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Grassland root communities: species distributions and how they are linked to aboveground abundance

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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 (YNP). Aboveground biomass was measured, and soil cores (2 cm in diameter) were collected to depths of 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 species at a dry grassland and a mesic grassland, respectively. By contrast, there was an average of 6.7 and 14.1 species per 0.5 m^2, determined by the presence of shoot material, 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 *Festuca idahoensis* segregated from *Artemisia tridentata* and *Pseudoroegnaria 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, USA.

Introduction

As sessile organisms, plants capture resources and interact with neighbors within the aboveground (sward) and belowground (root) zones that they occupy. 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 interspecific 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

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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 of examining root distributions, misses fine roots that are usually the most physiologically active. Other studies that rely on morphological differences to distinguish roots of different species in soil samples, often collected by coring, are limited to a small subgroup of species found in the community. 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 communities of plants, particularly in grasslands, which support many coexisting 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 complement 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 fluorescent-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 Yellowstone National Park. 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 at Mammoth Hot Springs in the northwest corner of YNP was 370 mm, with 62% falling during the April–Sept growing season, and mean temperature was 4.9°C. 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 g/m^2 [dry] vs. 235 g/m^2 [mesic]) 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 × 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 diameter root cores were collected at 1-m intervals starting at the beginning of each transect (15 cores per 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. As much care as possible was taken to prevent roots of different intervals from contaminating one 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 × 0.71 m) 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 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 >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 trnL 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, *Tetradymia canescens* and *Chrysothamnus viscidiflorus*, 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: *Aster adscendens* and *Solidago multiradiata* (261 bp), *Cirsium arvense* and *Equisetum laevigatum* (269 bp), and *Phleum pratense* and *Poa 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: there were 3.5–5.7 species among depths at the dry site and 2.1–4.3 species at the mesic site (Table 1). There was an average of 3.9 and 2.8 species per 31 cm^3 volume of soil across all depths at dry and mesic sites, respectively. There was a maximum of 8 species found in a 0–5 cm core (15.5 cm^3) at the dry site and a maximum of 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 of which could represent two species in a sample, were found in 31%, 28%, and 38% of the soil core samples, respectively (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, species richness determined by shoot material was 6.7 ± 0.5 species and 14.1 ± 0.6 species per 0.5 m^2 (mean $\pm$ SE) 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) and soil depth ($P > 0.10$) for either grassland. However, because the wet mass of the root samples declined exponentially with depth (Fig. 1), we found a positive relationship between the number of species divided by wet root mass, and root depth, for the two grasslands combined (there was no significant difference in the functions between sites), described by the linear relationship $\log_{10}(\text{no. species})/\text{root mass} = 0.96 \log_{10}(\text{root depth}) - 2.3$ ($r^2 = 0.79$, $P < 0.00010$.

TABLE 1. Average, maximum, and minimum number of species found in soil depth intervals and averaged across intervals.

	Dry site (Crystal Bench)			Mesic site (Mammoth)		
Soil depth (cm)	Average (*n*)	Maximum	Minimum	Average (*n*)	Maximum	Minimum
0–5	4.5 (15)	8	2	3.2 (15)	6	1
5–10	3.8 (15)	6	1	2.4 (15)	4	1
0–10 (pooled)	5.7 (15)	8	3	4.3 (15)	7	2
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 10-cm intervals	3.9	7.0	1.2	2.8	5.3	0.9

Notes: Values for 0–5 cm and 5–10 cm depths appear in the first two rows, and pooled values for the 0–10 cm depth appear in the third row. The variable *n* represents the number of root core samples. Sample sizes are in parentheses.

