Journal of Vegetation Science **27** (2016) 587–595

A framework for partitioning plant rooting profiles from neighbours using multiple data types

Heather Kropp, Kiona Ogle & Martin F. Wojciechowski

Keywords
Bayesian modelling; Below-ground competition; Molecular identification; Plant–plant interactions; Root biomass; Root ecology; Stable isotopes; Vertical root distribution

Nomenclature
USDA PLANTS (http://plants.usda.gov; accessed on 31 Aug 2012)

Received 19 August 2015
Accepted 15 November 2015
Co-ordinating Editor: Zaal Kikvidze

Kropp, H. (corresponding author, hkropp@asu.edu)[1],
Ogle, K. (Kiona.Ogle@nau.edu)[1,2],
Wojciechowski, M.F. (Martin.Wojciechowski@asu.edu)[1]

[1]School of Life Sciences, Arizona State University, PO Box 874601, Tempe, 85287-6899 AZ, USA;
[2]Informatics and Computing Program, Northern Arizona University, 1395 S. Knoles Drive PO Box 5693, Flagstaff, AZ, USA

Abstract

Aim: Vertical root distributions ('profiles') influence plant water use and productivity, and the differentiation of root profiles between neighbouring species can indicate the degree of plant interactions and niche partitioning. However, quantifying multiple species' root distributions in the field can be labour intensive and highly destructive to the soil and plants. We describe a method for partitioning multiple species roots using minimally destructive methods to determine if neighbour interactions alter the root profile of a common desert shrub, *Larrea tridentata* (creosote bush).

Location: Sonoran Desert, central Arizona, USA.

Methods: We obtained root and soil samples from soil cores collected around *Larrea* growing alone and next to three different neighbouring species. Bulk root mass was measured for each soil sample, and *Larrea* and neighbouring species root presence was determined with molecular identification methods. Water extracted from the soil and paired stem samples was analysed for its stable isotope composition (D and ^{18}O). Species-specific (i.e. *Larrea* and neighbouring species) root biomass and fractional active root area were estimated through a hierarchical statistical modelling approach that combined all three data sets and accounted for detection errors.

Results: The combined data model successfully partitioned *Larrea* root biomass from neighbouring plants and provided biologically relevant estimates of rooting profiles with greater certainty than individual analyses of each data source. The data model results indicate that plant neighbours alter *Larrea*'s root profile; *Larrea* growing under tree species had significantly higher root biomass in shallow soil layers than *Larrea* growing alone.

Conclusions: Our framework requires minimally destructive sampling methods, and accounts for sampling errors associated with different methods. We demonstrate the utility of our approach with a common desert shrub species, which illustrated that plant neighbours can alter the *Larrea* vertical root profile. Our approach is useful in problematic study systems fraught with sample collection issues or supporting species with inhibitory compounds that prohibit the use of more sophisticated molecular methods to identify the presence of other species' roots.

Introduction

Vertical root distributions ('profiles') play an integral role in plant survival and productivity, influencing the ability of plants to acquire water and nutrients from the soil (Ogle & Reynolds 2004). The degree of overlap of roots between neighbouring plants can be an important factor determining competition for below-ground resources (Casper & Jackson 1997; Schenk 2006). Studies of species' co-existence and competition in plant communities are often interested in the influence of vertical differentiation of root distributions on competition for soil water or nutrient pools (Ogle & Reynolds 2004; Mommer et al. 2008). However, studies of vertical root differentiation require the quantification of root profiles for multiple species, an often

Doi: 10.1111/jvs.12377 © 2016 International Association for Vegetation Science

difficult undertaking (Jackson et al. 1996; Mommer et al. 2011).

Root distribution studies are typically limited in scope as a result of methodological challenges that involve trade-offs between the spatial extent of the root system studied, time investment and level of destruction to the plant and soil. Excavation techniques are commonly used (Bohm 1979; Brisson & Reynolds 1994; Jackson et al. 1996) because they offer a detailed assessment of vertical and horizontal rooting patterns. However, excavations are time consuming, highly destructive and prohibit simultaneous above-ground studies on the plants (Danjon & Reubens 2007).

Molecular genetic techniques (e.g. polymerase chain reaction [PCR]) offer an alternative approach to identify species in mixtures of root samples collected from minimally destructive soil cores (Mommer et al. 2011), and have been applied in diverse ecosystems, from temperate to alpine systems (Bobowski et al. 1999; Jackson et al. 1999; Brunner et al. 2001). However, high concentrations of PCR inhibitors in roots (Mommer et al. 2011), combined with the small cross-sectional area of a typical soil core, may under-estimate root presence, and previous studies have not addressed these sources of uncertainty in subsequent data analysis (Bobowski et al. 1999; Brunner et al. 2001; Mommer et al. 2008).

