

# Root Distribution of Temperate Forage Species Subjected to Water and Nitrogen Stress

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## ABSTRACT

Root allocation and distribution patterns can influence forage yield during periods of moisture or nitrogen stress, as deep-rooted species access water and nutrients found deeper in the soil profile. In a greenhouse study, we examined rooting characteristics to a depth of 50 cm for 21 cool-season forage species (9 grasses, 6 legumes, and 6 forbs) exposed to drought and low N conditions. The goal of this research was to compare root distribution under uniform growing conditions for common grass, legume and non-leguminous forb species found in humid-temperate pastures of the northeast United States. Nitrogen or water stressed grasses generally had greater root biomass and a greater proportion of roots in the 30 to 40 cm soil layer than did stressed legumes or forbs. Low N significantly reduced root weight, but to a lesser extent than the decrease in shoot weight, resulting in an increase in root/shoot ratio. Drought stress reduced shoot weight but had no effect on root weight, also resulting in a greater root/shoot ratio. Drought stress also increased the proportion of deep roots compared with controls, whereas, N stress did not. Comparisons with previously published field studies suggested that information from container-grown plants could provide insights into field results, and also suggested that inherent species differences in root distribution could explain some, but not all, rooting patterns observed in multi-species forage mixtures.

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**W**ATER AND NUTRIENTS are typically not homogeneously distributed within the soil profile. Non-mobile nutrients such as P are usually concentrated near the soil surface, whereas mobile nutrients such as nitrate can move deep into the soil profile. Drought stress often develops from the top down as the soil surface dries, while adequate soil moisture can remain deep in the soil profile after surface soils have dried. Given the differences in water and nutrient availability, partitioning of root growth to different soil layers can have a profound effect on the ability of plants to acquire these resources. Ho et al. (2005) suggested that root architectural traits that increase topsoil foraging for P may incur tradeoffs for the acquisition of deep resources such as water. They found that shallow-rooted common bean (*Phaseolus vulgaris* L.) cultivars grew best under P stress, whereas deep-rooted cultivars grew best under drought. However, rooting depth is not necessarily a fixed characteristic of individual plant species or cultivars and a great deal of plasticity exists for root allocation. For example, root proliferation in nutrient-rich soil micro-sites is a well-documented phenomenon (Hodge 2004). Soil drying can also cause root proliferation at lower depths where moisture remains plentiful (Skinner et al., 1998; Skinner, 2008).

Root systems of temperate grassland species can grow to great depths. In a global analysis, Canadell et al. (1996) found

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a maximum rooting depth of  $2.6 \pm 0.2$  m for temperate grasslands. However, the majority of roots are found at much shallower depths. Temperate grasslands allocate about 40% of their roots to the top 10 cm of the soil profile, and 80 to 90% occur to the top 30 cm (Jackson et al., 1996). Schenk (2008) suggested that a number of ecological factors favor shallow over deep roots and that root profiles tend to be as shallow as possible while still being as deep as needed to fulfill evapotranspirational demands.

Previous studies of northeastern USA pasture mixtures have found that increasing the number of forage species in a mixture increased the overall proportion of deep roots in the soil profile (Skinner et al., 2004, 2006). Wardle and Peltzer (2003) found that plant species experiencing competition altered root distribution in the soil profile in different ways. In some cases, an increase in the ratio of deep to shallow roots occurred as competition led to suppression of root growth in the uppermost part of the soil profile. In other cases, the ratio decreased as plant vigor was reduced to the point that deep root systems were not produced to exploit resources at lower depths. Finally, they suggested that there were some instances where competition may have induced coexisting species to root at different depths relative to each other, thus improving resource partitioning among species.

In a maize (*Zea mays* L.)/wheat (*Triticum aestivum* L.) intercropping system in China, roots of intercropped wheat extended under the maize and occupied a larger soil volume than monoculture wheat. In contrast, maize roots responded to intercropping by growing deeper into the soil profile (Li et al., 2006). The author's suggested that increased yield and nutrient acquisition of both species under the intercropping system was due to the compatibility of their spatial root distributions. Berendse (1979) proposed that a stable equilibrium can exist among species, even in homogeneous environments, merely because of different rooting depths.

The primary goals of this research were to characterize the production and vertical distribution pattern of roots within the soil profile for common grass, legume and nonleguminous forb species found in humid-temperate pastures of the northeast United States, and determine the root allocation and vertical distribution responses to low N and drought. Secondly, we took advantage of information gained from this controlled environment study to investigate if inherent species differences in root vertical distribution assessed in the greenhouse could explain root distribution patterns of species mixtures from previously published field studies.

## MATERIAL AND METHODS

### Species and Cultivars

We examined a broad list of 21 species (9 grasses, 6 legumes, and 6 forbs) that are either sown into, or commonly found in Northeastern U.S. pastures (Table 1). Additional cultivars of tall fescue (*Lolium arundinaceum*) and birdsfoot trefoil (*Lotus corniculatus* L.) were included to examine attributes of interest within those

species. Those attributes include endophyte presence or absence in tall fescue, and rhizomatous vs. non-rhizomatous birdsfoot trefoil. Species examined covered a range of tolerances to defoliation frequency, drought, and nitrogen availability (USDA-NRCS 2002).

