ECOLOGICAL SOCIETY OF AMERICA

Ecology/Ecological Monographs/Ecological Applications

PREPRINT

The doi for this manuscript and the correct format for citing the paper are given at the top of the online (html) abstract.

Once the final published version of this paper is posted online, it will replace the preliminary version at the specified doi.

GRASSLAND ROOT COMMUNITIES: SPECIES DISTRIBUTIONS AND HOW THEY ARE LINKED TO ABOVEGROUND ABUNDANCE

Douglas A. Frank, Alyssa W. Pontes, Eleanor M. Maine, Julie Caruana, Ramesh Raina, Surabhi Raina, Jason D. Fridley

Department of Biology, 107 College Place, Syracuse University, Syracuse, NY 13244

ABSTRACT

There is little comprehensive information on the distribution of root systems among coexisting species, despite the expected importance of those distributions in determining the composition and diversity of plant communities. This gap in knowledge is particularly acute for grasslands, which possess large numbers of species with morphologically indistinguishable roots. In this study we adapted a molecular method, fluorescent fragment length polymorphism, to identify root fragments and determine species root distributions in two grasslands in Yellowstone National Park. Aboveground biomass was measured and soil cores (2 cm diam) were collected to 40 cm and 90 cm in an upland, dry grassland and a mesic, slope-bottom grassland, respectively, at peak foliar expansion. Cores were subdivided and species that occurred in each 10 cm interval were identified. The results indicated that the average number of species in 10 cm intervals (31 cm^3) throughout the sampled soil profile was 3.9 and 2.8 at a dry and a mesic grassland, respectively. By contrast, average species number per 0.5 m^2 determined by the presence of shoot material was 6.7 and 14.1 at dry and mesic sites, respectively. There was no relationship between soil depth and number of species per 10 cm interval in either grassland, despite the exponential decline of root biomass with soil depth at both sites. There also was no relationship between root frequency (i.e., the percentage of samples in which a species occurred) and soil depth for the vast majority of species at both sites. The preponderance of species were distributed throughout the soil profile at both sites. Assembly analyses indicated that species root occurrences were randomly assorted in all soil intervals at both sites, with the exception that *F. idahoensis* segregated from *A. tridentata* and *P. spicata* in 10-20 cm soil at the dry grassland. Root frequency throughout the entire sampled soil profile was positively associated with shoot biomass among species. Together these results indicated the importance of large, well

proliferated root systems in establishing aboveground dominance. The findings suggest that spatial belowground segregation of species probably plays a minor role in fostering resource partitioning and species coexistence in these YNP grasslands.

KEY WORDS: Fluorescent fragment length polymorphism, FFLP, grassland, plant competition, roots, Yellowstone National Park

INTRODUCTION

As sessile organisms, plants capture resources and interact with neighbors within the aboveground (sward) and belowground (root) zones that they occupy. Knowledge of canopy characteristics, including canopy size, shape, and leaf orientation and density, are relatively easy to measure and have been critical to progress in understanding whole - plant light absorption (Horn 1971, Weiner 1982, Johansson and Keddy 1991, Miller 1994), aboveground intra- and inter-specific plant competition (Grime 1977), and plant community assembly and composition (Grime 1977, Givnish 1982, Goldberg and Barton 1992). In contrast, the study of plant interactions belowground largely has proceeded with little empirical information on the structure of whole root communities under natural conditions.

Plant ecologists have long considered resource partitioning an important requisite for plant species coexistence (Hutchinson 1959, Tilman 1988). Compared to the single resource, light, obtained aboveground, roots acquire numerous resources from the soil, including water and as many as 17 essential nutrients (Marschner 1995). Nutrient addition experiments have revealed that coexisting species can partition belowground resources by being limited by different combinations of nutrients (e.g. N, phosphorus, potassium, Harpole and Tilman 2007) and differentiating the form and timing of nitrogen uptake (McKane et al. 1990, 2002). In addition, and of particular interest in this study, coexisting species partition belowground resources by segregating their root systems (Weaver 1919, Casper and Jackson 1997, Schenk et al. 1999).

However, important shortcomings are associated with methods typically used to measure root distributions in the field. Excavating roots, perhaps the most common method to examine root distributions, misses fine roots that are usually the most physiologically active. Other studies that rely on morphological differences of roots to distinguish roots of different species in

soil samples, often collected by coring, are limited to species that can be distinguished morphologically. Isotope methods have been used to isolate individually labeled plants from neighbors (Baldwin et al. 1971, Baldwin and Tinker 1972, Fusseder 1983, Milchunas et al. 1992), but cannot be scaled up to isolate populations of different species in diverse communities. As a consequence, there is no comprehensive information on the spatial properties of root systems of whole plant communities, particularly in grasslands, which support species that produce indistinguishable fine root systems.

The inability to identify plant roots comprehensively in grasslands has prevented the resolution of basic questions about community organization. For instance, how do root zone distributions and sizes vary among co-occurring species? Is root zone size related to canopy size? How is root zone size associated with nutrient uptake capacity? In addition, the dearth of information on the full compliment of coexisting species has stalled progress on exploring how root segregation may contribute to soil resource partitioning among species.

Molecular identification methods have great potential for providing the necessary information to address these questions. Researchers have developed the use of restriction fragment length polymorphism (RFLP) analysis of plastid genes and the rDNA internal transcribed spacer (ITS) region to identify root species in woodland, savanna, alpine, and grassland sites (Bobowski et al. 1999, Linder et al. 2000, Brunner et al. 2001, Ridgway et al. 2003). Ridgway *et al*. (2003) also described an alternative method with the potential to be more efficient than RFLP analysis. This latter technique identifies species based on direct analysis of fluorescently-tagged DNA amplification products (FFLP) from the plastid tRNA-Leu (*trnL*) gene.

We have examined the root community structure of two grasslands in Yellowstone National Park (YNP), one upland, dry site, and a second slope-bottom, mesic site. Roots were identified using FFLP analysis of species diagnostic portions of the *trn*L gene. This technique allowed us to determine, for the first time that we are aware, the root distributions of the preponderance of the coexisting species in grasslands under natural conditions. We addressed two specific questions: (1) How segregated (horizontally and by depth) were the root systems of coexisting grassland species? (2) Was aboveground biomass and the volume of soil exploited by species related in these grasslands?

MATERIALS AND METHODS

Field methods We examined the root distributions of co-occurring plant species at two grasslands on the northern winter range of YNP. YNP's northern winter range, a mostly rolling grassland and shrub-grassland, is grazed by herds of elk (*Cervus elaphus*), bison (*Bison bison*), and pronghorn (*Antilocapra americana*), primarily during October – April each year. The climate of the northern winter range is characterized by long, cold winters and short, dry summers. Thirty-year (1977 – 2007) mean annual precipitation and temperature at Mammoth Hot Springs in the northwest corner of YNP were 370 mm, with 62% falling during the April – Sept growing season, and 4.9°C, respectively. Soils of the northern winter range were derived from mostly tertiary and quaternary volcanic materials that have been glaciated several times after their deposition.