Another minimally destructive technique for quantifying root profiles involves the evaluation of stable isotopes in plant and soil water samples, which offer insights into the depths from which roots actively acquire water (Dawson et al. 2002). Stable isotope data are typically analysed with simple linear mixing (SLM) models (Phillips & Gregg 2003) that do not provide direct estimates of rooting profiles. However, linking SLM models with a model of root water uptake can provide estimates of the root area profile (Ogle et al. 2004, 2013).

This study applies a novel modelling approach that partitions root biomass between neighbouring species through the combination of three distinct data sets – molecular identification of roots, bulk root biomass and stable isotopes – while accounting for method-based detection and measurement errors in a hierarchical Bayesian framework. Individual data sets are limited in scope, offering incomplete information on the root profile of a species. For example, molecular identification indicates species' presence throughout the soil profile, but does not provide information on the overall magnitude of root biomass. Likewise, stable isotope data indicate the relative distribution of a species' active roots, but are not a direct measure of biomass. Finally, bulk root biomass data consist of a mixture of multiple species' roots that must be partitioned to examine the root biomass of singular species. However, the combined data sets offer information on the root profiles of a target species and its surrounding neighbours, allowing for the quantification of a species' fractional distribution of active roots and root biomass throughout the soil profile.

We describe and illustrate our framework by applying it to data collected for a common desert shrub (Barbour 1969), *Larrea tridentata* (Sessé & Moc. ex DC.) Coville (creosote bush). *Larrea*'s root distribution can be impacted by competition with neighbouring shrubs (Brisson & Reynolds 1994). We quantified *Larrea*'s vertical root distribution under different neighbourhood associations that could represent different competition environments (Yeaton et al. 1977): *Ambrosia deltoidea* (Torr.) W.W. Payne (triangle-leaf bursage), *Olneya tesota* A. Gray (desert ironwood) and *Prosopis velutina* Wooton (velvet mesquite). *Ambrosia* is a drought-deciduous small shrub (Szarek 1977), and *Olneya* and *Prosopis* are deep-rooted tree species (Suzan et al. 1996). We apply our analysis framework to quantify *Larrea*'s root profile to address two questions: (1) does *Larrea*'s root profile vary depending on plant neighbour identity; and (2) to what degree do *Larrea*'s roots overlap (vertically) with roots of neighbouring species? We assess rooting profiles through indices of both root biomass and active root area for water uptake.

Methods

Site description and root collection

Root samples were collected in the Sonoran Desert near Phoenix, Arizona, at the McDowell Mountain Regional Park (33.7261N, -111.6987W, 476 m a.s.l.). The site is dominated by *L. tridentata*, *A. deltoidea*, *P. velutina* and *O. tesota*, with many shrubs growing in close proximity with overlapping canopies. Mean annual precipitation (1981–2010) was 29.6 cm and mean daily temperature ranged from 11.5 °C (Dec) to 33 °C (Jul) (WRCC 2013).

Plants with overlapping canopies were considered to be neighbours, and *Larrea* growing with a canopy separated by at least 1 m from another plant's canopy was considered to be growing alone. Four neighbourhood associations were studied: *Larrea* growing near *Ambrosia*, *Olneya* or *Prosopis*, and *Larrea* growing alone. Soil cores for both root identification and stable isotope analysis were collected on 20–21 Aug 2012 in five soil layers (0–10, 10–20, 20–30, 30–40, 40–60 cm), and a full description of the field sample collection is included in the Supporting Information (Appendix SI). The analysis of soil and stem water samples for stable isotope composition is also described in Appendix SI.

Molecular identification

Genomic DNA was extracted using a method developed for roots with high concentrations of PCR inhibitors,

Doi: 10.1111/jvs.12377 © 2016 International Association for Vegetation Science

such as polysaccharides and polyphenolics (Brunner et al. 2001). We highlight the methods here and provide a full description in Appendix SI. Despite the targeted DNA extraction protocol to remove inhibitors, viscous brown material sometimes persisted in samples after extraction, indicating the presence of potential PCR inhibitors (Paterson et al. 1993; Lodhi et al. 1994). High concentrations of phenolics and tannins, known PCR inhibitors, have been observed in *Larrea* roots (Hyder et al. 2002). Thus, additional purification steps were adapted from Paterson et al. (1993). The nuclear rDNA Internal Transcribed Spacer region (ITS1-5.8S gene-ITS2; Baldwin et al. 1995) was amplified with primers ITS4 and ITS5 (White et al. 1990). All PCR products were digested with restriction endonuclease RsaI and samples from the *Prosopis* pair were also digested with BssHII; the distinct fragment lengths from digestions with RsaI and BssHII (Thermo-Fisher Scientific, Waltham, MA, US) are shown in (Appendix S2) for each species. Extensive testing of the methods (described in Appendix SI) was conducted since the PCR methods described above have not previously been applied to roots of the desert species in this study.

