference since last collection date. Hereafter, data from woody plants (trees and shrubs) will be referred to as trees for simplicity. Data from trees that died during the study were removed because dendrometer installation appeared to damage some trees. Dendrometer data was collected approximately four times per year.

*Plant Root Responses (rhizotron):* Root image analyses and a hydrologic tracer experiment were used to assess plant root responses to treatments. Two-meter-long, clear, cellulose acetate butyrate tubes were installed at a 30° angle in each of the twelve plots.

Root images were taken at 2 cm increments to produce at least 50 images in each plot approximately four times per year in the 2010, 2011, and 2012 seasons using a BTC-100x video microscope camera (Bartz Technology Co, Carpentaria, CA, USA). Root photos were taken at 15x magnification with a 2-cm image width.

*Plant Root Responses (tracer experiment):* To measure vertical patterns of water uptake by grasses and trees in treatment and control plots, a tracer experiment was conducted. Five treatment and five control plots were assigned to a target depth (5, 15, 30, 45, and 60 cm), and a tracer solution was injected to that depth. This was repeated during an early-season sampling (December 2011) and a late-season sampling (April 2012). In each plot, 10 mm-width pilot holes were drilled to an assigned depth in a 15 cm by 15 cm grid. Two ml of 70 % D<sub>2</sub>O tracer (70 % deuterium, 30 % hydrogen; Cambridge Isotopes, MA, USA) followed by two ml of tap water was injected into each hole using custom needles (16-gauge, regular width hypodermic tubing; Vita Needle, Needham, MA, USA), after which each pilot hole was refilled with soil and left for two days [28, 40, 51]. Following the injection and uptake period, non-transpiring grass and tree samples were collected in the plots. Grass samples were composited by species so that each sample included plant material from several individuals. Tree samples were composited by species and each sample included plant material from several twigs from one to three individuals.

Results from 100 December 2011 samples were published previously. Here we report results from a total of 600 samples from the December 2011 and April 2012 pulsing campaigns. This experiment represented the first time plots were reused in a depth-controlled tracer experiment (*i.e.*, December 2011 and April 2012) so it was

important to determine if any tracer from the first injection campaign persisted in the second injection campaign. To test for this effect and to confirm the location of tracer injections, soil samples from both pulse campaigns were taken one week after injection.

All soil and plant samples from the tracer experiment were immediately sealed in custom-made glass tubes with parafilm and transported on ice to a freezer within 6 hours. Water was extracted from soil and plant samples by cryogenic distillation within two weeks of sampling, and shipped directly to cold storage [28, 52]. Samples were refrigerated at 4° C until November 2015, where they were analyzed using a cavity ringdown spectrometer (CRDS—Picarro Instruments, Santa Clara, CA, USA) with vaporization module running at 110° C. A subset of 100 samples were analyzed in November 2012 for Deuterium (<sup>2</sup>H) and <sup>18</sup>O concentrations at the University of Alaska Anchorage ENRI lab, and results described in Kulmatiski and Beard [40]. Isotope values from the subset samples re-analyzed in 2015 were highly correlated with values determined in 2012 ( $R^2 = 0.97$ ; see SI).

#### **Data Analyses and Statistics**

*Soil moisture:* PAW data are reported but not tested statistically because samples were taken from one treatment and one control plot.

*Aboveground Growth Analyses:* Disc pasture meter and tree circumference data were analyzed using linear mixed models with a two-way factorial design. For DPM, we used a split plot in time design. For tree circumference we used a randomized design with repeated measures. Data were averaged by plot and date and square root transformed prior to analysis to better meet assumptions of normality. While both datasets were analyzed using linear models, specific model selection was based on the nature of data

distribution for each. Fixed effects were treatment type, date, and the interaction of treatment and date, and plot was a random effect. Time was considered a fixed effects factor because we were concerned with how our shelter treatment affected grass, tree, and root growth, and because it would presumably change in a meaningful way from the beginning of our treatment installation to the end of the study.

*Plant Root Analyses (rhizotron):* The number, diameter, and length of plant roots in each minirhizotron 'window' (a proxy for soil depth) in each plot on each sampling date was assessed using RootFly software (Birchfield and Wells, Clemson University, Clemson, SC, USA) [40]. This analysis was redone for the 2010 and 2011 growing seasons alongside the unanalyzed data from 2012. To find mean root number by depth for each treatment, root counts by window were averaged in 10 cm-depth increments (*i.e.*, windows 1-10 went into the 10 cm depth average) with increments running 10-100 cm. Continuous growth in mean root volume was calculated by depth using the length and diameter for each root, then averaged in 10 cm-depth increments from 10-100 cm. All rhizotron data were analyzed by depth using a linear mixed model with a two-way factorial in a split-plot in time design. Fixed effects were treatment, date, and the interaction of treatment and date. These data were log transformed prior to analysis to more adequately meet assumptions of normality.

*Plant Root Analyses (tracer experiment):* Isotope concentrations of plant and soil water samples from the spectrometer were calibrated to standards of known concentration using Picarro Chemcorrect software and reported in delta ( $\delta$ ) notation [51]. To control for natural isotopic enrichment, for example due to evaporation, the deuterium excess (*i.e.*, relative to <sup>18</sup>O) value was calculated:

$$\boldsymbol{D}_{excess} = \boldsymbol{D} - (\boldsymbol{8} \times {}^{18}\boldsymbol{O})$$

where D is the calibrated deuterium value (per mil) for a given sample, and <sup>18</sup>O is the calibrated <sup>18</sup>O value for the same sample [53–55].

Several steps were taken to prepare isotope data for analyses. First, we tested samples for injected tracer by comparing  $D_{excess}$  values to 2 SDs above mean ambient deuterium concentrations. Second, deuterium concentrations in plant samples were used to calculate the proportion of tracer uptake by depth. Proportion and not concentration data were used to control for differences in tracer concentrations among plant species due to traits such as stored water volume [28]. Proportional uptake by depth was calculated for each treatment and functional group (*i.e.*, 5 cm, control, grass). Proportion of tracer uptake was averaged by depth and calculated as:

$$P_{di} = \frac{\overline{S_i}}{\sum_{n=i}^J S_d}$$

where  $S_i$  is the  $D_{excess}$  value for each sample,  $S_d$  is the mean  $D_{excess}$  value of samples from soil depth d (*i.e.*, 5 cm). The  $S_d$  values for each soil depth were summed from 1-j (*i.e.*, 5 -60 cm). Mean  $P_{di}$  values by depth are reported. Finally, every injection depth represented a range of the soil profile, with roots in each range taking up tracer from that injection. We divided by the number of cm each depth represented (*i.e.*, 5 cm represented 0-10 cm), thus converting root water uptake to per-cm basis, and allowing us to determine the depth at which 50 % uptake occurred [29].