Rolling topography on the northern winter range creates steep gradients of soil moisture, organic carbon and nitrogen, and plant productivity and composition. In this study, we contrasted root distributions of coexisting plant species in two grasslands, a relatively dry upland

grassland situated on a large bench above Crystal Creek, and a mesic grassland located at the base of a slope in a large shallow depression above Mammoth Hot Springs. The two sites differed markedly in aboveground production (116 gm^{-2} [CB] vs 235 gm^{-2} [M]) and soil N (0.23% vs 0.78%) and C (2.4% vs 10.4%) content (Frank 2007).

Shoot samples (> 1 g) of all visible plant species were collected in August, 2005, and June and July, 2006, to provide material for molecular identification of species roots. In most cases shoot material from multiple (2 – 5) conspecific individuals was collected to explore the possibility of polymorphism within species (Appendix A). Shoot biomass was determined and root cores were collected in June and July of 2006 at the dry and mesic sites, respectively, after shoots had reached peak biomass in each grassland. A 3 x 4 m plot of homogeneous vegetation was established at each site. Within the plot, three parallel 4 m transects spaced 1 m apart were established and five, 2 cm diam root cores were collected at 1 m intervals starting at the beginning of each transect (15 cores /site). Soil was cored to 90 cm at M. At CB, large subsurface rocks limited the depth of soil cores to 30 or 40 cm. Each core was separated into 0-5 cm, 5-10 cm, and, thereafter, 10 cm intervals. Care was taken to as much as possible limit roots of one interval contaminating another. Soil cores were stored at -20°C until processed for root identification.

At 0, 2, and 4 m distances along each transect, shoot biomass was estimated in a 0.5 m^2 (0.71 x 0.71m) quadrat using the canopy intercept method that related biomass to the number of times a species was contacted by a pin passed through the canopy at a fixed angle (Frank and McNaughton 1990). We recorded contacts with 50 randomly placed pins per 0.5 m^2 quadrat. The root cores were removed from the center of each quadrat after shoot biomass was sampled.

Molecular methods To identify roots, we first generated a library of the tRNA-Leu (*trnL*) gene sequences from each species present at our field sites. Next, we identified a subregion within the *trnL* intron that we could use to identify species via the fluorescent fragment length polymorphism (FFLP) method of Ridgway *et al*. (2003). Note that this procedure identified the presence of species in root samples, not the abundance of species in those samples. Detailed molecular methods are provided in Appendix A.

Statistical methods The frequency that a species was found in soil core samples was used as a measure of the volume of soil that was occupied by that species. Linear and quadratic functions were used to explore the relationship between root frequency and soil depth for each species at each site. Relationships between overall root frequency and shoot biomass among species also were explored with linear and quadratic functions. A quadratic term was added only if it was found to explain an additional significant ($\alpha = 0.05$) amount of the variation in the dependent variable. Variables were log-transformed to achieve homoscedasticity.

Analysis of species segregation patterns for canopy co-occurrence data and root cores across the soil rooting depth gradient was performed using the approach of Sanders et al. (2003), which calculated a standardized 'C-score' that represented the degree to which species co-occurred more or less often than expected by chance. The quasi-swap algorithm is a method of matrix randomization that preserves row (sample richness) and column (species abundance) totals with minimum bias compared to other swap algorithms (Miklós and Podani 2004). A value between +/- 1.96 standard deviations does not reject the null hypothesis that a community is randomly assembled ($P<0.05$), while a value above 1.96 indicates a significant negative species association (i.e., segregation). Standardized C-scores were calculated for each root depth

strata and canopy data separately using the 'quasi-swap' algorithm with 500 permutations in the VEGAN statistical package (Oksanen et al. 2007) for R version 2.6.

RESULTS

Effectiveness of using *trn*L to identify roots At the dry site, all 19 species for which leaf tissue had been sampled for *trn*L analysis possessed unique fragment lengths, with the exception of two shrub species, *T. canescens* and *C. viscidiflous*, which could not be discriminated from one another (257 bp, Appendix A). An unidentified fragment of 280 bp was detected in 8 of the 76 root samples. This fragment may represent a species that was active early in the spring and was not detected aboveground when leaf tissue was sampled for *trn*L analysis, or may represent a polymorphism at the *trn*L region for another species. This fragment was not included in any of the statistical analyses.

At the mesic grassland, the fragment length for each of the 23 species for which leaf material had been collected was unique, except in the case of three species pairs; *A. adscendens* and *S. multiradiata* (261 bp), *C. arvense* and *E. laevigatum* (269 bp), and *P. pratense* and *P. pratensis* (376 bp) (Appendix A). Members of each species pair, consequently, could not be distinguished from one another.

Species richness The average number of species per 10 cm core interval (31 cm^3, 0-5 and 5-10 samples were pooled for this analysis) varied 3.5 – 5.7 species among depths at the dry site and 2.1 – 4.3 species at the mesic site (Table 1). The average number of species per 31cm^3 volume of soil across all depths was 3.9 and 2.8 at dry and mesic sites , respectively. There was a maximum eight species found in a 0-5 cm core (15.5 cm^3) at the dry site and a maximum 7 species found in 10-20 cm and 60-70 cm cores at the mesic site. No roots were found in one 50-

60 cm core from the mesic site; however for most intervals at both sites the most species-depauperate soil volume was occupied by a single species (Table 1). Because the members of certain pairs of species at each site could not be discriminated from one another, maximum, average, and minimum values for the sites are probably conservative. For example, at the mesic site, where derived belowground species richness values were likely most conservative, 261 bp, 269 bp, and 376 bp fragments, each which could represent two species in a sample, were found in 31%, 28%, and 38% of the soil core samples, repectively (Appendix B). At the dry site, the 257 bp fragment that could not distinguish a pair of species was found in 64% of the root samples (Appendix B). As a comparison to the root species richness values, the mean ($\pm$ SE) number of species per 0.5 m^2 determined by shoot material was 6.7 ($\pm$0.5) and 14.1 ($\pm$0.6) at the dry and mesic sites, respectively.

There was no significant relationship between average, maximum, or minimum number of species per soil 0-10 cm interval (0-5 cm and 5-10 cm samples were combined) with soil depth ($P > 0.10$) for either grassland. However, because the wet weight of the root samples declined exponentially with depth ($P < 0.008$ for both sites, Fig. 1), we found a positive relationship between the number of species to wet root weight (SP#/RT WT) with root depth (RD) for the two grasslands combined (there was no significant difference in the functions between sites), described by the linear relationship $\log \text{SP\#/RT WT} = 0.96(\log \text{RD}) - 2.3$ ($r^2 = 0.79$, $P < 0.0001$), where "log" denoted common logarithms.