Overview of modelling approach

Root profiles were quantified with a latent variable, the fraction of active root area (f), a relative measure of the vertical distribution of functional roots. We estimated f based on three linked sub-models: (1) a biophysical model of root water uptake informed by stable isotope data, (2) root presence informed by molecular identification, and (3) an empirical root biomass model that pairs bulk biomass data with presence data (Appendix S3). Ogle et al. (2004, 2013) consider f to be a mixture of normalized gamma distributions (Eq. 1) that allows for a continuous, flexible root profile that can be either unimodal or bimodal. Relevant to all three data sets, for neighbour association j (j = 1, 2, 3, 4) and soil depth z (z = 1, 2, …, 60 cm), *Larrea*'s active root profile is modelled as:

$$f_{j,z} = w_j \mathrm{Gamma}(z|a_1, m_{1_j}) + (1 - w_j)\mathrm{Gamma}(z|a_2, m_{2_j}) \quad (1)$$

The mixture weight, w, represents the relative importance of roots in the shallow layers, and the mean depths of the shallow and deep roots (m_1 and m_2, respectively; *i.e.* means of the associated gamma distributions) vary among neighbour associations. Conversely, for simplicity, a_1 and a_2, which influence the shape of the gamma distributions, are assumed to be the same across neighbour associations. A description of model parameters is included in Table 1.

Water uptake model and stable isotopes

Following Ogle et al. (2004), a biophysical model of root water uptake (Ogle et al. 2013) was paired with stable isotope data using a process-based isotope mixing model that informs f. The predicted stem isotope values ($\delta^{\mathrm{pred}}_{\mathrm{stem},m,j}$) for each neighbour association j (j = 1,…4), for both deuterium (δD, m = 1) and δ^{18}O (m = 2) were considered to be a mixture of the observed soil water isotope values ($\delta^{\mathrm{obs}}_{\mathrm{soil},m,j,i}$) based on the proportion of water (p) obtained from each soil layer i (i = 1 [0–10 cm], 2 [10–20 cm], …, 5 [40–60 cm]):

$$\delta^{\mathrm{pred}}_{\mathrm{stem},m,j} = \sum p_{j,i}\delta^{\mathrm{obs}}_{\mathrm{soil},m,j,i} \quad (2)$$

The observed soil isotope values were averaged across soil cores within each depth increment and neighbour association, and the observed stem isotope values were assumed to be normally distributed around the predicted value in Eq. 2.

We model the layer-specific p for each neighbourhood association as *Larrea*'s predicted water uptake from each layer (q), normalized by the total water uptake from all layers such that:

$$p_{j,i} = \frac{q_{j,i}}{\sum_{i=1}^{5} q_{j,i}} \quad (3)$$

The predicted water uptake is based on a biophysical model of root water uptake:

$$q_{j,i} = f_{j,i}\frac{K_{\mathrm{soil},j,i}K_{\mathrm{root},j}}{K_{\mathrm{soil},j,i} + K_{\mathrm{root},j}}(\Psi_{\mathrm{soil},j,i} - \Psi_{\mathrm{tloss}}) \quad (4)$$

With the exception of the latent quantity f, quantities in Eq. 4 were obtained from field data where Ψ_{soil} is the soil water potential, Ψ_{tloss} is the root water potential at the turgor loss point, and K_{soil} and K_{root} are the hydraulic conductance of the soil and roots, respectively (described in Appendix SI). Note that $f_{j,i}$ above is the fraction of roots in layer i, which is obtained by summing $f_{j,z}$ (Eq. 1) over all z within layer i, for each neighbour association j.

Root presence model

A model of *Larrea*'s root presence from molecular identification was motivated by occupancy models that explicitly incorporate detection probabilities associated with imperfect sampling (Mackenzie et al. 2002). False negatives could be generated through two different processes, and the first source was associated with soil core sampling. Roots in desert systems tend to be sparsely

distributed across space (Wilcox et al. 2004), and thus the soil auger may under-sample roots within its small cross-sectional area. The second source of false negatives results from the imperfect nature of PCR as a result of high concentrations of inhibitors and/or low concentrations of DNA.