To test whether or not tracer was injected to target depths and whether or not tracer from the first campaign was present during the second campaign, we measured tracer concentration by depth in all plots. To test for differences in tracer concentration among plots, sample depth was standardized:

$$S = d_n - d_n$$

where *S* is the standardized depth increment,  $d_n$  is the depth from which the sample was taken, and  $d_p$  is the pulse injection depth (*i.e.*, a sample from 5-10 cm where injection depth was 5 cm became 0-5 cm). This allowed us to compare uptake proportions by soil depth for each plot. Proportions were calculated as:

$$P = \frac{D_{excess}}{D_{total}}$$

where  $D_{excess}$  is the amount of deuterium in the sample and  $D_{total}$  is the summed  $D_{excess}$ across all samples in a given plot date. Proportions were averaged at each depth across all plots for both campaigns. Proportional tracer uptake by depth was meant to peak at 0 cm after standardization. Differences in tracer concentration among depths were tested using a one-way linear mixed model with a first order autoregressive covariance structure to accommodate spatial autocorrelation among depths. Data were log transformed prior to analysis to better meet assumptions of normality.

Because there was only one plot assigned to each target depth, results from this tracer experiment do not provide inference to water uptake on the landscape. Rather, results provide inference only to the plots in this experiment. Samples within plots, therefore, were used as replicates of each experimental plot. Plant-derived tracer data was grouped by injection campaign (December 2011 or April 2012), functional group, and treatment. Data were analyzed using a linear mixed model with fixed effects of treatment type, depth, and the interaction of treatment and depth. All statistical analyses were performed in SAS JMP 9.4 (SAS Institute, Inc., Cary, NC, USA). Differences are considered significant at the  $\alpha = 0.05$  level throughout.

#### RESULTS

#### Soil Moisture

PAW was greater in the treatment than control plot by 14, 53, 80, 235, 61, and 19 % in the pretreatment year and five subsequent growing seasons, respectively (Fig 2a-f).



Fig 2. Annual plant available water (PAW) by depth. Measurements were taken in one treatment and one control plot for the (a) 2008 (pretreatment), (b) 2009, (c) 2010, (d) 2011, (e) 2012, and (f) 2013 growing seasons. Water was assumed plant available when  $\Psi > -3$  MPa.

*Grass:* DPM measurements of grass growth were greater in control than treatment plots (P < 0.001). Measurements were greater on control than treatment plots on several dates in the 2009, 2010, and 2011 growing seasons and on one date early in the 2012 growing season (Fig 3).



Fig 3. Grass height measured using a disc pasture meter. Treatment plots with increased precipitation intensity are denoted with dashed lines, open circles, and control plots with ambient precipitation are denoted with solid lines, filled circles. Vertical dotted lines separate growing seasons. Mean  $\pm 1$  S.E. shown. Significant differences between treatment and control averages are denoted with an asterisk ( $\alpha = 0.05$ ).

*Tree:* Circumference increment measurements were greater in treatment than

control plots in the 2011 growing season and early in the 2012 growing season.

Circumference increment did not differ on prior or subsequent dates. This resulted in 15 % greater cumulative circumference increment in treatment than control plots by the end of the study (P < 0.001; Fig 4).



Fig 4. Circumference increment of trees. Treatment plots with increased precipitation intensity are denoted with dashed lines, open circles, and control plots with ambient precipitation are denoted with solid lines, filled circles. Inset shows cumulative circumference increment growth throughout the study period from pretreatment through growing season five. Vertical dotted lines separate growing seasons. Mean  $\pm 1$  S.E. shown. Significant differences between treatment and control averages are denoted with an asterisk ( $\alpha = 0.05$ ).

#### **Root Growth**

In the 2010, 2011, and 2012 growing seasons, root recruitment differed by depth between treatment types (P < 0.001, P < 0.001, P = 0.006, respectively; Fig 5a-c). In the

2010 and 2012 growing seasons, mean root volume differed by depth between treatment types (P < 0.001 and P < 0.001), but did not during the 2011 growing season (Fig 5d-f). In the 2010 growing season, root recruitment between growing seasons was greater in treatment than control plots in 20-60 cm soil depths, and root volume was greater in control than treatment plots at 50, 70, and 100 cm soil depths. In the 2011 growing season, root recruitment was greater in treatment than control plots at 30 and 40 cm soil depths, and greater in control than treatment plots at 80 cm. In the 2012 growing season, root recruitment was greater in control than treatment plots at 90 cm, and root volume was greater in control than treatment plots in 70-80 cm soil depths.



**Fig 5. Number of new roots and mean root volume.** New roots (a-c) and root volume (d-f) by depth in treatment (dashed lines, open circles) and control plots (solid lines, filled circles) during the observed 2010 (a, d), 2011 (b, e), and 2012 (c, f) growing seasons. Mean  $\pm 1$  S.E. Asterisks indicate differences between treatment and control values at the  $\alpha = 0.05$  level.

Across treatments, 197 grass samples, 316 tree samples, and 87 soil samples were analyzed for isotope concentrations. Analysis was based on extracted water volumes, which ranged from one to four ml. Tracer was constrained to 10 cm above and 15 cm below target depths with the greatest concentrations within 10 cm of the target depth (P < 0.001; Fig 6).



Fig 6. Tracer concentrations ([tracer]) relative to injection depth. Positive numbers on the x-axis indicate soil depths lower than the injection point, negative numbers indicate shallower soil depths. Mean  $\pm 1$  S.E.

*Grass Uptake:* There was no difference in tracer uptake between treatment and control plots at any soil depth (Fig 7a-b). Grass uptake patterns were consistently greater

in shallow soils in December and greater in deeper soils in April in both treatment (P < 0.001) and control (P < 0.001) plot comparisons. Fifty percent of grass tracer uptake occurred in the top 12 and 41 cm of soil in December and April, respectively.

*Tree Uptake:* Trees in treatment plots absorbed more late-season tracer from 60 cm than trees in control plots (Fig 7c-d). Fifty percent of tree uptake occurred in the top 27 and 32 cm of soil in December and April, respectively. Tree uptake differed between December and April in both treatment (P = 0.02) and control (P = 0.003) plots, where more tracer was absorbed from shallow depths in December, and more tracer was absorbed from greater soil depths in April.



**Fig 7. Deuterium tracer uptake.** The proportion of deuterium tracer uptake by depth in treatment and control plots for grasses (a, b) and trees (c, d), December 2011(a, c) and April 2012 (b, d). Treatment plots indicated with dashed lines, open circles and control plots indicated with solid lines, filled circles. Tracer proportion reported on a by-cm-depth-increment proportion. Mean  $\pm 1$  S.E. shown.

#### DISCUSSION

We conducted a five-year experiment that collected precipitation and redeposited that precipitation as 1 cm events. A previous study revealed that during the first three treatment years, small increases in precipitation intensity 'pushed' water deeper into the soil profile, decreased grass growth, and increased tree growth [40]. Here we report results from the following two years. During the last two growing seasons, most natural precipitation occurred as events that were greater than 1 cm, and as a result treatments had very little effect on PAW with depth. This allowed an opportunity to test grass and tree responses to treatment removal. Treatment differences in grass and tree growth rates quickly disappeared. Grasses and trees, therefore, responded rapidly (*i.e.*, within a single growing season) to changes in precipitation intensity and demonstrated little to no legacy effect of treatments on growth rate. However, there was a legacy effect of treatments on total tree growth. At the end of the experiment, trees were 15 % larger in treatment than control plots and this reflected a large treatment response in the third year of the experiment when treatments exerted their greatest effect on PAW.