Species root distributions Fifteen of the 19 species that were collected around the plot at the dry site for *trn*L analysis were found in the root samples, and 13 of those 15 species occurred aboveground within the quadrats sampled for shoot biomass (Appendix B). Roots of the

remaining two species presumably grew from stems located outside the quadrats. The frequency that species were present in root samples across all depth intervals (0-40 cm) at the dry site ranged from 1% for *A. cernuum* and *A. frigida* to 73% for *P. sandbergii* (Appendix B). Root frequency was unrelated to soil depth for any species at the dry site, with the exception of *P. sandbergii*, whose root frequency declined linearly with depth (Fig. 2). Thirteen of the 15 species for which roots appeared in cores at the dry site were present in the deepest soil interval (30-40 cm); the two remaining species were rare belowground and only found in one soil sample each (Appendix B).

At the mesic site, all 19 species and species pairs identified with *trn*L fragments were found aboveground or belowground (Appendix B). There were five unidentified species in the shoot biomass quadrats that represented 8% of the total shoot biomass present at the mesic site. Those five species were not identified to species at the time of sampling and tissue was not collected for later identification or for *trn*L analysis. There were no "unknown" fragments detected in root samples that did not correspond to a characterized species or species pair. Therefore, the roots of each of the five unknown species either, by chance, were not represented in the core samples or the fragment size of the unknown species was the same as another fragment-identified species.

Percent root frequency across all depths (0-90 cm) at the mesic site ranged from 0% for three species (*Potentilla anserina, Senecio sp., Viola adunca*) rarely sampled aboveground to 38% for the species pair *Phleum pratense/ Poa pratensis*, which together were abundant aboveground (Appendix B). Three species, or species pairs, varied significantly with depth: 1) the pair, *Circium arvense/ Equisetum laevigatum*, was unimodally related to depth, with root frequency peaking approximately 40-50 cm, (2) *Fragaria virginiana* was negatively, linearly related to depth, and (3) *Trifolium repens* was positively related to soil depth (Fig 3). Thirteen of

254 the 16 species whose roots were identified in samples were detected at the deepest sampled depth
255 (80-90 cm); two of the remaining three species (*Sisyrinchium angustifolium, Taraxacum*
256 *officinale*) were found as deep as 70 – 80 cm and the third species (*Iris missouriensis*) was very
257 rare and found in only one soil sample (Appendix B).
258
259 Relationship between root frequency and shoot biomass Log-transformed shoot biomass was
260 positively related to log-transformed root frequency (calculated for the entire sampled soil
261 profile) in both grasslands. At the dry site, the relationship was linear (Fig. 4a). The slope of the
262 log – log relationship did not differ from unity ($P = 0.71$), indicating that the relationship
263 between the untransformed variables did not depart from linearity. The seven most common
264 species that produced > 1.2 gm^2 of shoot material had the seven highest root frequencies (>
265 20%). At the mesic site, there was an increasing quadratic relationship between log shoot
266 biomass and log root frequency (Fig. 4b).
267
268 Aboveground and belowground species associations Plant species were associated randomly
269 aboveground and belowground at the dry site, except for a statistically significant amount of
270 species segregation that occurred in the 10-20 cm soil interval (Fig. 5). Correlation analyses
271 examining the presence and absence of all species pairs at that interval revealed significant
272 negative associations of *F. idahoensis* with *A. tridentata* and *P. spicata*. ($P < 0.002$ for both). *P.*
273 *spicata* and *A. tridentata* were found in six and nine of the 10, 0 - 40 cm cores in which *F.*
274 *idahoensis* roots were identified. Consequently the negative relationship between *F. idahoensis*
275 and *A. tridentata*, in particular, was not a result of the two species having been horizontally
276 separated in the sampling plot. A re-analysis of species association patterns for 10-20 cm

samples without *F. idahoensis* resulted in the remaining species being randomly associated, indicating that the distribution of *F. idahoensis* roots was responsible for the significant segregation signature for roots in 10 – 20 cm soil in the original analysis. At the mesic site, plant species were randomly associated aboveground and at each soil depth interval (Fig. 5)

DISCUSSION

Root segregation The role of resource partitioning in promoting the diversity of plant communities has been a long-term topic of great interest to plant ecologists (Hutchinson 1959, Schoener 1974, Berendse 1979, Chesson 1994). Results from a number of studies indicate that species spatially differentiate their root systems, which is considered a prominent mechanism used by coexisting species to partition soil resources (Casper and Jackson 1997, Schenk et al. 1999). However, most of these root investigations have been hampered by important limitations of the standard methods that are usually used to measure root distributions. Excavating roots, for instance, results in the loss of fine roots, which often are the most physiologically active roots. Other studies that rely on visually discriminating roots of different species are limited to the proportionally few co-existing species that can be morphologically distinguished (Vogt et al. 1989, Harper et al. 1991, Casper and Jackson 1997, Schenk et al. 1999). Moreover, we are unaware of any root study that has included a random null model to explore spatial co-occurrence.

In this study we used a molecular method to identify root fragments picked from soil cores in order to determine the distribution of the roots of plant species in two YNP grasslands. Results suggest that root segregation played a relatively minor role in resource partitioning among the great majority of the species in these grasslands. Roots picked from 31 cm^3 soil volumes (10 cm

intervals) usually included mixed species. An average of 3.9 and 2.8 species, with maximum numbers of 8 and 7 species, were found at dry and mesic sites, respectively. Analysis of belowground community assembly (Fig. 5) revealed random sorting among species at each soil depth at dry and mesic sites, with the exception of a statistically significant segregation signal among species occurring at the 10-20 cm soil interval at the dry grassland. Further analysis indicated that the segregation signature for that interval was due to *F. idahoensis* spatially differentiating its roots from *A. tridentata*, a shrub, and *P. spicata*, a grass. The segregation of two common grasses, *F. idahoensis* and *P. spicata*, that have similar mid-season aboveground production pulses is consistent with results of McKane et al. (1990), who found belowground spatial differentiation between two dominant grasses that possessed similar phenologies in an old-field community.

There also was limited support for species segregating according to depth in both YNP grasslands. We found that the root frequencies of a single species (*Poa sandbergii*) at the dry grassland (Fig. 2) and two species (F. *virginiana, T. repens*) and a species pair (*C. arvense/ E. laevigatum*) at the mesic grassland (Fig. 3) were related to soil depth. However, root frequency was unassociated with depth for the great majority of the species in both grasslands. There also was no relationship between species number and soil depth, even though root biomass declined exponentially with depth. Finally, the preponderance of species was found throughout the entire soil profile that was sampled at each site, indicating that the vast majority of species exploited all soil depths.

The results indicating that multiple species occupied a relatively small volume of soil, the widespread random sorting of species, and the limited evidence for differentiation by depth among species in both grasslands suggests that root segregation probably played a relatively

minor role in maintaining species coexistence. Instead, differentiating the form (e.g., NH_4^+ vs NO_3^-) or timing that a nutrient is taken up (McKane et al. 1990, 2002) or differences in the combinations of limiting soil resources (Harpole and Tilman 2007) may largely be responsible for resource partitioning among co-occurring plant species. Of course, this study has not addressed the effects of pathogens (Dobson and Crawley 1994, Mitchell 2003, Kulmatiski et al. 2008), herbivory (Grime 1979, Milchunas et al. 1988, Grace and Jutila 1999), and disturbance (Pickett and White 1985, Picket et al. 1999), which also can be major determinants of plant community composition and may have played important roles in the shoot and root community properties in YNP grassland.