The likelihood of observing a species' roots (r = 1, root is present; r = 0, root is not present) in each soil core sample is assumed to depend on the probability that a species' has roots in a given layer in the soil, ϕ, and the probability of detecting the presence of roots, p_d, given the soil sampling and PCR methods. Thus, the likelihood of observed r = 1 or r = 0 in each soil sample n (n = 1, 2, …, 95) is:

$$\begin{aligned} P(r=1|\phi,p_d) &= p_d\phi \\ P(r=0|\phi,p_d) &= \phi(1-p_d)+(1-\phi) \end{aligned} \quad (5)$$

p_d can be defined as the product of two probability terms (see Appendix SI for full description): β, the probability of collecting roots in a sample, and γ, the probability of PCR successfully detecting the presence of a species' roots given the sample contains roots:

$$p_d = \beta\gamma \quad (6)$$

Finally, the probability that *Larrea* has roots in a given layer i, ϕ, describes the presence of roots within the soil profile, with values near 1 indicating a high likelihood of *Larrea* root presence. Thus, we expect ϕ to be directly related to the fraction of *Larrea*'s active roots, f, such that:

$$\phi_{j,i} = \phi^*(1-e^{-af_{j,i}}) \quad (7)$$

Here, ϕ^* is the probability that would occur as f goes to infinity, but since $0 \leq f_{j,i} \leq 1$, the maximum probability that *Larrea* has roots in any layer i is $\phi^*(1-e^{-a})$.

We used a simpler model with coarser soil layer resolution for the probability of non-*Larrea* species (v = 1 [*Prosopis*], 2 [*Olneya*], 3 [*Ambrosi*a] or 4 [other unidentified species]) rooting in a soil layer, ϕ, since there were no additional data to help inform the root distributions (i.e. no stable isotopes). We assumed that ϕ declines with distance (d) to the closest shrub of that species (d is relative to the target *Larrea* shrub, l) for shallow soil layers (g = 1, 0–20 cm) and deep layers (g = 2, 20–60 cm):

$$\text{logit}(\phi_{v,g,l}) = b_v - \alpha_{v,g} \times d_l \quad (8)$$

The parameters, b and α, varied with each species v since vertical and lateral root distributions are expected to vary by species (Schenk & Jackson 2002; Ogle & Reynolds 2004). Additionally, the lateral extent of shallow vs deep roots is expected to differ (Jackson et al. 1996), and thus α varied by g.

Bulk root biomass model

The observed bulk root biomass (R) collected from each soil sample n (n = 1, 2, …, 95) was assumed to be normally distributed:

$$R_n \sim \text{Normal}(\mu_{R_{i,l}}, \sigma_R) \quad (9)$$

where the mean root biomass (μ_R) varies by soil layer i and *Larrea* shrub l (l = 1…13) associated with each sample; μ_R was modelled as a mixture of roots from *Larrea* and its neighbours:

$$\mu_{R_{i,l}} = \left(p_{\text{larrea}_l} f_{j,i} + (1-p_{\text{larrea}_l}) f_{o_i}\right) R_{\text{tot}_l} \quad (10)$$

Again, f (Eq. 1) is the fractional active root profile of *Larrea*, and f_o is the root profile of 'other' neighbouring species, which is based on summing the f_o's in Eq. 12 over depths z corresponding to each layer i. R_{tot} is the observed total root biomass in the soil profile below each *Larrea* shrub, and p_{larrea} is the relative proportion of *Larrea* roots compared to all other species. Soil samples without roots or at depths that could not be sampled were treated as missing data and are estimated from Eq. 9, and these estimates were subsequently used to compute R_{tot}.

The proportion of *Larrea*'s roots under each shrub is based on the probability of *Larrea* and the neighbouring species having roots in a soil layer, ϕ, for each neighbour association j associated with shrub l:

$$p_{\text{larrea},l} = \frac{\sum_i \phi_{i,j}}{\sum_i \phi_{i,j} + \sum_g \sum_v \lambda_{v,g,l}} \quad (11)$$

Since soil samples were collected around *Larrea* canopies, the contribution of root biomass from neighbouring plants is expected to consist mainly of lateral roots that occur more frequently in surface layers (Schenk & Jackson 2002), and thus the fractional rooting area of neighbouring plants (f_o) was expected to decline exponentially with depth z (z = 1, 2, …, 60 cm) with rate parameter (ρ) that varies by neighbour association j.

$$f_{o,j,z} = \rho_j e^{-\rho_j z} \quad (12)$$

Data model performance

We compared the degree of uncertainty and model fit in the analysis of each individual data set (*e.g.* stable isotope, molecular identification, bulk root biomass) to the combined model to evaluate the improvement in estimates

Doi: 10.1111/jvs.12377 © 2016 International Association for Vegetation Science

and inference with the multiple data sets approach. The process-based isotope mixing model was compared to a simpler mixing model that did not include a biophysical water uptake model (described in Appendix SI). Model fit was evaluated by computing the coefficient of determination (R^2) from a regression of the observed vs predicted stem isotope data, for both the simple model (isotope only data) and the combined, process-based model (all three data sets).

The simplified analysis of the molecular data was conducted using an occupancy model applied only to the molecular data, with a uniform, U(0,1), prior for ϕ (see Eq. 7). For both the simple and combined model, model fit was assessed by computing the prediction accuracy (percentage of the model predicted samples with *Larrea* roots present compared to the observed samples with *Larrea* roots present).