The rapid disappearance of treatment effects on current growth supported conclusions by Kulmatiski and Beard [40]. That study concluded that after three years of treatments, tree growth increased with precipitation intensity. The fact that grass and tree growth rate responses quickly disappeared when treatments were effectively removed provides further support for the idea that observed plant growth responses were caused by increased precipitation intensity and not shelter effects or random differences in plant growth among plots. The rapid disappearance of treatment effects also suggests that grass and tree growth rates in savannas are highly resilient from short-term (*i.e.*, three-year)

changes in precipitation intensity but that occasionally deep soil water infiltration can produce long-lasting benefits to woody plant biomass.

Grass growth responded quickly to treatments (Fig 3). During the first three years of the experiment, grass growth was shorter in treatment than in control plots. By the end of the 2012 and into the 2013 growing season, when treatments had small effects on plant available water, grasses showed no treatment effects. The trends we saw in grass growth were evident even in the first two growing seasons when trees showed no response to treatments, suggesting a direct effect on grasses, rather than an indirect effect through competition with trees. Woody plants were either slower to respond or required larger changes in plant available water to respond to increased precipitation intensity (Fig 4). While significant woody plant growth in treatment plots was detected in the largest treatment season (2011; Fig 2d), differences in tree growth rate between treatment types disappeared soon after the beginning of the 2012 growing season. This response suggests a minimal-intensity threshold for woody growth rate and a potential time lag of several months between treatment removal and woody growth rate response.

Root recruitment and volume (*i.e.*, rhizotron data) were determined for the 2010 and 2011 seasons of expected treatment effects on soil moisture, and the 2012 season of decreasing treatment effects on soil moisture. Consistent with this, rhizotron data revealed a greater difference in the distribution of new roots in the 2010 and 2011 than in the 2012 season. Interestingly, total root number across depths was consistent between treatments in all 3 seasons, suggesting that total root mass is constrained, and that plants have to be selective about where new roots are added. In addition, it appeared that plants from treatment plots had less deep root mass (50-100 cm) and greater shallow root recruitment (10-40 cm). Since grasses create the majority of root biomass in this system [56], rhizotron data suggest grasses are recruiting roots between 20-40 cm depth at the expense of roots between 50-100 cm depth. While the effect disappears in 2012, grasses may be emphasizing root recruitment at 10 cm depth in an effort to get access to more nutrient-rich soils in this no-treatment year.

We are aware of only one study that has combined depth-specific tracer data with rhizotron data and that study included only 100 tracer samples and one year of rhizotron data [40]. Here we provide a more robust comparison that includes six times as much isotope data from two sampling campaigns and three years of rhizotron data. While root volume showed no difference between treated and control plots, root recruitment and D<sub>2</sub>O tracer uptake revealed consistent patterns for grasses. Both root recruitment from the 2012 growing season and the tracer experiment implemented that same season show a peak in tracer uptake around 40 cm, and greater uptake in treatment plots above 40 cm. Interestingly, root volume did not show a peak at 40 cm, further highlighting the importance of root recruitment over root volume. Regarding the tracer experiment, it is also worth mentioning that the ability of treatment plot trees to significantly increase deep water uptake presumably allowed them to take advantage of increased infiltration resulting from shelter treatments (Fig 7a-d; [29, 56]), even in a weak treatment season.

Interestingly, we found rare evidence for a theory posed by Heinrich Walter [57] that plant functional groups change water-use strategy between early and late growing season. Grass roots were more active in shallow soils (5 and 15 cm) early in the growing season, and shifted to lower soil depths (45 and 60 cm) later in the season. Calculations of 50% uptake depths reflected this pattern. Of three studies using this technique [28, 29,

40] this is the only study to show grasses changing uptake patterns within a year. Also noteworthy, trees in control plots were, like the grasses, more active in shallow soils (5 cm) early in the season, and trees in treatment plots were more active in deeper soils (60 cm) late in the season (see SI).

Results show that small increases in precipitation intensity can result in large increases in plant available soil water. In addition to causing these large changes in soil water, we provide evidence that removal of small precipitation events (*i.e.*, < 5 mm), which are one of the most important factors contributing to water balance in semi-arid ecosystems [58], could cause changes in water uptake by functional group from early season to late season. These rapid precipitation intensity-influenced plant growth patterns could also play a role in facilitating woody plant encroachment and increasing the rate of aquifer recharge by driving water deeper into the soil where it is less available to grass roots [40]. Our study provides evidence that savannas respond quickly to small increases in mean precipitation intensity, and that continued climate change is likely to increase shrub encroachment in many parts of the world.

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APPENDICES



**Fig A.1. Daily precipitation events recorded at the Satara Rest Camp.** Events were measured over the 5 years (2008-2013) of the study period. Dark markers represent events larger than our treatment-imposed event size of 1 cm. Dates are shown in month-year format.

Species	Common/Alt. Names	Family	<b>Growth Form</b>
Ormocarpum trichocarpum	Hairy caterpillar-pod	Fabaceae	Tree
Combretum imberbe	Leadwood	Combretaceae	Tree
Cynodon sp.	Bermuda grass, dog's tooth grass	Poaceae	Grass
Dichrostachys sineria	Stickle bush	Fabaceae	Tree
Lannea schweinfurthii	False marula	Anacardiaceae	Tree
Sclerocarya birrea	Marula, maroela	Anacardiaceae	Tree
Maerua angolensis	Bead-bean	Capparaceae	Tree
Colophospermum mopane	Mopane, balsam tree, butterfly tree	Fabaceae	Tree
Senegalia nigrescens	Acacia nigrescens, knob thorn	Fabaceae	Tree
Panicum sp.	Panic grass	Poaceae	Grass
Ehretia rigida	puzzle bush	Boraginaceae	Tree
Themeda triandra	Red grass, red oat grass	Poaceae	Grass
Heteropogon contortus	Black speargrass, tanglehead	Poaceae	Grass
Eragrostis cilianensis	Stinkgrass, candy grass, gray lovegrass	Poaceae	Grass
Vachellia tortilis	Acacia tortilis, umbrella thorn	Fabaceae	Tree
Flueggea virosa	Securinega virosa, white-berry bush	Euphorbiaceae	Tree
Dalbergia melanoxylon	African blackwood	Fabaceae	Tree
Bothriochloa radicans	Smelly grass	Poaceae	Grass
Digitaria eriantha	Fingergrass, crabgrass	Poaceae	Grass
panicum maximum	Guinea grass	Poaceae	Grass
Albizia havein	A. harveyi, Common False-thorn	Fabaceae	Tree
Cassia abbreviata	Sjambok Pod	Fabaceae	Tree
Euclea divinorum	Magic guarri	Ebenaceae	Tree
Grewia monticola	Grey raisin, silver raisin	Malvaceae	Tree
Grewia flavescens	Donkey-berry	Malvaceae	Tree
Brachystegia spiciformis	Zebrawood	Fabaceae	Tree
Commiphora sp.	Myrrh	Burseraceae	Tree
Ximenia sp.	Large sourplum	Olaceae	Tree

Table A.1. Studied species and their respective common names, families, and growth forms.