Shoot - root relationships There has been considerable interest in factors that control the variation in shoot vs root allocation among species and the importance of shoot vs root competition in structuring plant communities (Cahill 1999, 2002, de Kroon et al. 2002). A number of studies have indicated that belowground competition, in general, is stronger than aboveground competition (Fowler 1986, Wilson 1988, Casper and Jackson 1997), in particular in habitats primarily limited by soil resources, such as water as in the case of YNP grasslands that were examined in this study.

Competition aboveground is generally considered to be asymmetrical because of the ability of taller plants to cast shade on their understory neighbors (Weiner 1990, Casper and Jackson 1997, de Kroon et al. 2002). Whether or not belowground competition is symmetrical or asymmetrical is still unclear. Several studies that have experimentally varied root biomass in the greenhouse and field have concluded the existence of size-symmetric root competition (Gerry and Wilson 1995, Weiner et al. 1997, Cahill and Casper 2000). However, there is evidence that

346 under some conditions root competition may be asymmetrical (Fransen et al. 2001). In addition,
347 it has been proposed that asymmetrical root competition is most likely to occur in nutrient rich
348 soils where the soil volume is completely occupied by roots and resources become available in a
349 patchy manner as organic material is mineralized. Under such circumstances, species with more
350 widely distributed roots may have a disproportionate competitive advantage over other species
351 that are less proliferated throughout the soil (Fransen et al. 2001, de Kroon et al. 2002).
352 Root frequency, a measure of the soil volume occupied by a species, was positively related
353 to shoot biomass in both YNP grasslands. This indicated that the capacity of a species to
354 produce shoot biomass was associated with the volume of soil exploited by its roots. At the dry
355 site, the seven most abundant plants aboveground possessed the seven most proliferated root
356 systems, suggesting the importance of relatively extensive root systems in establishing
357 aboveground dominance. In addition, the relationship between shoot biomass and root frequency
358 at the dry grassland (Fig. 4a) indicated that the ability of a root system to support shoot biomass
359 did not vary with the volume of soil exploited by species. If one defines the competitiveness of a
360 species as the ability of that species to obtain resources, and it is further assumed that the amount
361 of shoot biomass that is supported by root system volume (i.e., frequency) is a measure of the
362 capacity of a unit of root system to supply soil resources to shoots, then competition among
363 species at the dry grassland was symmetrical; the amount of shoot biomass supported by roots
364 increased linearly as the amount of soil exploited by roots increased. In contrast, at the mesic
365 site, shoot biomass increased quadratically, at a rate greater than linear (Fig. 4b). For instance,
366 the ratio, shoot biomass : root frequency at the mesic site increased from 0.05 to 0.4, by 700%,
367 for species with 10% and 30% root frequency. The increase in the capacity of a unit root
368 distributed in the soil to supply shoot biomass as the size of a species root system increases

suggests that belowground competition at the mesic site was operating asymmetrically. Evidence for symmetric competition at the relatively dry and infertile grassland vs asymmetric competition at the relatively mesic and fertile grassland supports the notion that asymmetric competition may be more common in resource-rich compared to resource-poor environments (Fransen et al. 2001, de Kroon et al. 2002).

However, care needs to be exercised when interpreting the results of this study for several reasons. First, this study examined the presence and absence of species in soils, not their abundance. Plant species have been found to allocate root biomass differently, some producing finer roots or proliferating through the soil more diffusely than others (Eissenstat and Caldwell 1988). Consequently, the results do not provide information on the distribution of root biomass of species. Second, we have treated all roots equivalently, even though roots will differ in function, with some providing more of an anchoring function while other roots will primarily function to take up resources (Robinson et al. 2002). Third, we did not measure grazing in this study and therefore we do not know at our sites which species lost more aboveground biomass than others to consumers and how herbivory may have influenced the distribution of roots of species.

Nevertheless this study provides novel information on species root distributions, community belowground assembly, and the linkages between belowground and aboveground allocation strategies in grassland. The results revealed a limited amount of spatial, including depth, segregation of species in YNP grassland. We also found that shoot biomass was positively related to the soil volume exploited by a species, indicating the importance of the soil occupied by a root system in establishing aboveground dominance in semi-arid grassland. These findings

suggest that the maintenance of grassland diversity in YNP is primarily a function of factors other than the spatial segregation of species root systems.

ACKNOWLEDGEMENTS

We thank D. Springer and I. Panagopoulos for early help developing molecular methods to identify roots and E. Hellquist and V. Green for assistance with collecting the soil core samples and shoot measurements. This study was funded by the SU College of A&S and NSF grant DEB-0318716.

414 LITERATURE CITED

415 Berendse, F. 1979. Competition between plant populations with different rooting depths I.
416 Theoretical considerations. Oecologia 43:19-26.
417
418 Baldwin, J.P. and P.B. Tinker. 1972. A method for estimating the lengths and spatial patterns of
419 two interpenetrating root systems. Plant and Soil 37:209-213.
420
421 Baldwin, J.P. and P.B. Tinker and F.H.C. Marriott. 1971. The measurement of length and
422 distribution of onion root in the field and the laboratory. Journal of Applied Ecology 8: 543-554.
423
424 Bobowski, B.R., Hole, D., Wolf, and P.G., Bryant, L. 1999. Identification of roots of woody
425 species using polymerase chain reaction (PCR) and restriction fragment length polymorphism
426 (RFLP) analysis. Mol Ecol 8:485-491.
427
428 Brunner, I., S. Brodbeck, U. Büchler, and C. Sperisen. 2001. Molecular identification of fine
429 roots of trees from the Alps: reliable and fast DNA extraction and PCR-RFLP analyses of plastid
430 DNA. Mol Ecol 10:2079-2087.
431
432 Cahill, J.F. Jr. 1999. Fertilization effects on interactions between above- and belowground
433 competition in an old field. Ecology 80: 466-480.
434
435 Cahill, J.F. Jr. 2002. Interactions between root and shoot competition vary among species.
436 Oikos 99: 101-112.

Cahill, J.F, and B.B. Casper. 2000. Investigating the relationship between neighbor root biomass and belowground competition: field evidence for symmetric competition belowground. Oikos 90: 311-320.

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

Chesson, P. 1994. Multispecies competition in variable environments. Theoretical Population Biology 45: 227-276.

De Kroon, H., L. Mommer, and A Nishiwaki. 2002. Root competition: Towards a mechanistic understanding. Pages 215-234 *in* H. de Kroon, E.J.W. Visser, editors. Root ecology. Springer-Verlag, New York.

Dobson, A. and M. Crawley. 1994. Pathogens and the structure of plant communities. TREE 9:393-398.

Eissenstat, D.M. and M.M. Caldwell. 1988. Seasonal timing of root growth in favorable microsites. Ecology 69: 870-873.

Fowler, N. 1986. The role of competition in plant communities in arid and semiarid regions. Annual Review of Ecology and Systematics 17:89-110.