The simplified analysis of root biomass data involved comparing the bulk root biomass estimates to a more traditional model of root mass profiles where the bulk fractional root biomass declines non-linearly with depth (Gale & Grigal 1987; Jackson et al. 1996). The mean model of bulk root biomass (Eq. 9) was modified such that R_{tot} was only scaled by f in Eq. 13, where f was defined as:

$$f_{j,z} = 1 - \kappa_j^z \qquad (13)$$

and is summed over all z within layer i for application to Eq. 10. κ describes the decline of bulk root biomass with depth. For both the simple (biomass data only) and combined models, model fit was assessed by calculating the R^2 from a regression of the observed and predicted root biomass.

Implementation

The above models were implemented in OpenBUGS (v 3.2.3), and posterior estimates are presented as posterior means with 95% credible intervals (2.5th and 97.5th percentiles). A full description of the prior distributions, model implementation and model code is included in Appendix SI and S3).

Results

Model evaluation

The model that analysed all data sets simultaneously was able to partition root biomass between *Larrea* and neighbouring plants, and generally yielded more precise estimates of parameters associated with *Larrea*'s root profile in comparison to analyses of individual data sets (Table 2). Moreover, the predicted ^{18}O composition of stem water obtained from the combined model had a slightly higher R^2 (Table 2) than the isotopes-only data model. However, the isotopes-only data model predictions of the δD composition of stem water had a higher R^2 than the combined data model, but the former produced unrealistic estimates of water uptake, allowing

Table 1. Description of parameters and variables in each sub-model of the combined, process model.

Symbol	Description	Source/Type
Fractional Rooting Area		
f	Fractional area of active roots	Eq. 1
w	Proportion of surface roots	Parameter
a_1, a_2	Describes shape of vertical root distributions	Parameter
m_1, m_2	Average rooting depths for deep and shallow roots	Parameter
Water Uptake		
p	Proportional water uptake	Eq. 3
$\delta_{\text{soil}}^{obs}$	Soil water isotope abundance	Data
$\delta_{\text{stem}}^{\text{pred}}$	Stem water isotope abundance	Parameter
K_s	Soil hydraulic conductance	Computed from data*
K_r	Root hydraulic conductance	Computed from data*
Ψ_{tloss}	Turgor loss water potential	Computed from data*
Ψ_s	Soil water potential	Computed from data*
Root Presence		
β	Probability of sampling roots in the soil core	Parameter
γ	Probability of PCR success	Parameter*
ϕ	Probability of root presence	Parameter*
ϕ_*	Maximum probability of root presence	Parameter
a	Slope of probability of root presence as fractional active root area increases	Parameter
r	Presence of root in sample	Data
d	Distance of closest shrub	Data
b	Intercept for logit ϕ (non-*Larrea* species)	Parameter
α	Slope for logit ϕ (non-*Larrea* species)	Parameter
Root Biomass		
μ_R	Mean root biomass in each soil layer	Eq. 10
R_{tot}	Total root biomass under each shrub	Data
p_{larrea}	Proportion of *Larrea* roots in root mixtures	Eq. 11
f_o	Fractional root of non-*Larrea* species	Eq. 12
ρ	Decay of root area with depth of non-*Larrea* species	Parameter
Simple Linear Mixing Model		
p_{slm}	Fractional water uptake from each layer	Eq. S7*
s	Soil water scaled proportional water uptake	Parameter*
Simple Root Biomass Model		
κ	Shape parameter describing root decline with depth	Parameter

*Additional information on data collection and parameterization is included in Appendix SI.

Table 2. Evaluation of model performance between the combined, process model approach and simpler, individual analyses of data sets.

Data Set	Model	Model Fit	Measure of Fit
Stable Isotope (Stem ^{18}O)	Isotopes-only	0.18	R^2
	Combined data sets	0.21	R^2
Stable Isotope (Stem D)	Isotopes-only	0.49	R^2
	Combined data sets	0.15	R^2
Molecular	Molecular-only	67%	Prediction accuracy
	Combined data sets	79%	Prediction accuracy
Root Biomass	Biomass-only	0.06	R^2
	Combined data sets	0.26	R^2

water to be taken up from layers in the soil that had water potentials below *Larrea*'s estimated turgor loss point (in Appendix S4).

Importantly, the full model that combined all three data sets provided estimates with narrower credible intervals for the probability of *Larrea* root presence (ϕ) compared to analysing each data set independently with relatively non-informative priors (in Appendix S3). On average, the combined data model predicted the presence of *Larrea* in 79% of the observed samples containing *Larrea*, and the simple analysis using only the molecular data predicted *Larrea*'s presence in 67% of the actual observed samples containing *Larrea* (Table 2). The root biomass model of the combined data set explained more variation compared to the traditional, nonlinear model (Eq. 13), which had a lower fit compared to the gamma mixture model (Eq. 1; Table 2).