**Fig A.2. Comparison of isotope samples.** Measurements reported are (a) Deuterium concentration and (b) Deuterium excess above <sup>18</sup>O values and ambient isotope concentrations. Samples were measured repeatedly 3 years apart.

Standard Name	δ <sup>18</sup> O Mean	δD <sup>2</sup> H Mean
Dummy	-16.06	0
Low	-16.06	83.23
Medium	-16.06	291.32
High	-16.06	707.54

Table A.2. Calibration standards used with the Picarro CRDS.



Fig A.3. Deuterium tracer uptake patterns for notable species. Included are (a) Dichrostachys sinerea, and (b) Flueggea virosa measured in treatment plots (dashed lines, open circles) and control plots (solid lines, filled circles). Mean  $\pm 1$  S.E. shown.

Depth (cm)	10	20	30	40	75	100+
Bulk density	0.8	0.9	1	1.1	1.2	1.3
Porosity	0.698	0.660	0.623	0.585	0.547	0.509

Table A.3. Bulk density and porosity.

Measurements were taken near the Satara Rest Camp, Kruger National Park, South Africa, and data were measured down as low as possible, with bedrock present intermittently starting at approximately 1.1 meters in depth.



Fig A.4. Comparison of tracer uptake. Grasses (a-b) and trees (c-d) were measured between injection dates on control plots (a, c) and treatment plots (b, d). April 2012 uptake indicated with dashed lines, open circles and December 2011 uptake indicated with solid lines, filled circles. Tracer proportion reported on a by-cm-depth-increment proportion. Mean  $\pm 1$  S.E. shown.

## Appendix B. STATISTICAL RESULTS

## Table B.1(a-d). Statistics for grass DPM data.

a. Grass DPM: Fit Statistics			
-2 Res Log Likelihood	28.54		
AIC (smaller is better)	32.54		
AICC (smaller is better)	32.59		
BIC (smaller is better)	33.51		
CAIC (smaller is better)	35.51		
HQIC (smaller is better)	32.19		
Generalized Chi-Square	10.74		
Generalized Chi-Square / DF	0.04		

b. Grass Disc Pasture Meter: Least Squares Means						
Treatment Type Estimate S.E. DF t-value P-value						
Control	3.2394	0.03657	10	88.57	< 0.0001	
Treatment	3.0355	0.03654	10	83.07	< 0.0001	

c. Grass Disc Pasture Meter: Type III tests of fixed effects						
EffectNum DFDen DFF-valueP-value						
Treatment	1	10	15.56	0.0028		
Date	24	239	76.83	< 0.0001		
Trt*Date	24	239	3.93	< 0.0001		

d. Grass DPM: Tests of Effect Slices for Treatment*Date Sliced by Date						
Date	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>
01-Dec-07	4.09	0.108	4.42	0.101	0.38	0.5377
12-Jan-09	7.86	0.331	11.93	0.285	26.2	< 0.0001
26-Feb-09	10.68	0.269	15.19	0.309	23.95	< 0.0001
17-Mar-09	11.76	0.358	14.08	0.302	7.01	0.0086
01-Apr-09	10.60	0.293	14.39	0.291	17.64	< 0.0001
24-Apr-09	9.13	0.244	13.47	0.259	25.68	< 0.0001
19-Jun-09	8.30	0.289	10.18	0.246	5.97	0.0153
02-Nov-09	3.68	0.125	4.98	0.126	5.87	0.0161
22-Dec-09	7.64	0.194	8.11	0.174	0.44	0.5054
14-Jan-10	9.37	0.238	9.82	0.288	0.14	0.7125
05-Mar-10	11.16	0.269	11.72	0.261	0.43	0.5149
14-Apr-10	10.79	0.258	12.54	0.303	3.87	0.0503
03-Dec-10	7.05	0.152	7.84	0.138	1.31	0.2526
08-Mar-11	12.18	0.369	14.66	0.375	6.5	0.0114
19-Apr-11	10.94	0.298	13.52	0.357	8.41	0.0041
28-Oct-11	4.32	0.155	4.58	0.138	0.16	0.6923
06-Mar-12	9.58	0.271	11.88	0.364	7.32	0.0073
30-Mar-12	9.37	0.254	9.19	0.245	0.03	0.8603
30-Oct-12	6.86	0.224	7.10	0.222	0.1	0.7552
03-Dec-12	6.70	0.302	7.44	0.253	1.3	0.2562
24-Jan-13	9.78	0.329	9.32	0.238	0.25	0.6184
08-Mar-13	13.32	0.464	13.12	0.449	0	0.9627
26-Apr-13	15.63	0.441	14.14	0.409	2.28	0.1327
27-May-13	13.90	0.531	13.31	0.389	0.33	0.5654
11-Jun-13	13.38	0.402	13.65	0.300	0.1	0.7489

## Table B.2(a-d). Statistics for tree dendroband data.

a. Tree Dendroband: Fit Statistics			
-2 Res Log Likelihood	364.22		
AIC (smaller is better)	368.22		
AICC (smaller is better)	368.26		
BIC (smaller is better)	369.19		
CAIC (smaller is better)	371.19		
HQIC (smaller is better)	367.86		
Generalized Chi-Square	39.49		
Generalized Chi-Square / DF	0.14		

b. Tree Dendroband: Type III Tests of Fixed Effects						
Effect	Num DF Den DF F-value P-value					
Treatment	1	10	2.65	0.1346		
Date	29	276	23.73	< 0.0001		
Trt*Date	29	276	1.09	0.3428		

c. Tree Dendroband: Least Squares Means						
Treatment TypeEstimateS.E.DFt-valueP-value						
Control	1.8974	0.05667	10	33.48	< 0.0001	
Treatment	2.0284	0.05706	10	35.55	< 0.0001	