Frank D.A. 2007 Drought effects on above- and belowground production of a grazed temperate grassland ecosystem. Oecologia 152: 131-139.

Frank, D.A. and S.J. McNaughton. 1990. Aboveground biomass estimation with the canopy intercept method: a plant growth form caveat. *Oikos* 57:57-60.

Fransen, B, H. de Kroon, and F. Berendse. 2001. Soil nutrient heterogeneity alters competition between perennial grass species. Ecology 82: 2534-2546.

Fusseder, A. 1983. A method for measuring length, spatial distribution and distances of living roots *in situ*. Plant and Soil 73: 441-445.

Gerry, A.K. and Wilson, S.D. 1995. The influence of initial size on the competitive responses of six plant species. Ecology 76: 272-279.

Givnish, T.J. 1982. On the adaptive significance of leaf height in forest herbs. American Naturalist 120:353-381.

Goldberg, D.E. and Barton, A.M. 1992. Patterns and consequences of interspecific competition in natural communities: A review of field experiments with plants. American Naturalist 139: 771-801.

Grace, J.B., and H. Jutila. 1999. The relationship between species density and community biomass in grazed and ungrazed coastal meadows. Oikos 85:398–408

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169-1194.

Grime, J.P. 1979. Plant strategies and vegetation processes. Wiley, London

Harper, J.L., Jones, M. and Sackville Hamilton, N.R. 1991. The evolution of roots and the problem of analyzing their behaviour. Pages 2-22 *in* D. Atkinson, editor, Plant root growth: An ecological perspective. Blackwell Scientific Publications, Oxford.

Harpole, W.S. and D. Tilman. 2007. Grassland species loss resulting from reduced niche dimension. Nature 446: 791-793.

Horn, H.S. 1971. The adaptive geometry of trees. Princeton University Press, New Jersey.

Hutchinson, G.E. 1959. Homage to Santa Rosalia: or, why are there so many kinds of animals. American Naturalist 93: 145-159.

Jackson RB, Moore LA, Hoffmann WA, Pockman WT, Linder CR. 1999. Ecosystem rooting depth determined with caves and DNA. Proc Natl Acad Sci USA 96:11387-11392.

505 Johansson, M.E. and Keddy, P.A. 1991. Intensity and asymmetry of competition between plant
506 pairs of different degrees of similarity: an experimental study on two guilds of wetland plants.
507 Oikos 60:27-34.
508
509 Kulmatiski, A., K.H. Beard, J.R. Stevens, and S.M. Cobbold. 2008. Plant-soil feedbacks: a meta-
510 analytical review. Ecology Letters 11: 980-992.
511
512 Linder CR, Moore LA, Jackson RB. 2000. A universal molecular method for identifying
513 underground plant parts to species. Mol Ecol 9:1549-1559.
514
515 McKane, R.B., D.F. Grigal, and M.P. Russelle. 1990. Spatiotemporal differences in ^{15}N uptake
516 and the organization of an old-field plant community. Ecology 71: 1126-1132.
517
518 McKane, R.B., L.C. Johnson, G.R. Shaver, K.J. Nadelhoffer, E.B. Rastetter, B. Fry, A.E. Giblin,
519 K. Kielland, B.L. Kwiatkowski, J.A. Laundre, and G, Murray. 2002. Resource-based niches
520 provide a basis for plant species diversity and dominance in arctic tundra. Nature 415: 68-71.
521
522 Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, NY.
523
524 Miklós, I. and Podani, J. (2004). Randomization of presence-absence matrices: comments and
525 new algorithms. Ecology 85, 86–92.
526

527 Milchunas D.G., O.E. Sala, and W.K. Lauenroth. 1988. A generalized model of the effects of
528 grazing by large herbivores on grassland community structure. Am Nat 132:87–106
529
530 Milchunas, D.G., C.A. Lee, W.K. Lauenroth and D.P. Coffin. 1992. A comparison of ^{14}C, ^{86}Rb,
531 and total excavation for determination of root distributions of individual plants. Plant and Soil
532 144: 125-132.
533
534 Miller, T.E. 1994. Direct and indirect species interactions in an early old-field plant community.
535 American Naturalist 143: 1007-1025.
536
537 Mitchell, C.E. 2003. Trophic control of grassland production and biomass by pathogens.
538 Ecology Letters 6: 147-155.
539
540 Oksanen, J., Kindt, R., Legendre, P., O'Hara, B. and Stevens, M. H. H. 2007. VEGAN:
541 Community Ecology Package (R package version 1.8-7)
542
543 Pickett, S.T.A. and White, P.S. 1985. The ecology of natural disturbance as patch dynamics.
544 Academic Press, New York.
545
546 Pickett, S.T.A., J. Wu, and M.L. Cadenasso. 1999. Patch dynamics and the ecology of disturbed
547 ground: A framework for synthesis. Pages 707-722 *in* L.R. Walker, editor. Ecosystems of
548 disturbed ground. Elsevier, Amsterdam.
549

Ridgway, KP, Duck JM, Young JPW. 2003. Identification of roots from grass swards using PCR-RFLP and FFLP of the plastid *trn*L (UAA) intron. BMC Ecology 3:8.

Robinson, D., A. Hoidge and A. Fitter. 2002. Constraints on the form and function of root systems. Pages 1-32 *in* H. de Kroon, and E.J.W. Visser, editors. Root ecology. Springer-Verlag, New York.

Sanders, N. J., Gotelli, N. J., Heller, N. E. & Gordon, D. M. 2003. Community disassembly by an invasive species. *Proc. Natl Acad. Sci. USA* 100, 2474-2477.

Schenk, H.J., Callaway, R.M. and Mahall, B.E. 1999. Spatial root segregation: Are plants territorial? Advances in Ecological Research 28:145-180.

Schoener, T.W. 1974. Resource partitioning in ecological communities. Science: 185: 27-39

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton

Vogt, K.A., Vogt, D.J., Moore, E.E., and Sprugel, D.G. 1989. Methodological considerations in measuring biomass, production, respiration and nutrient resorption for tree roots in natural ecosystems. Pages 217-232 *in* J.G. Torrey and L.J. Winship, editors, Applications of continuous and steady-state methods to root biology. Kluwer Academic Publishers, Dordrecht.

573 Weaver, J.E. 1919. The ecological relations of roots. Carnegie Institution of Washington.
574 Washington, DC.
575
576 Weiner, J. 1982. A neighborhood model of annual-plant interference. Ecology 63: 1237-1241.
577
578 Weiner, J. 1990. Asymmetric competition in plant populations. Trends in Ecology and
579 Evolution 360-364.
580
581 Weiner, J., Wright, D.B. and Castro, S. 1997. Symmetry of below-ground competition between
582 *Kochia scoparia* individuals. Oikos 79: 85-91.
583
584 Wilson, S.D. 1988. Shoot competition and root competition. Journal of Applied Ecology 25:
585 279-296
586
587

588 Table 1. Average, maximum, and minimum number of species found in soil intervals and

589 averaged across intervals. Values for 0-5 and 5-10 cm data are both pooled and left separated.