Model application: effect of neighbours on Larrea's root profile

Larrea's active root area (*f*, Fig. 1) and root biomass (μ_R, Fig. 2) profiles varied based on neighbourhood association. *Larrea* growing next to the tree species (*Prosopis* and *Olneya*) had higher *f* and root biomass in shallower depths (Fig. 1a,c) compared to *Larrea* growing alone (Fig. 1d) or next to *Ambrosia* (Fig. 1b). Moreover, *f* of *Larrea* growing next to *Prosopis* peaked between 30–40-cm depths (Fig. 1c), and *Larrea*'s predicted root biomass was significantly higher when growing near *Prosopis* (Fig. 2a) compared to *Larrea* in other neighbourhood associations (Fig. 2b–d). *Larrea*'s root biomass growing alone and next to *Ambrosia* (Figs. 2a,c, respectively) was generally low between 0–40 cm, with comparatively high biomass between 40–60 cm. Summing over depths, *Larrea*'s total root biomass was significantly larger when growing next to *Olneya* or *Prosopis* than when growing next to *Ambrosia* or alone. *Larrea's* root biomass when growing alone or next to *Ambrosia* was minimal at 0–10 cm (Fig. 2b,d), and the root biomass of neighbouring species' was largest in the upper 10 cm of the soil (Fig 2f,h), indicating minimal overlap between *Larrea* roots and neighbouring species. However, *Larrea* growing next to tree species had vertical root distributions that had higher overlap with neighbouring species root biomass compared to *Larrea* growing next to *Ambrosia* (Fig. 2a,c,e,g).

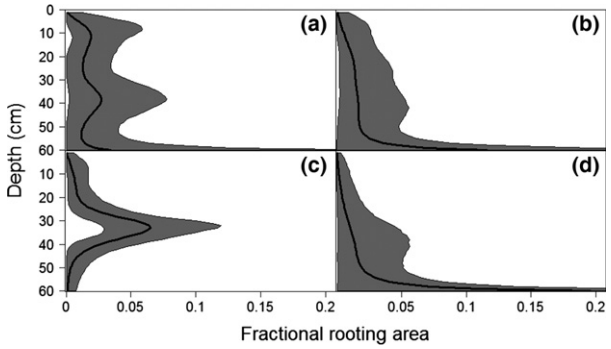

Fig. 1. The posterior estimates (mean and 95% credible regions) for fractional active root area (*f*) of *L. tridentata* under four different neighbourhood associations, with *Larrea* growing next to: (**a**) *O. tesota*, (**b**) *A. deltoidea* or (**c**) *P. velutina*, or (**d**) growing alone.

Discussion

Model performance and applications

We demonstrate that the combination of multiple data types can offer a more detailed quantification of root systems. Stable isotopes are commonly used to estimate depths of active water uptake, and the prevalence of stable isotope laboratories and technologies allows for straightforward sample preparation and analysis (Dawson et al. 2002; Ogle et al. 2004). Our multi-data set approach that linked *Larrea*'s active rooting area to stable isotope data produced biologically realistic and more precise estimates of proportional soil water uptake with finer depth resolution compared to a simpler mixing model approach that was only informed by isotope data and soil water content.

The combined model approach allowed for the partitioning of *Larrea*'s root biomass from other species and improved estimates of the presence of species' roots relative to the molecular-only data analysis. Mommer et al. (2008) presented a method to partition the proportion of biomass belonging to each species using real time PCR (qPCR). However, in systems such as our study system, where plant roots contain high amounts of inhibitory compounds, method development for qPCR requires additional costs and time commitment, and samples may be subject to higher failure rates (Mommer et al. 2011). Our approach of pairing simpler PCR methods with a stable isotope analysis for studying below-ground rooting distributions provides an alternative to more labour intensive approaches such as qPCR. We also demonstrate the impor-