d. Tree Dendroband: Tests of Effect Slices for Treatment*Date Sliced by Date							
Date	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>	
01-Dec-07	4.20	0.160	4.22	0.237	0	0.9792	
12-Jan-09	1.44	0.327	2.03	0.441	0	0.9474	
28-Jan-09	1.47	0.394	2.50	0.523	0.24	0.6217	
26-Feb-09	4.54	0.934	5.84	0.738	0.57	0.4521	
17-Mar-09	1.03	0.239	1.30	0.212	0	0.9576	
01-Apr-09	0.52	0.220	0.32	0.165	0.26	0.6074	
24-Apr-09	-0.52	0.175	-0.39	0.350	0.04	0.8444	
19-Jun-09	-0.34	0.234	-0.35	0.373	0.04	0.8399	
26-Oct-09	2.24	0.351	2.04	0.153	0.01	0.9048	
02-Nov-09	0.63	0.126	0.52	0.078	0	0.9658	
17-Nov-09	0.24	0.101	0.29	0.146	0	0.982	
22-Dec-09	4.00	0.340	4.50	0.478	0.12	0.7295	
14-Jan-10	3.99	0.717	4.25	0.711	0	0.9655	
02-Feb-10	1.42	0.462	1.08	0.379	0.71	0.4012	
05-Mar-10	1.07	0.202	0.84	0.218	0.24	0.6264	
14-Apr-10	2.83	0.250	2.07	0.249	0.6	0.438	
20-Oct-10	2.45	0.227	2.14	0.162	0.04	0.8421	
03-Dec-10	2.19	0.402	1.74	0.524	0.64	0.425	
20-Dec-10	11.23	1.399	7.80	1.404	10.92	0.0011	
08-Mar-11	13.63	1.499	9.45	1.640	25.17	< 0.0001	
19-Apr-11	1.93	0.278	1.26	0.229	0.7	0.4048	
08-Dec-11	4.56	0.999	3.85	1.412	1.48	0.2244	
10-Mar-12	0.28	0.045	0.23	0.050	0	0.9668	
31-Oct-12	2.05	0.394	2.04	0.521	0	0.9614	
03-Dec-12	2.42	0.317	1.82	0.217	0.47	0.4941	
24-Jan-13	4.22	1.042	3.21	1.018	8.51	0.0038	
08-Mar-13	3.44	0.719	2.46	0.816	2.14	0.1447	
26-Apr-13	1.14	0.345	1.93	0.920	0.4	0.5281	
27-May-13	0.33	0.198	-0.04	0.259	0.01	0.9232	
11-Jun-13	-0.07	0.130	0.19	0.152	1.35	0.2459	

a. Rhizotron 09-10: Fit Statistics			
-2 Res Log Likelihood	1173.81		
AIC (smaller is better)	1177.81		
AICC (smaller is better)	1177.83		
BIC (smaller is better)	1178.78		
CAIC (smaller is better)	1180.78		
HQIC (smaller is better)	1177.45		
Generalized Chi-Square	256.09		
Generalized Chi-Square / DF	0.51		

 Table B.3(a-d). Statistics for rhizotron root recruitment data from the 2009-2010

 growing season.

b. Rhizotron 2009-2010: Type III tests of fixed effects							
Effect	Num DF Den DF F-value P-valu						
Treatment	1	10	10.06	0.01			
Depth	10	490	13.21	< 0.0001			
Trt*Depth	10	490	2.67	0.0034			

c. Rhizotron 2009-2010: Least Squares Means						
Treatment TypeEstimateS.E.DFt-valueP-value						
Control	1.8871	0.1302	10	14.5	< 0.0001	
Treatment	2.4799	0.1342	10	18.48	< 0.0001	

d. Rhizotron 09-10: Tests of Effect Slices for Treatment*Date Sliced by Date						
Depth		TRT		CTRL		
( <b>cm</b> )	Treatment	S.E.	Control	S.E.	<b>F-value</b>	<b>P-value</b>
10	8.433333333	1.710	7.333	1.412	1.48	0.2243
20	22.06666667	3.557	8.833	1.185	14.28	0.0002
30	17.83333333	2.765	9.067	1.181	7.49	0.0064
40	23.43333333	4.067	12.933	2.263	13.7	0.0002
50	20.33333333	2.68	10.5	1.038	10.12	0.0016
60	14.46666667	2.007	11.033	1.373	3.91	0.0485
70	8.766666667	1.698	6.933	0.837	0.64	0.4243
80	6.766666667	1.257	7.433	0.913	0.94	0.3335
90	6.766666667	1.358	6.167	0.711	0.63	0.4286
100	6.3	1.386	3.3	0.666	0.65	0.4214

Table B.4(a-d). Statistics for rhizotron root recruitment data from the 2010-2011 growing season.

a. Rhizotron 10-11: Fit Statistics				
-2 Res Log Likelihood	1313.96			
AIC (smaller is better)	1317.96			
AICC (smaller is better)	1317.98			
BIC (smaller is better)	1318.93			
CAIC (smaller is better)	1320.93			
HQIC (smaller is better)	1317.6			
Generalized Chi-Square	342.05			
Generalized Chi-Square / DF	0.68			

b. Rhizotron 2010-2011: Type III tests of fixed effects						
Effect	Num DF Den DF F-value P-value					
Treatment	1	10	0.02	0.8847		
Depth	9	490	14.21	< 0.0001		
Trt*Depth	9	490	3.96	< 0.0001		

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c. Rhizotron 2010-2011: Least Squares Means						
Treatment Type Estimate S.E. DF t-value P-value						
Control	1.7644	0.1408	10	12.53	< 0.0001	
Treatment	1.7346	0.1423	10	12.19	< 0.0001	

d. Rhizotron 10-11: Tests of Effect Slices for Treatment*Depth Sliced by Depth						
		TRT		CTRL		
Depth (cm)	Treatment	S.E.	Control	S.E.	<b>F-value</b>	<b>P-value</b>
10	4.40	1.120	4.83	0.954	0.3	0.5823
20	14.27	2.122	12.27	1.752	0	0.9905
30	16.97	1.685	12.53	2.430	4.23	0.0403
40	16.43	2.168	9.77	1.540	4.29	0.0388
50	9.30	1.768	10.50	2.049	0	0.9488
60	6.07	0.826	10.30	1.720	0.82	0.3647
70	4.37	0.947	6.70	0.962	2.71	0.1001
80	2.47	0.428	8.50	1.090	8.87	0.003
90	3.03	0.522	4.07	0.706	0.02	0.8871
100	3.20	0.753	2.93	0.705	0.03	0.8723

a. Rhizotron 11-12: Fit Statistics			
-2 Res Log Likelihood	1450.11		
AIC (smaller is better)	1454.11		
AICC (smaller is better)	1454.13		
BIC (smaller is better)	1455.08		
CAIC (smaller is better)	1457.08		
HQIC (smaller is better)	1453.75		
Generalized Chi-Square	437.22		
Generalized Chi-Square / DF	0.86		

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Table B.5(a-d). Statistics for rhizotron root recruitment data from the 2011-2012	2
growing season.	