		Dry site (Crystal Bench)				Mesic site (Mammoth)			
Soil depth (cm)		**Average (n)**		**Maximum**	**Minimum**	**Average (n)**		**Maximum**	**Minimum**
0 - 10 (pooled)	*0 -5*	**5.7 (15)**	*4.5 (15)*	**8**	***2***	**4.3**	*3.2 (15)*	**7**	**2**
	5 -10		*3.8 (15)*	*6*	***1***		*2.4 (15)*	*4*	*1*
10-20		**3.8 (15)**		**7**	**1**	**2.7 (14)**		**7**	**1**
20 – 30		**3.8 (15)**		**7**	**1**	**3.1 (15)**		**5**	**1**
30 – 40		**3.5 (12)**		**7**	**1**	**3.4 (15)**		**6**	**1**
40 – 50						**2.6 (15)**		**4**	**1**
50 – 60						**2.6 (14)**		**5**	**0**
60 – 70						**3.4 (15)**		**7**	**1**
70 – 80						**2.7 (15)**		**5**	**1**
80 - 90						**2.1 (15)**		**4**	**1**
Average among 10cm intervals		**3.9**		**7.0**	**1.2**	**2.8**		**5.3**	**0.9**

590

591

592

593

594

595

FIGURE LEGEND

Fig. 1. The relationships of the average number of species per root sample and wet root weight with soil depth for two YNP grasslands, (a) the dry site (Crystal Bench) and (b) the mesic site (Mammoth). Species number and root weight values are per 2 cm diam by 10 cm soil depth increment. Sample sizes are 15, except for the 40 - 50 cm interval at the mesic grassland, which is provided in parentheses (see Appendix A for explanation). Wet root weight (RW) declined exponentially with soil depth for the dry site by $RW = 877e^{-0.14(SD)}$ ($r^2 = 0.98$, $P < 0.008$) and for the mesic site by $RW = 243e^{-0.09(SD)}$ ($r^2 = 0.94$, $P < 0.0001$).

Fig. 2. Relationship between root frequency (RT FREQ) and root depth (RT DEPTH) for *Poa sandbergii* at the dry site (Crystal Bench). Root frequency is the percentage of the samples in which the species was identified.

Fig. 3. Relationships between root frequency and root depth for (a) the species pair *Cirsium arvense – Equisetum laevigatum* and the species (b) *Fragaria virginiana* and (c) *Trifolium repens* at the mesic site (Mammoth).

Fig. 4. The relationship between common log-transformed shoot biomass and common log-transformed root frequency among species at the (a) dry and (b) mesic grasslands.

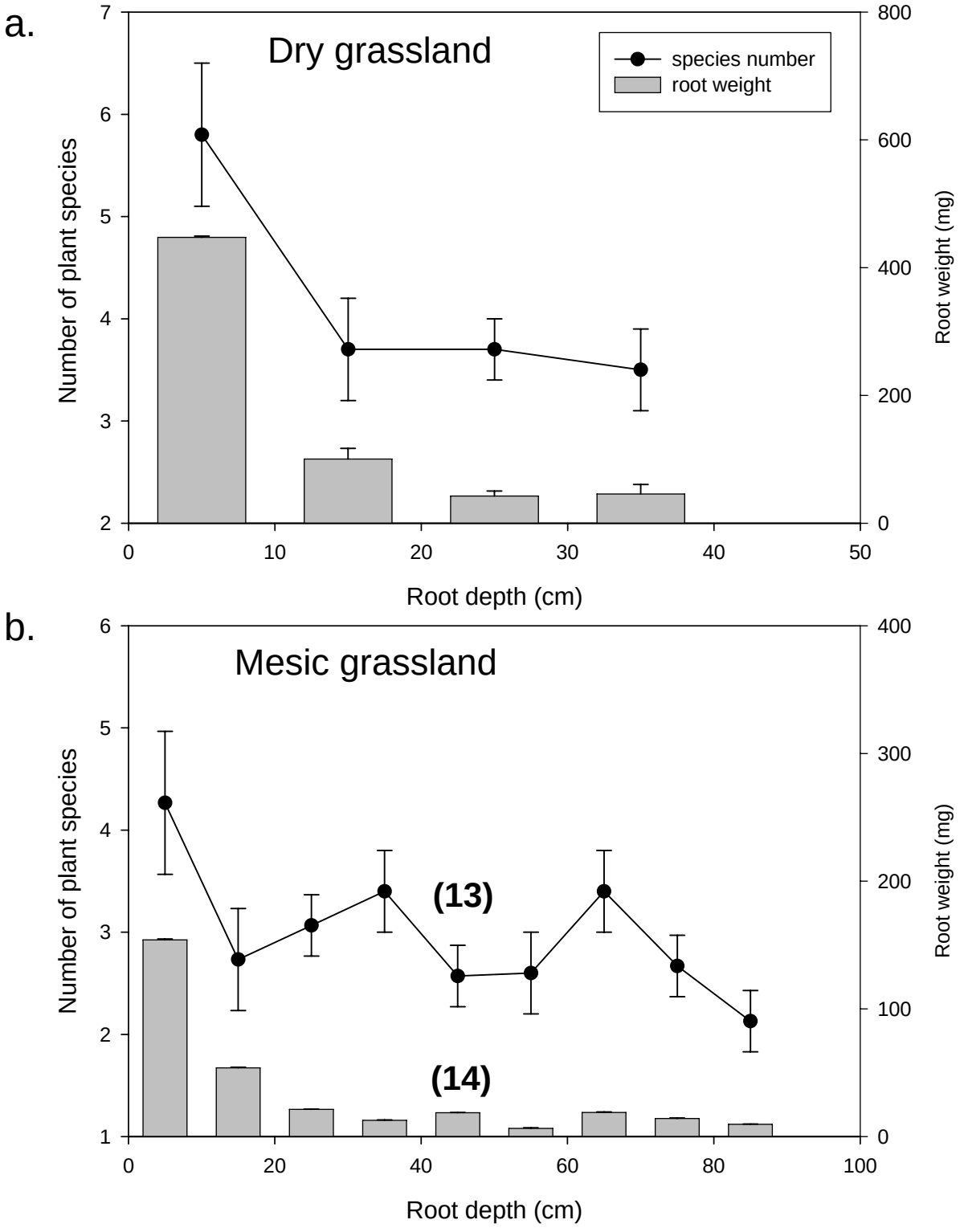
Fig. 5. Species associations aboveground and at 10 cm soil intervals belowground at the dry (Crystal Bench, black) and mesic (Mammoth, grey line) grasslands. Values within dashed grey

619 lines indicate random associations; values above 1.96 indicate a significant negative association

620 ($P<0.05$)