Species root distributions

Fifteen of the 19 species that were collected in 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 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

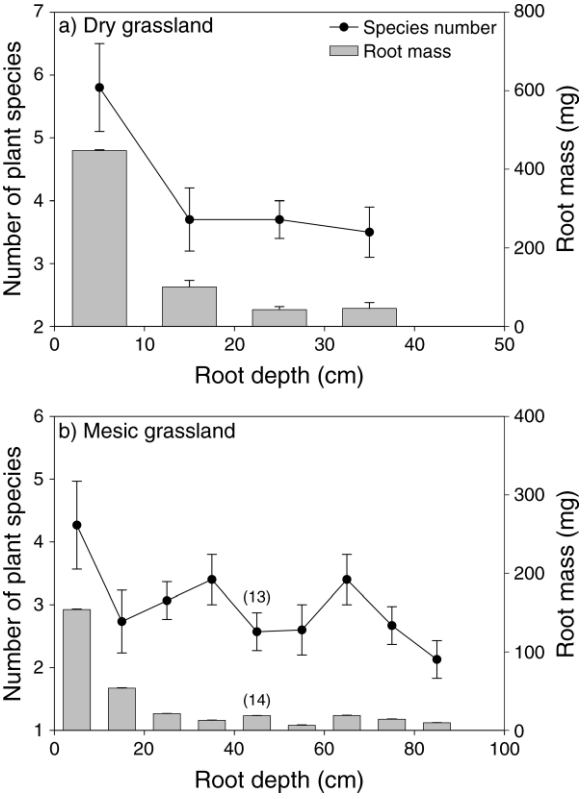

FIG. 1. The average number of species per root sample and wet root mass with soil depth for two Yellowstone National Park grasslands, (a) the dry site (Crystal Bench) and (b) the mesic site (Mammoth). Species number and root mass values are per 2 cm diameter by 10 cm soil depth increment. Sample sizes are 15, except for the 40–50 cm interval at the mesic grassland, which is provided in parentheses (see Appendix A for an explanation). The error bars represent ±SE. Wet root mass (RM) declined exponentially with soil depth (SD) for the dry site by $RM = 877e^{-0.14(SD)}$ ($r^2 = 0.98$, $P < 0.008$) and for the mesic site by $RM = 243e^{-0.09(SD)}$ ($r^2 = 0.94$, $P < 0.0001$).

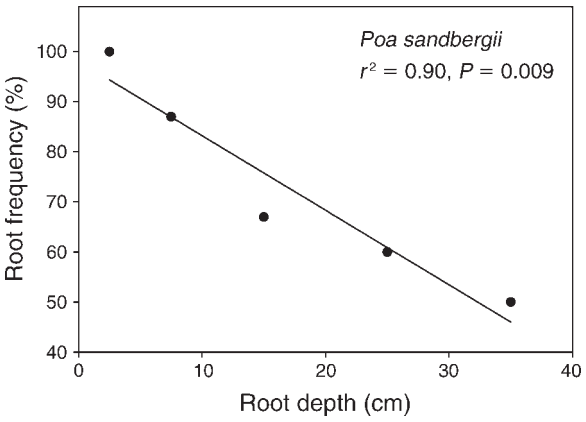

FIG. 2. Relationship between root frequency and root depth for *Poa sandbergii* at the dry site (Crystal Bench). Root frequency = −1.5(root depth) + 98. Root frequency is the percentage of the samples in which the species was identified.

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 at ~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 the 16 species whose roots were identified in samples were detected at the deepest sampled depth (80–90 cm); two of the remaining three species (*Sisyrinchium angustifolium*, *Taraxacum officinale*) were found as deep as 70–80 cm, and the third species (*Iris missouriensis*) was very rare and found in only one soil sample (Appendix B).

Relationship between root frequency and shoot biomass

Log-transformed shoot biomass was positively related to log-transformed root frequency (calculated for the entire sampled soil profile) in both grasslands. At the dry site, the relationship was linear (Fig. 4a). The slope of the log–log relationship did not differ from unity ($P = 0.71$), indicating that the relationship between the untransformed variables did not depart from linearity. The seven most common species that produced >1.2 g/m^2 of shoot material had the seven highest root frequencies (>20%). At the mesic site, there was an increasing quadratic relationship between log(shoot biomass) and log(root frequency) (Fig. 4b).