b. Rhizotron 2011-2012: Type III tests of fixed effects								
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>				
Treatment	1	10	0.05	0.8333				
Depth	9	497	11.01	< 0.0001				
Trt*Depth	9	497	2.63	0.0057				

c. Rhizotron 2011-2012: Least Squares Means								
Treatment Type Estimate S.E. DF t-value P-								
Control	1.9766	0.1665	10	11.87	< 0.0001			
Treatment	1.9258	0.1665	10	11.56	< 0.0001			

d. Rhizotron 11-12: Tests of Effect Slices for Treatment*Depth Sliced by Depth										
Depth (cm)	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>				
10	12.93	4.685	5.17	1.499	0.47	0.4932				
20	13.63	3.229	9.70	1.233	0.64	0.4257				
30	16.50	3.333	12.23	2.074	1.44	0.2305				
40	19.27	4.331	18.73	3.310	0.09	0.7647				
50	12.17	2.373	14.43	2.430	0.01	0.9128				
60	7.00	1.819	10.33	1.732	1.2	0.2738				
70	6.97	1.770	8.97	1.916	0.28	0.5994				
80	3.20	0.725	6.87	1.013	1.7	0.1933				
90	2.40	0.464	9.23	1.756	6.98	0.0085				
100	3.93	0.894	3.30	0.898	1.09	0.2974				

 Table B.6(a-d). Statistics for rhizotron mean volume data from the 2009-2010 growing season.

a. Rhizo Vol 09-10: Fit Statistics					
-2 Res Log Likelihood	25103.24				
AIC (smaller is better)	25107.24				
AICC (smaller is better)	25107.24				
BIC (smaller is better)	25108.21				
CAIC (smaller is better)	25110.21				
HQIC (smaller is better)	25106.88				
Generalized Chi-Square	15206.79				
Generalized Chi-Square / DF	2.21				

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h Rhizo V	<u>al 2009-2010</u>	• Tyne III te	ests of fixed	effects
Effect	Num DF	Den DF	F-value	P-value
Treatment	1	10	7.75	0.0193
Depth	10	6861	10.97	< 0.0001
Trt*Depth	10	6861	4.02	< 0.0001

c. Rhizo Vol 2009-2010: Least Squares Means								
<b>Treatment Type</b>	Estimate S.E. DF		t-value	<b>P-value</b>				
Control	-4.7878	0.09224	10	-51.9	< 0.0001			
Treatment	-5.1484	0.09098	10	-56.59	< 0.0001			

d. Rhizo Vol 09-10: Tests of Effect Slices for Treatment*Date Sliced by Date										
		TRT		CTRL						
Depth (cm)	Treatment	S.E.	Control	S.E.	<b>F-value</b>	<b>P-value</b>				
10	0.020	0.003	0.015	0.004	1.61	0.2043				
20	0.028	0.008	0.023	0.003	1.45	0.2284				
30	0.024	0.003	0.027	0.005	2.11	0.1461				
40	0.020	0.003	0.024	0.004	1.12	0.2898				
50	0.016	0.002	0.043	0.012	10.4	0.0013				
60	0.020	0.003	0.032	0.005	2.93	0.0867				
70	0.021	0.004	0.036	0.008	9.33	0.0023				
80	0.025	0.004	0.023	0.003	0.57	0.4504				
90	0.024	0.004	0.055	0.011	2.83	0.0926				
100	0.019	0.003	0.044	0.009	11.05	0.0009				

Table B.7(a-d). Statistics for	rhizotron mean	volume data	from the 201	10-2011
growing season.				

a. Rhizo Vol 10-11: Fit Statistics					
-2 Res Log Likelihood	18092.97				
AIC (smaller is better)	18096.97				
AICC (smaller is better)	18096.97				
BIC (smaller is better)	18097.94				
CAIC (smaller is better)	18099.94				
HQIC (smaller is better)	18096.61				
Generalized Chi-Square	10183.98				
Generalized Chi-Square / DF	2				

b. Rhizo Vol 2010-2011: Type III tests of fixed effects									
Effect	Nun	n DF	De	n DF	F-val	ue	P-val	ue	
Treatment		1		10	0	.05	0.8	332	
Depth		10		5070	4	.49	< 0.0	001	
Trt*Depth		10		5070	0	.95	0.4	816	
c. Rhizo Vol 2010-2011: Least Squares Means									
Treatment Type Estin		Estim	ate	S.E.	DF	t-v	value	P-v	alue
Control		-3.73	306	0.1222	2 10	-	30.53	<0	.0001
Treatment		-3.76	579	0.1223	3 10	-	30.81	<0	.0001

d. Rhizo Vol 10-11: Tests of Effect Slices for Treatment*Date Sliced by Date									
Depth		TRT		CTRL					
(cm)	Treatment	S.E.	Control	S.E.	<b>F-value</b>	<b>P-value</b>			
10	0.116	0.028	0.196	0.086	0.41	0.5208			
20	0.120	0.042	0.139	0.029	0.14	0.7046			
30	0.112	0.020	0.116	0.025	0.21	0.6486			
40	0.081	0.018	0.183	0.058	1.2	0.2726			
50	0.080	0.023	0.127	0.028	0.51	0.4746			
60	0.038	0.006	0.072	0.010	1.5	0.2214			
70	0.125	0.070	0.088	0.027	0.2	0.6544			
80	0.061	0.014	0.170	0.067	0.05	0.822			
90	0.072	0.025	0.083	0.016	0.1	0.7551			
100	0.199	0.075	0.133	0.043	1.32	0.2503			

 Table B.8(a-d). Statistics for rhizotron mean volume data from the 2011-2012

 growing season.

a. Rhizo Vol 11-12: Fit Statistics				
-2 Res Log Likelihood	22005			
AIC (smaller is better)	22009			
AICC (smaller is better)	22009.01			
BIC (smaller is better)	22009.97			
CAIC (smaller is better)	22011.97			
HQIC (smaller is better)	22008.65			
Generalized Chi-Square	13921.35			
Generalized Chi-Square / DF	2.35			

b. Rhizo Vol 2011-2012: Type III tests of fixed effects							
Effect	<b>P-value</b>						
Treatment	1	10	0.38	0.5495			
Depth	11	5909	1.7	0.0666			
Trt*Depth	10	5909	3.96	< 0.0001			

c. Rhizo Vol 2011-2012: Least Squares Means						
Treatment Type Estimate S.E. DF t-value P-valu						
Control	-	-	-	-	-	
Treatment	-3.6598	0.1709	10	-21.41	< 0.0001	

d. Rhizo Vol 11-12: Tests of Effect Slices for Treatment*Date Sliced by Date							
Depth		TRT		CTRL			
(cm)	Treatment	S.E.	Control	S.E.	<b>F-value</b>	<b>P-value</b>	
10	0.218	0.071	0.197	0.073	1.79	0.1805	
20	0.146	0.030	0.191	0.054	0.13	0.7166	
30	0.271	0.068	0.152	0.032	0.13	0.7172	
40	0.147	0.034	0.116	0.027	1.49	0.2222	
50	0.177	0.055	0.289	0.099	0.44	0.5057	
60	0.137	0.029	0.518	0.197	3.55	0.0597	
70	0.187	0.045	0.253	0.052	3.9	0.0482	
80	0.252	0.098	0.209	0.054	5.35	0.0207	
90	0.134	0.049	0.255	0.055	0.03	0.8675	
100	0.146	0.035	0.327	0.099	0.24	0.6235	