Doi: 10.1111/jvs.12377 © 2016 International Association for Vegetation Science

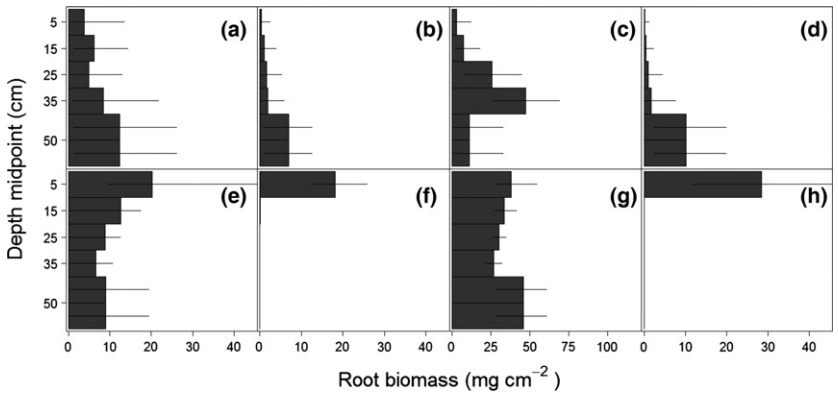

Fig. 2. Posterior estimates (mean and 95% credible interval) of root biomass (μ_R) partitioned into each soil layer for *L. tridentata* (**a**–**d**) and all other neighbouring species combined (**e**–**h**), for four neighbourhood associations, with *Larrea* growing next to *O. tesota* (**a**, **e**), *A. deltoidea* (**b**, **f**) or *P. velutina* (**c**, **g**), and growing alone (**d**, **h**).

tance of accounting for detection issues associated with PCR, and our PCR success rate of 0.54 [0.48, 0.60] indicates that false negatives are common and must be explicitly considered. Previous studies have not specified a statistical framework for accounting for false negatives, and our occupancy-inspired model framework may be useful in other PCR-based studies (Bobowski et al. 1999; Brunner et al. 2001).

We also demonstrate the usefulness of incorporating uncertainty and process information in estimating latent (unobservable) species-specific active root area or root biomass. Our model predicts species-specific root biomass in each soil layer, accounts for limitations in sampling, and allows for flexibility in the vertical root distribution (Ogle et al. 2004). Models of root biomass typically assume an exponential or nonlinear decline with depth, but woody plant roots may not necessarily be concentrated at the surface (Gale & Grigal 1987; Ogle et al. 2004). In arid or semi-arid systems, deep roots may be more important for physiological activity during dry periods than shallow roots (Schwinning et al. 2002; Ogle & Reynolds 2004).

Ecological application: Larrea tridentata*'s root profile*

Our statistical framework for partitioning the root profiles of *L. tridentata* and select neighbouring species demonstrates that plant neighbours alter *Larrea*'s vertical root distribution. *Larrea* had larger root biomass and more active roots at shallow depths (<40 cm) when growing next to tree species neighbours. *Olneya* and *Prosopis* are known to have facilitative effects on understorey shrubs via canopy shading, and *Larrea*'s roots may be more active in shallow layers as a result of increased water availability (Suzan et al. 1996; Schade et al. 2003).

Under stressful drought conditions, arid shrubs can shift water uptake and root activity to deeper soil layers (Schwinning et al. 2002; Reynolds et al. 2004). *Larrea* growing alone or next to *Ambrosia* had very little biomass or root area in shallow soil layers, and these neighbour associations may experience higher limitations in surface soil water as a result of increased evaporation due to decreased canopy shading (Suzan et al. 1996). *Larrea* and *Ambrosia* roots have been shown to avoid overlap (Yeaton et al. 1977; Brisson & Reynolds 1994), and little vertical overlap in root biomass was also observed in this study. The variation in *Larrea*'s root profile arising from plant neighbour associations highlights a source of variation in *Larrea* root profiles, in addition to previously reported variation across deserts (Ogle & Reynolds 2004).

Variation in root profiles arising from plant neighbour associations can affect transpiration, competition for soil water and ultimately plant productivity (Casper & Jackson 1997; Reynolds et al. 2004), and our study indicates that studies interested in below-ground competition or plant water use should account for the effect of neighbours on root profiles. Our framework for analysing root distributions provides a method for analysing variation in species root profiles from neighbours that uses minimally destructive sampling, and is particularly useful in ecological systems suffering from methodological difficulties, such as arid regions (Jackson et al. 1996; Mommer et al. 2011).

Acknowledgements

This project was partially supported by the Arizona State University Graduate and Professional Student Association's JumpStart Grant Program. Additional support was provided by the School of Life Sciences at Arizona State University to KO and MFW.

References

Baldwin, B.G., Sanderson, M.J., Porter, J.F., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

Barbour, M. 1969. Age and space distribution of the desert shrub *Larrea* Div *Aricata*. *Ecology* 50: 679–685.

Bobowski, B., Hole, D., Wolf, P. & Bryant, L. 1999. Identification of roots of woody species using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. *Molecular Ecology* 8: 485–491.

Bohm, W. 1979. *Methods of studying root systems*. Springer, Berlin, DE.

Brisson, J. & Reynolds, J.F. 1994. The effect of neighbors on root distribution in a Creosotebush (*Larrea tridentata*) population. *Ecology* 75: 1693–1702.

Brunner, I., Brodbeck, S., Buchler, U. & Sperisen, C. 2001. Molecular identification of fine roots of trees from the Alps : reliable and fast DNA extraction and PCR – RFLP analyses of plastid DNA. *Molecular Ecology* 10: 2079–2087.