Aboveground and belowground species associations

Plant species were associated randomly aboveground and belowground at the dry site, except for a statistically significant amount of species segregation that occurred in the 10–20 cm soil interval (Fig. 5). Correlation analyses examining the presence and absence of all species pairs at that interval revealed significant negative associations of *F. idahoensis* with *A. tridentata* and *P. spicata*. ($P < 0.002$ for both). *P. spicata* and *A. tridentata* were found in 6 and 9 of the 10, 0–40 cm cores in which *F. idahoensis* roots were identified. Consequently, the negative relationship between *F. idahoensis* and *A. tridentata*, in particular, was not a result of the two species having been horizontally 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

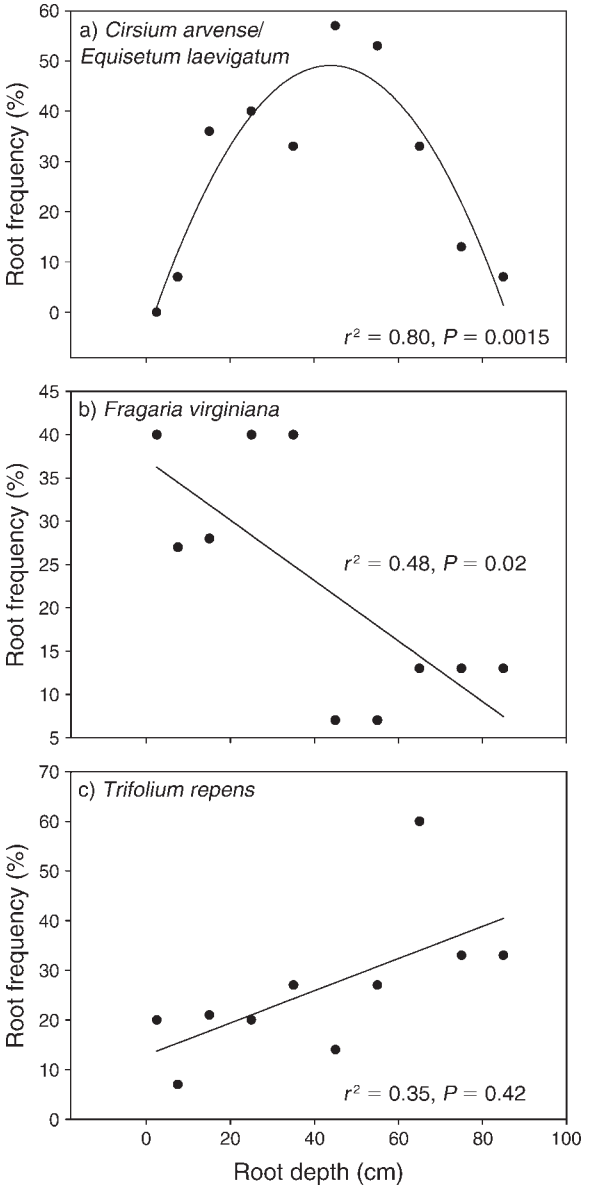

FIG. 3. Relationships between root frequency and root depth for (a) the species pair *Cirsium arvense*/*Equisetum laevigatum* (root frequency = 2.5[root depth] − 0.03[root depth]^2 − 5.0); the species (b) *Fragaria virginiana* (root frequency = 37.1 − 0.35[root depth]); and (c) *Trifolium repens* at the mesic site (Mammoth) (root frequency = 12.9 − 0.32[root depth]).