Table B.9(a-c). Statistics for standardized tracer pulse accuracy from soil data.

a. TPA: Fit Statistics					
-2 Res Log Likelihood	348.42				
AIC (smaller is better)	352.42				
AICC (smaller is better)	352.52				
BIC (smaller is better)	353.83				
CAIC (smaller is better)	355.83				
HQIC (smaller is better)	352.4				
Generalized Chi-Square	141.09				
Generalized Chi-Square / DF	1.17				

b. TPA: Type III tests of fixed effects							
Effect	Effect Num DF Den DF F-value P-value						
Depth	20	107	4.28	< 0.0001			

c. Conservative T Grouping for depth Least Squares Means (Alpha=0.01)				
LS-I	means with	the		
signifi	e letter are	HOU Pren	f	
denth	Estimate			
0	-1.4166		Α	
-5	-1.4521		A	
5	-2.0166	В	A	
-10	-2.3183	B	A	
10	-2.6896	B		
15	-3.1355	B		
-55	-3.5329	В		
-50	-3.6879	В		
30	-3.7023	В		
35	-3.7217	В		
20	-3.8795	В		
25	-3.8833	В		
-45	-4.0706	В		
-15	-4.1766	В		
-25	-4.2986	В		
-20	-4.2995	В		
-40	-4.3159	В		
40	-4.356	В		
-35	-4.3668	В		
-30	-4.4186	В		
45	-4.435	В		

Table B.10(a-d)	. Statistics for	<sup>•</sup> grass tracer u	ptake data	from De	cember 2011.
		Simply indexia	prane aara		

a. D2O Grass Dec: Fit Statistics				
-2 Res Log Likelihood	102.64			
AIC (smaller is better)	124.64			
AICC (smaller is better)	128.31			
BIC (smaller is better)	151.38			
CAIC (smaller is better)	162.38			
HQIC (smaller is better)	135.39			
Generalized Chi-Square	16.69			
Generalized Chi-Square / DF	0.2			

b. D2O Grass Dec: Type III Tests of Fixed Effects							
Effect	Num DF Den DF F-value F						
Treatment	1	74	0.12	0.7313			
Depth	4	74	43.5	< 0.0001			
Trt*Depth	4	74	1.42	0.2359			

c. D2O Grass Dec: Least Squares Means							
Treatment TypeEstimateS.E.DFt-valueP-value							
Control	5.5206	0.07816	74	70.63	< 0.0001		
Treatment	5.5561	0.06697	74	82.96	< 0.0001		

d. D2O Grass Dec: Tests of Effect Slices for Treatment*Depth Sliced by Depth								
Depth (cm)	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>		
5	0.040	0.0108	0.049	0.0092	1.3	0.2586		
15	0.026	0.0090	0.020	0.0036	0.41	0.5219		
30	0.009	0.0008	0.006	0.0013	3	0.0873		
45	0.006	0.0015	0.006	0.0012	0	0.9791		
60	0.003	0.0002	0.004	0.0001	0.98	0.3257		

Table B.11(a-d).	<b>Statistics for</b>	grass tracer u	ptake data fro	m April 2012.
		<b>a</b>		

a. D2O Grass Apr: Fit Statistics				
-2 Res Log Likelihood	219.92			
AIC (smaller is better)	241.92			
AICC (smaller is better)	244.54			
BIC (smaller is better)	271.93			
CAIC (smaller is better)	282.93			
HQIC (smaller is better)	254.1			
Generalized Chi-Square	46.33			
Generalized Chi-Square / DF	0.41			

b. D2O Grass Apr: Type III Tests of Fixed Effects						
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>		
Treatment	1	103	3.89	0.0511		
Depth	4	103	7.59	< 0.0001		
Trt*Depth	4	103	0.2	0.9401		

c. D2O Grass Apr: Least Squares Means						
Treatment Type Estimate S.E. DF t-value P-valu					<b>P-value</b>	
Control	5.4689	0.102	103	53.64	< 0.0001	
Treatment	5.7261	0.0812	103	70.52	< 0.0001	

d. D2O Grass Apr: Tests of Effect Slices for Treatment*Depth Sliced by Depth						
Depth (cm)	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>
5	0.010	0.0017	0.0084	0.0013	0.35	0.558
15	0.017	0.0071	0.0084	0.0015	2.38	0.1263
30	0.011	0.0017	0.0066	0.0020	1.58	0.2121
45	0.024	0.0053	0.0356	0.0198	0.43	0.5112
60	0.011	0.0015	0.0118	0.0031	0.21	0.6515

a. D2O Tree Dec: Fit Statistics				
-2 Res Log Likelihood	-38.08			
AIC (smaller is better)	.18.08			
AICC (smaller is better)	-16.01			
BIC (smaller is better)	9.54			
CAIC (smaller is better)	19.54			
HQIC (smaller is better)	-6.87			
Generalized Chi-Square	117			
Generalized Chi-Square / DF	1			

b. D2O Tree Dec: Type III Tests of Fixed Effects							
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>			
Treatment	1	42	0.57	0.4563			
Depth	4	27.68	4.54	0.006			
Trt*Depth	4	27.68	0.59	0.6724			

c. D2O Grass Dec: Least Squares Means						
Treatment Type	Estimate	S.E.	DF	t-value	P-value	
Control	4.8384	0.03865	20.06	122.02	< 0.0001	
Treatment	4.7989	0.03446	24.27	139.26	< 0.0001	

d. D2O Tre	d. D2O Tree Dec: Tests of Effect Slices for Treatment*Depth Sliced by Depth						
Depth (cm)	Treatment	TRT S.E.	Control	CTRL S.E.	<b>F-value</b>	<b>P-value</b>	
5	0.019	0.0007	0.0261	0.0056	2.23	0.1676	
15	0.017	0.0019	0.0171	0.0027	0.07	0.7932	
30	0.016	0.0043	0.0131	0.0014	0.16	0.6974	
45	0.013	0.0009	0.0116	0.0006	0.01	0.91	
60	0.011	0.0004	0.0104	0.0002	0.06	0.8079	

Table B.13(a-d). Statistics for tree tracer	uptake data	from April 2	012.

a. D2O Tree Trt: Fit Statistics				
-2 Res Log Likelihood	-29.56			
AIC (smaller is better)	-7.56			
AICC (smaller is better)	-5.48			
BIC (smaller is better)	24.72			
CAIC (smaller is better)	35.72			
HQIC (smaller is better)	5.56			
Generalized Chi-Square	6.58			
Generalized Chi-Square / DF	0.05			

b. D2O Tree Trt: Type III Tests of Fixed Effects						
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>		
Date	1	129	2.2	0.1406		
Depth	4	129	0.49	0.7395		
Date*Depth	4	129	2.99	0.0212		

c. D2O Tree Trt: Least Squares Means						
Treatment TypeEstimateS.E.DFt-valueP-value						
December	4.7989	0.02961	129	162.09	< 0.0001	
April	4.8611	0.02967	129	163.86	< 0.0001	

d. D2O Tree Trt: Tests of Effect Slices for Date*Depth Sliced by Depth						
Depth (cm)	December	Dec S.E.	April	Apr S.E.	<b>F-value</b>	<b>P-value</b>
5	0.019	0.0007	0.018	0.0009	0.69	0.4077
15	0.017	0.0019	0.014	0.0004	1.31	0.2544
30	0.016	0.0043	0.014	0.0022	0.1	0.7508
45	0.013	0.0009	0.014	0.0006	0.27	0.6039
60	0.011	0.0004	0.019	0.0038	11.48	0.0009