621

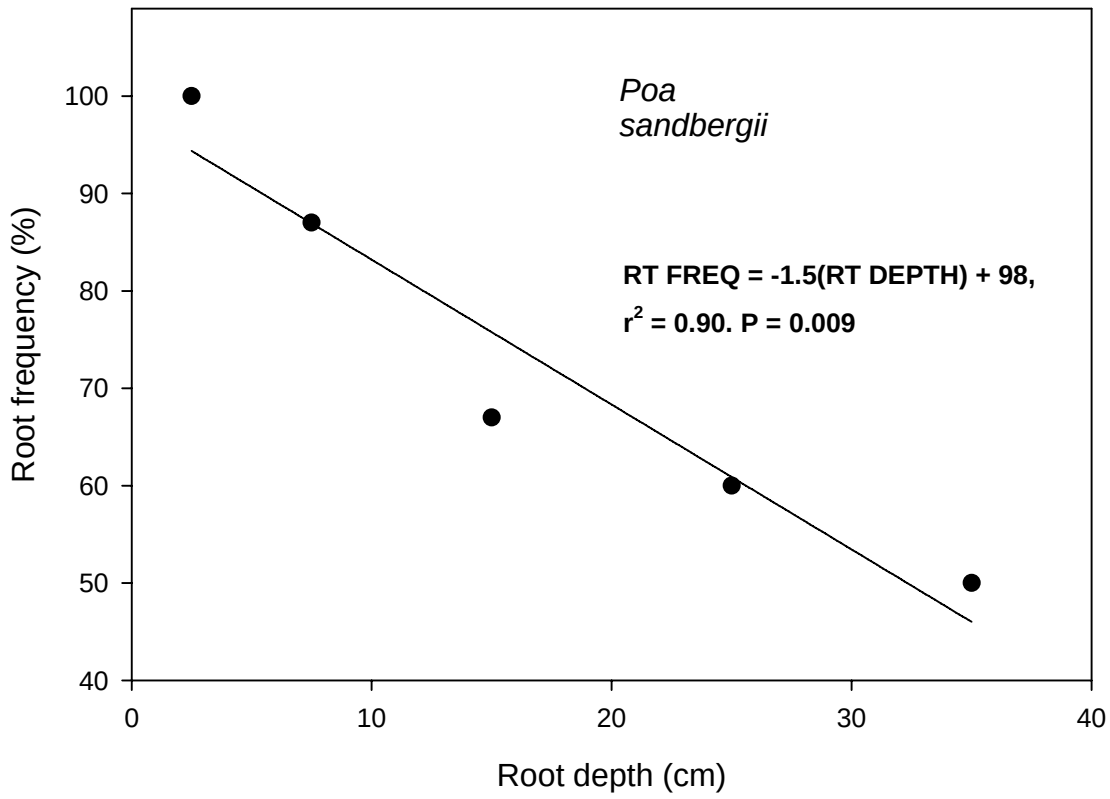
Fig. 1


a.
Dry grassland
species number
root weight
Number of plant species
Root weight (mg)
Root depth (cm)
b.
Mesic grassland
(13)
(14)
Number of plant species
Root weight (mg)
Root depth (cm)