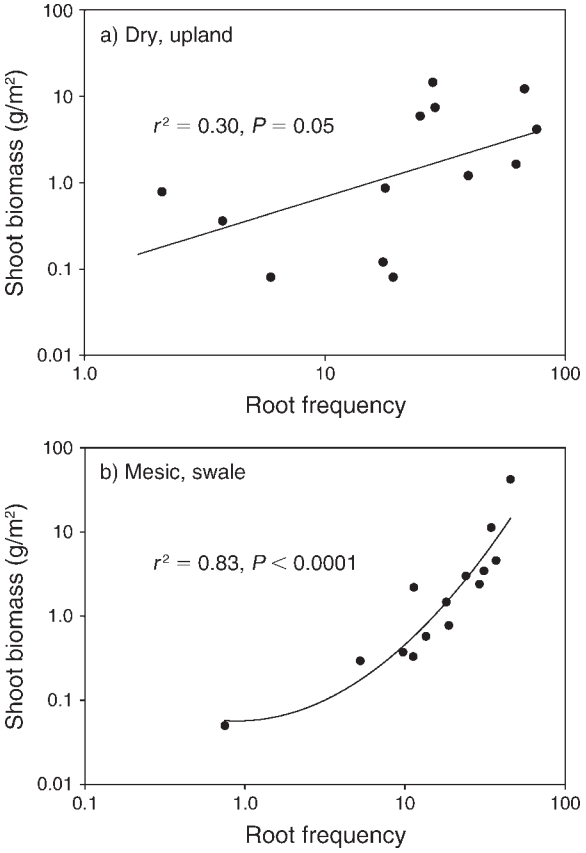

FIG. 4. The relationship between shoot biomass and root frequency among species at the (a) dry and (b) mesic grasslands. Note logarithmic scales on *x*- and *y*-axes.

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 midseason 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

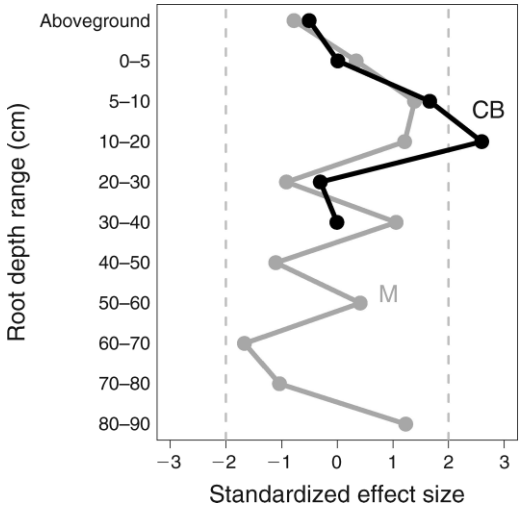

FIG. 5. Species associations aboveground and at 10-cm soil intervals belowground at the dry (Crystal Bench, black line) and mesic (Mammoth, gray line) grasslands. Values within the vertical dashed gray lines indicate random associations; values >1.96 indicate a significant negative association ($P < 0.05$).

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 were 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, Pickett 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 under some conditions root competition may be asymmetrical (Fransen et al. 2001). In addition, it has been proposed that asymmetrical root competition is most likely to occur in nutrient-rich soils where the soil volume is completely occupied by roots, and resources become available in a patchy manner as organic material is mineralized. Under such circumstances, species with more widely distributed roots may have a disproportionate competitive advantage over other species that are less proliferated throughout the soil (Fransen et al. 2001, de Kroon et al. 2002).

Root frequency, a measure of the soil volume occupied by a species, was positively related to shoot biomass in both YNP grasslands. This indicated that the capacity of a species to produce shoot biomass was associated with the volume of soil exploited by its roots. At the dry site, the seven most abundant plants aboveground possessed the seven most proliferated root systems, suggesting the importance of relatively extensive root systems in establishing aboveground dominance. In addition, the relationship between shoot biomass and root frequency at the dry grassland (Fig. 4a) indicated that the ability of a root system to support shoot biomass did not vary with the volume of soil exploited by species. If one defines the competitiveness of a species as the ability of that species to obtain resources, and it is further assumed that the amount of shoot biomass that is supported by root system volume (i.e., frequency) is a measure of the capacity of a unit of root system to supply soil resources to shoots, then competition among species at the dry grassland was symmetrical; the amount of shoot biomass supported by roots increased linearly as the amount of soil exploited by roots increased. In contrast, at the mesic site, shoot biomass increased quadratically, at a rate greater than a linear rate (Fig. 4b). For instance, the ratio of shoot biomass to root frequency at the mesic site increased from 0.05 to 0.4, by 700%, for species with 10% and 30% root frequency. The increase in the capacity of a unit root 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, the 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.

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APPENDIX A

The fragment length polymorphism method used in the study to identify root fragments in soil cores, with a table of the species-diagnostic fragment lengths (*Ecological Archives* E091-225-A1).

APPENDIX B

Root frequencies and measures of shoot biomass for two Yellowstone National Park grasslands (*Ecological Archives* E091-225-A2).