## Table B.14(a-d). Statistics for grass tracer uptake data comparing December control uptake to April control uptake.

a. D2O Grass Ctrl: Fit Statistics			
-2 Res Log Likelihood	128.47		
AIC (smaller is better)	150.47		
AICC (smaller is better)	154.19		
BIC (smaller is better)	177.07		
CAIC (smaller is better)	188.07		
HQIC (smaller is better)	161.16		
Generalized Chi-Square	22.85		
Generalized Chi-Square / DF	0.28		

b. D2O Grass Ctrl: Type III Tests of Fixed Effects						
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>		
Date	1	73	0.17	0.6787		
Depth	4	73	4.11	0.0046		
Date*Depth	4	73	16.18	< 0.0001		

c. D2O Grass Ctrl: Least Squares Means						
Treatment TypeEstimateS.E.DFt-valueP-value						
December	5.5206	0.09199	73	60.01	< 0.0001	
April         5.4689         0.08354         73         65.47         <0.00						

d. D2O Grass Ctrl: Tests of Effect Slices for Date*Depth Sliced by Depth						
Depth (cm)	December	Dec S.E.	April	Apr S.E.	<b>F-value</b>	<b>P-value</b>
5	0.049	0.0092	0.0084	0.0013	27.69	< 0.0001
15	0.020	0.0036	0.0084	0.0015	8.2	0.0055
30	0.006	0.0013	0.0066	0.0020	0.1	0.7497
45	0.006	0.0012	0.0356	0.0198	16.77	0.0001
60	0.004	0.0001	0.0118	0.0031	12	0.0009

# Table B.15(a-d). Statistics for grass tracer uptake data comparing December treatment uptake to April treatment uptake.

a. D2O Grass Trt: Fit Statistics			
-2 Res Log Likelihood	204.61		
AIC (smaller is better)	226.61		
AICC (smaller is better)	229.2		
BIC (smaller is better)	256.71		
CAIC (smaller is better)	267.71		
HQIC (smaller is better)	238.83		
Generalized Chi-Square	40.17		
Generalized Chi-Square / DF	0.35		

b. D2O Grass Trt: Type III Tests of Fixed Effects						
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>		
Date	1	104	2.12	0.1482		
Depth	4	104	4.08	0.0041		
Date*Depth	4	104	15.69	< 0.0001		

c. D2O Grass Trt: Least Squares Means							
Treatment TypeEstimateS.E.DFt-valueP-value							
December	5.5561	0.08918	104	62.3	< 0.0001		
April         5.7261         0.07528         104         76.07         <0.0001							

d. D2O Grass Trt: Tests of Effect Slices for Date*Depth Sliced by Depth						
Depth (cm)	December	Dec S.E.	April	Apr S.E.	<b>F-value</b>	<b>P-value</b>
5	0.040	0.0108	0.010	0.0017	17.56	< 0.0001
15	0.026	0.0090	0.017	0.0071	3.92	0.0504
30	0.009	0.0008	0.011	0.0017	0.05	0.8198
45	0.006	0.0015	0.024	0.0053	22.84	< 0.0001
60	0.003	0.0002	0.011	0.0015	20.92	< 0.0001

## Table B.16(a-d). Statistics for tree tracer uptake data comparing December control uptake to April control uptake.

a. D2O Tree Ctrl: Fit Statistics			
-2 Res Log Likelihood	-62.15		
AIC (smaller is better)	-40.15		
AICC (smaller is better)	-38.51		
BIC (smaller is better)	-5.46		
CAIC (smaller is better)	5.54		
HQIC (smaller is better)	-26.08		
Generalized Chi-Square	7.07		
Generalized Chi-Square / DF	0.04		

b. D2O Tree Ctrl: Type III Tests of Fixed Effects							
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>			
Date	1	163	1.7	0.1939			
Depth	4	163	4.54	0.0017			
Date*Depth	4	163	4.13	0.0033			

c. D2O Tree Ctrl: Least Squares Means							
Treatment TypeEstimateS.E.DFt-value					<b>P-value</b>		
December	4.8384	0.02525	163	191.6	< 0.0001		
April	4.7961	0.02042	163	234.9	< 0.0001		

d. D2O Tree Ctrl: Tests of Effect Slices for Date*Depth Sliced by Depth							
Depth (cm)	December	Dec S.E.	April	Apr S.E.	<b>F-value</b>	<b>P-value</b>	
5	0.0261	0.0056	0.0183	0.0004	10.68	0.0013	
15	0.0171	0.0027	0.0151	0.0004	3.22	0.0744	
30	0.0131	0.0014	0.0162	0.0015	2.06	0.1527	
45	0.0116	0.0006	0.0142	0.0005	1.21	0.2735	
60	0.0104	0.0002	0.0116	0.0002	0.02	0.8884	

# Table B.17(a-d). Statistics for tree tracer uptake data comparing December treatment uptake to April treatment uptake.

a. D2O Tree Trt: Fit Statistics				
-2 Res Log Likelihood	-29.56			
AIC (smaller is better)	-7.56			
AICC (smaller is better)	-5.48			
BIC (smaller is better)	24.72			
CAIC (smaller is better)	35.72			
HQIC (smaller is better)	5.56			
Generalized Chi-Square	6.58			
Generalized Chi-Square / DF	0.05			

b. D2O Tree Trt: Type III Tests of Fixed Effects							
Effect	Num DF	Den DF	<b>F-value</b>	<b>P-value</b>			
Date	1	129	2.2	0.1406			
Depth	4	129	0.49	0.7395			
Date*Depth	4	129	2.99	0.0212			

c. D2O Tree Trt: Least Squares Means							
Treatment TypeEstimateS.E.DFt-value				P-value			
December	4.7989	0.02961	129	162.09	< 0.0001		
April	4.8611	0.02967	129	163.86	< 0.0001		

d. D2O Tree Trt: Tests of Effect Slices for Date*Depth Sliced by Depth							
Depth (cm)	December	Dec S.E.	April	Apr S.E.	<b>F-value</b>	<b>P-value</b>	
5	0.019	0.0007	0.018	0.0009	0.69	0.4077	
15	0.017	0.0019	0.014	0.0004	1.31	0.2544	
30	0.016	0.0043	0.014	0.0022	0.1	0.7508	
45	0.013	0.0009	0.014	0.0006	0.27	0.6039	
60	0.011	0.0004	0.019	0.0038	11.48	0.0009	