623

624 Fig. 2

625



626
627

628

629

630

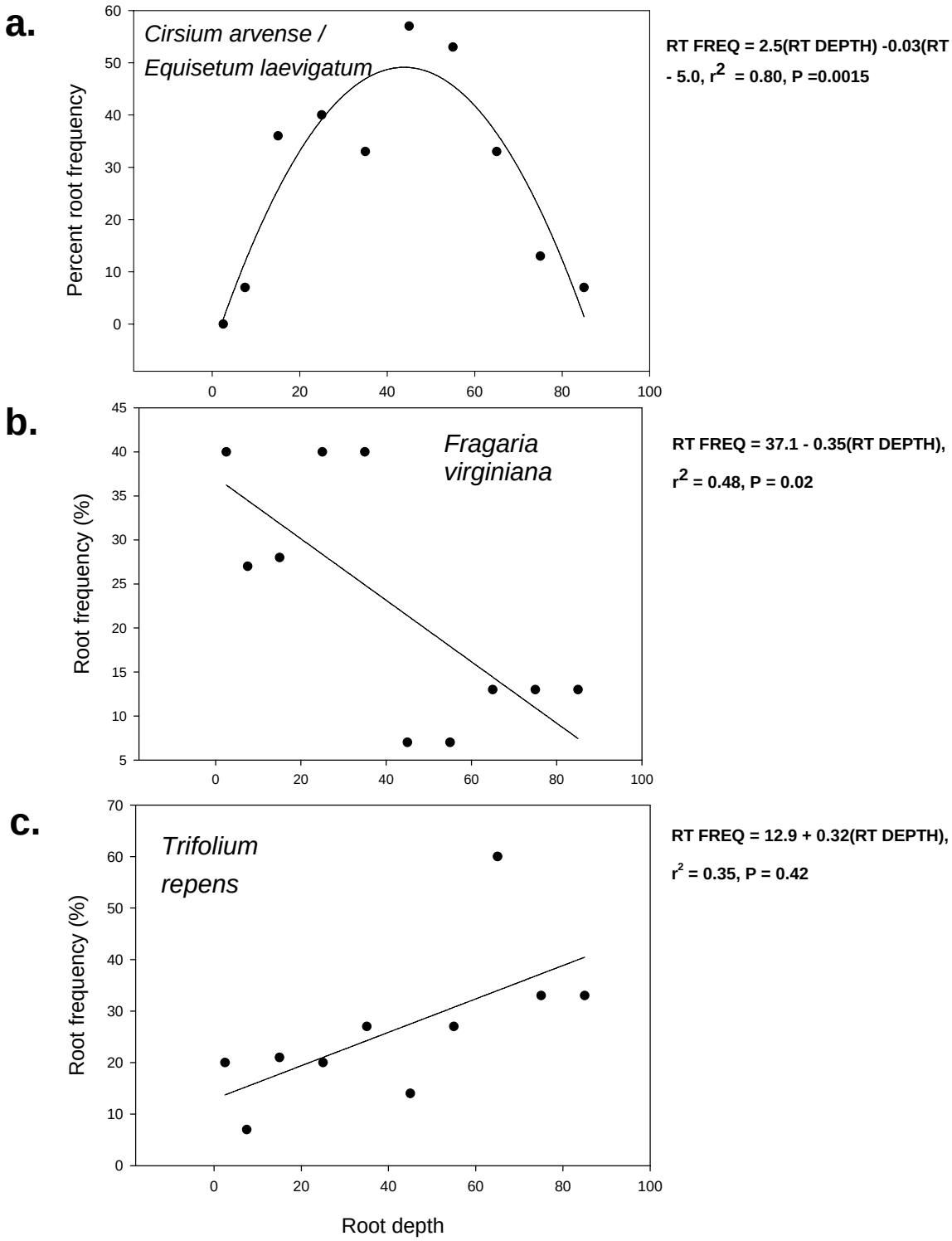
631

632

633

634

635 Fig. 3

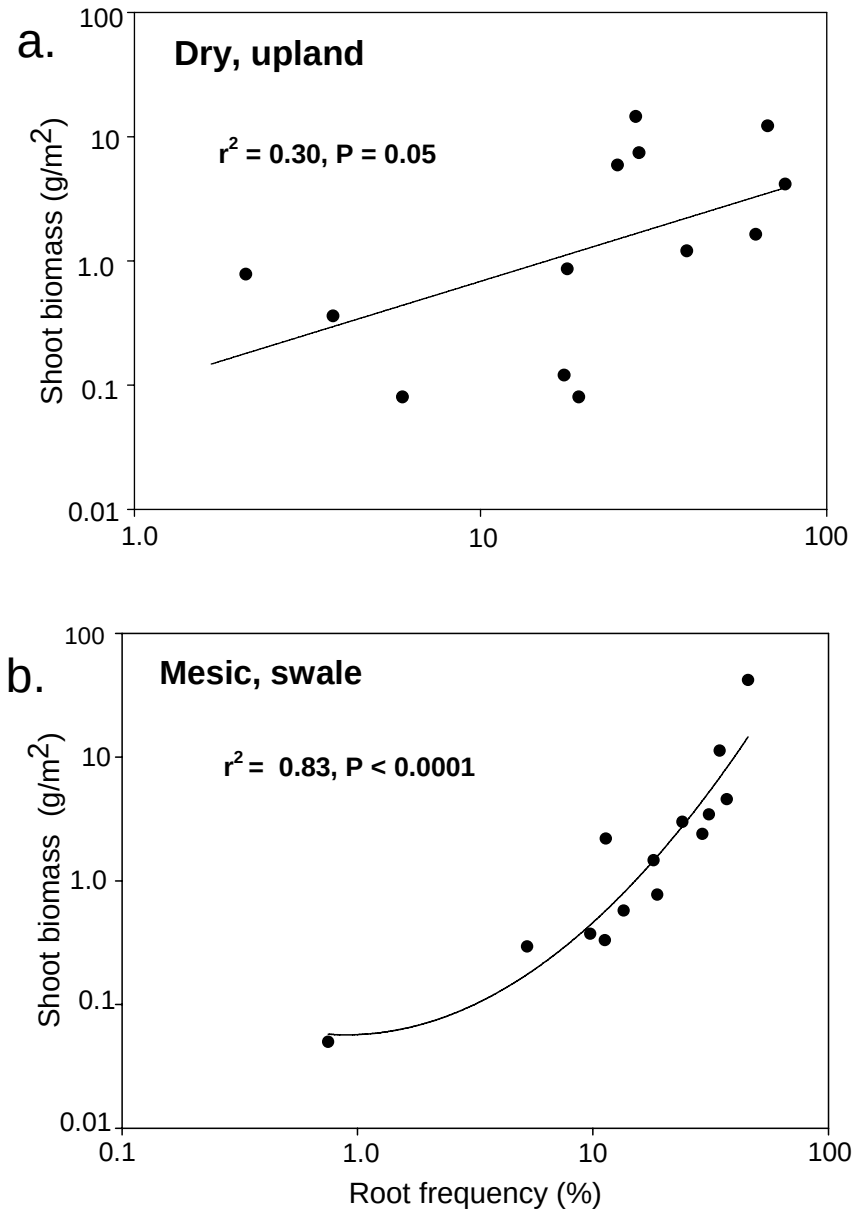


636

637

638 Fig. 4

639



640

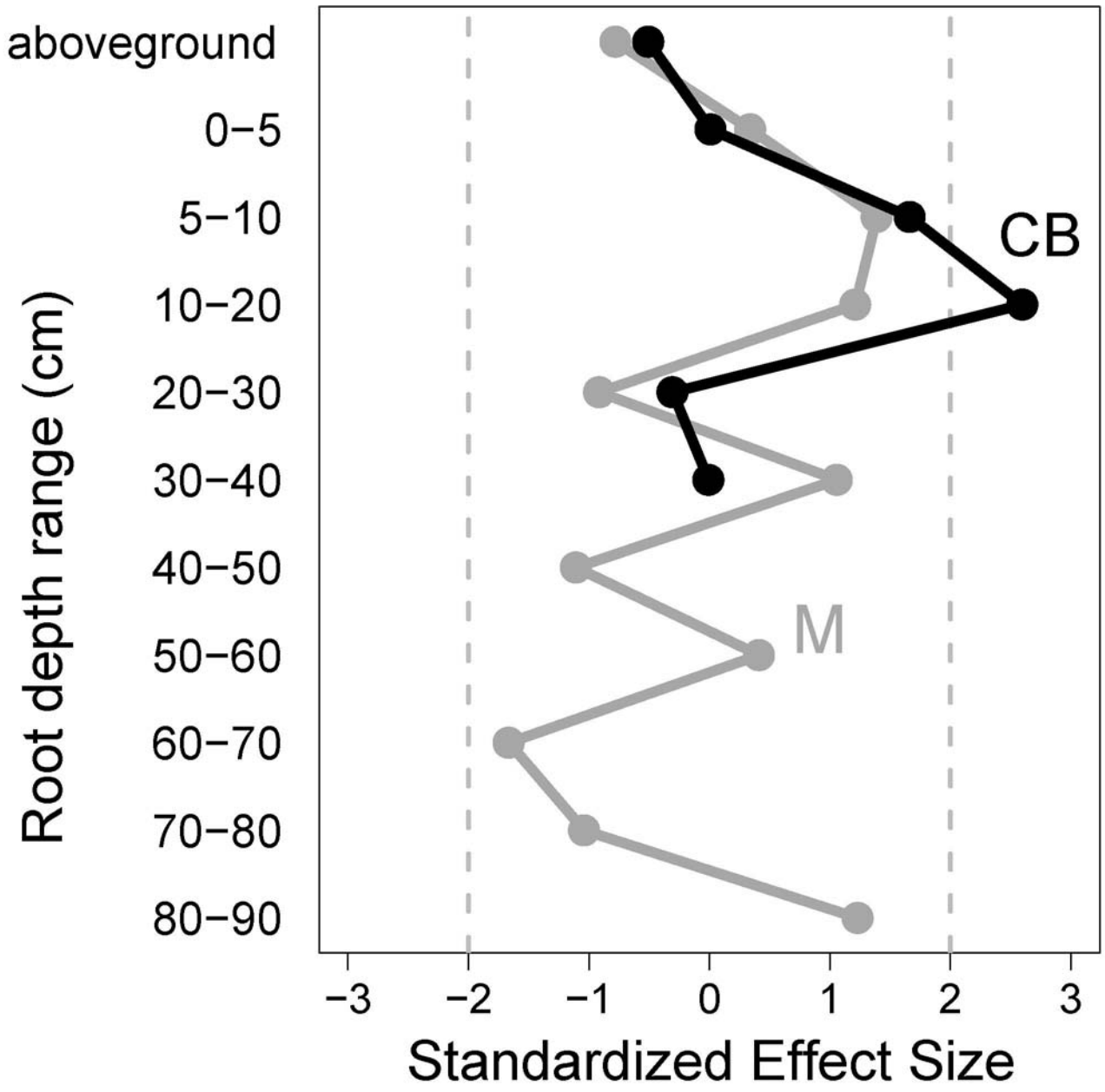
641

642

643

644

645 Fig. 5



646