Casper, B.B. & Jackson, R.B. 1997. Plant competition underground. *Annual Review of Ecology and Systematics* 28: 545–570.

Danjon, F. & Reubens, B. 2007. Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant and Soil* 303: 1–34.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H. & Tu, K.P. 2002. Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* 33: 507–559.

Gale, M.R. & Grigal, D.F. 1987. Vertical root distributions of northern tree species in relation to successional status. *Canadian Journal of Forest Research* 17: 829–834.

Hyder, P.W., Fredrickson, E., Estell, R.E., Tellez, M. & Gibbens, R.P. 2002. Distribution and concentration of total phenolics, condensed tannins, and nordihydroguaiaretic acid (NDGA) in creosotebush (*Larrea tridentata*). *Biochemical Systematics and Ecology* 30: 905–912.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E. & Schulze, E.D. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108: 389–411.

Jackson, R.B., Moore, L.A., Hoffman, W.A., Pockman, W.T. & Linder, C.R. 1999. Ecosystem rooting depth determined with caves and DNA. *Proceedings of the National Academy of Sciences of the United States of America* 96: 11387–11392.

Lodhi, M.A., Ye, G.-N., Weeden, N.F. & Reisch, B.I. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter* 12: 6–13.

Mackenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Royle, J.A. & Langtimm, C.A. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83: 2248–2255.

Mommer, L., Wagemaker, C.A.M., De Kroon, H. & Ouborg, N.J. 2008. Unravelling below-ground plant distributions: a real-time polymerase chain reaction method for quantifying species proportions in mixed root samples. *Molecular Ecology Resources* 8: 947–953.

Mommer, L., Dumbrell, A.J., Wagemaker, C.A.M. & Ouborg, N.J. 2011. Belowground DNA-based techniques: untangling the network of plant root interactions. *Plant and Soil* 348: 115–121.

Ogle, K. & Reynolds, J.F. 2004. Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays. *Oecologia* 141: 282–294.

Ogle, K., Wolpert, R.L. & Reynolds, J.F. 2004. Reconstructing plant root area and water uptake profiles. *Ecology* 85: 1967–1978.

Ogle, K., Tucker, C. & Cable, J.M. 2013. Beyond simple linear mixing models : process-based isotope partitioning of ecological processes. *Ecological Applications* 24: 181–195.

Paterson, A.H., Brubaker, C.L. & Wendel, J.F. 1993. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Molecular Biology Reporter* 11: 122–127.

Phillips, D.L. & Gregg, J.W. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261–269.

Reynolds, J.F., Kemp, P.R., Ogle, K., Fernández, R.J. & Fernandez, R.J. 2004. Modifying the "Pulse-Reserve" paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. *Oecologia* 141: 194–210.

Schade, J.D., Sponseller, R., Collins, S.L. & Stiles, A. 2003. The influence of *Prosopis* canopies on understorey vegetation: effects of landscape position. *Journal of Vegetation Science* 14: 743.

Schenk, H.J. 2006. Root competition: beyond resource depletion. *Journal of Ecology* 94: 725–739.

Schenk, H.J. & Jackson, B. 2002. Rooting depths, lateral root spreads, and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology* 90: 480–494.

Schwinning, S., Davis, K., Richardson, L. & Ehleringer, J. 2002. Deuterium enriched irrigation indicates different forms of rain use in shrub/grass species of the Colorado Plateau. *Oecologia* 130: 345–355.

Suzan, H., Nabhan, G.P. & Patten, D.T. 1996. The importance of *Olneya tesota* as a nurse plant in the Sonoran Desert. *Journal of Vegetation Science* 7: 635–644.

Szarek, S.R. 1977. Ecophysiological studies of Sonoran Desert plants II. Seasonal photosynthesis patterns and primary production of *Ambrosia deltoidea* and *Olneya tesota*. *Oecologia* 28: 365–375.

White, T.J., Bruns, T.L., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J. & White, T., (eds.) *PCR Protocols: a guide to methods and applications*, pp. 315–322. Academic Press, Orlando, FL, US.

Doi: 10.1111/jvs.12377 © 2016 International Association for Vegetation Science

Wilcox, C.S., Ferguson, J.W., Fernandez, G.C. & Nowak, R.S. 2004. Fine root growth dynamics of four Mojave Desert shrubs as related to soil moisture and microsite. *Journal of Arid Environments* 56: 129–148.

Yeaton, R.I., Travis, J. & Gilinsky, E. 1977. Competition and spacing in plant communities: the Arizona upland association. *Journal of Ecology* 65: 587–595.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Additional method description.

Appendix S2. Table of restriction enzyme analysis summary.

Appendix S3. Model comparison figures.

Appendix S4. Model Script.