### Experimental Conditions

Seedlings were germinated in a greenhouse in late February to early March 2002 in 164 mL cone-tainers (Stuewe & Sons, Corvallis, OR) filled with a 2:1 v/v mixture of washed no. 1 silica sand and screened silt-loam topsoil. Sand was added to the topsoil to improve drainage and facilitate root washing. Seed germination was staged among the species so that their germination would occur in concert. All legumes were inoculated with appropriate rhizobium bacteria suspended in water when plants were 2 wk old. Plants were fertilized with nutrient solution (7000  $\mu\text{M}$   $\text{NH}_4$ , 1000  $\mu\text{M}$   $\text{NO}_3$ , 3000  $\mu\text{M}$  K, 3080  $\mu\text{M}$  Ca, 1000  $\mu\text{M}$  P, 2.25  $\mu\text{M}$  S, 938  $\mu\text{M}$  Mg, 940  $\mu\text{M}$  Cl, 12.5  $\mu\text{M}$  B, 1  $\mu\text{M}$  Mn, 1  $\mu\text{M}$  Zn, 0.25  $\mu\text{M}$  Cu, 0.25  $\mu\text{M}$  Mo, 25  $\mu\text{M}$  Fe-EDTA; pH adjusted to 6.5) when seedlings were 3 wk old. Nutrient solution application was subsequently alternated with tap water as needed to maintain adequate soil moisture (daily or twice daily). The greenhouse received natural light, and temperatures were maintained at 24°C during the day and 15.5°C at night ( $\pm 2.5^\circ\text{C}$ ).

The experiment was replicated in 3 greenhouse sections that were transplanted at 1-wk intervals. Species and treatments were randomized within each replication. Eight-week-old plants were transplanted into PVC pots (15 cm diam.  $\times$  50 cm deep) with plywood fit at the bottom. Plywood bottoms had multiple holes to allow water drainage and were lined with a fine nylon mesh. PVC pots were filled with a 1:1 v/v mixture of the same soil and sand as the cone-tainers. Pots were filled by hand to a uniform depth and measurements of a subset of the pots suggested that weights differed by about  $\pm 2$  to 3%. Each pot was watered to saturation with tap water following transplanting. Diehard Endo Drench inoculant (Horticultural Alliance, Sarasota, FL) containing endomycorrhizal fungi, bacteria (species and relative abundance not indicated), soluble sea kelp, soluble humic acid, and soluble yucca plant extract was added to each pot as 0.36 g powder in 100 mL distilled water.

One week after transplanting, 50% of the leaf area of each plant was removed. At this time, 1 L of nutrient solution was added to all pots to re-saturate the soil and normalize soil moisture among the pots before initiating stress treatments. Control and drought stressed plants were given the same nutrient solution previously used for fertilizing seedlings. Nitrogen limited plants were given a reformulated solution to limit N (70  $\mu\text{M}$   $\text{NH}_4$ , 10  $\mu\text{M}$   $\text{NO}_3$ , 3000  $\mu\text{M}$  K, 2100  $\mu\text{M}$  Ca, 1000  $\mu\text{M}$  P, 990  $\mu\text{M}$  S, 938  $\mu\text{M}$  Mg, 940  $\mu\text{M}$  Cl, 12.5  $\mu\text{M}$  B, 1  $\mu\text{M}$  Mn, 1  $\mu\text{M}$  Zn, 0.25  $\mu\text{M}$  Cu, 0.25  $\mu\text{M}$  Mo, 25  $\mu\text{M}$  Fe-EDTA). The need to formulate nutrient solutions to create high and low N treatments inevitably affected other nutrients, in this case the low N solution had lower Ca and higher S. This formulation was chosen so that differences in nutrients other than N would have minimal effects. The tap water that alternated with nutrient solution applications also contain 1085  $\mu\text{M}$  Ca, reducing differences between N treatments. Plants are relatively insensitive to high S with effects of S mainly occurring through pH changes. Both nutrient solutions were adjusted to the same pH.

At 30 h after watering to saturation, six pots in each greenhouse section (one each of a fast- and slow-growing grass, legume and forb selected at random) were weighed to establish a base-line weight for well-watered pots. The same pots were continually weighed daily to monitor plant water usage. When the average weight of these pots fell at least 100 g, control and low N plants were given water alternating with nutrient solution in the amount needed to re-establish average base-line weight. Drought-treated plants were not given any water or nutrient solution after the initial flushing with nutrient solution until the last week of the experiment, when porometer readings from 16 control and drought-stressed plants indicated that mid-day transpiration rates in drought-stressed plants were on average 40% that of controls. Drought-stressed plants were then given 150 mL nutrient solution. Control and low N pots received a total of 1087 mL of water in 6 applications and 1126 mL nutrient solution in 5 applications during the 4-wk period. Control, drought stressed and low N plants received a total of 238, 129, and 2.4 mg N, respectively.

Four plants per replication of each species and cultivar were harvested 7 d following defoliation, separated into roots and shoots, and dried to obtain baseline weights. Roots and shoots of two plants per replication of each species and cultivar were harvested 28 d after clipping when plants were 13 wks old. At the second harvest, root systems were dissected into fine, coarse, and taproots. The terminal two root branches were considered fine roots. These were typically less than 1 mm in diameter. Stolon biomass was pooled with stems for stolon-producing species and rhizome biomass was pooled with coarse and taproots for rhizome-producing species. Unless otherwise indicated, all reported root data are for combined fine, coarse, and tap roots. Root distribution among 5 depth intervals within the pots (0–10 cm, 11–20 cm, 21–30 cm, 31–40 cm, and 41–50 cm) was determined. Roots from the 41–50 cm depth were not used in assessing proportional root distribution to avoid artifacts associated with roots accumulating at the bottom of the pot. Thus, allocation to deep roots was assessed as the proportion of roots in the 30 to 40 cm layer to that in the total 0 to 40 cm depth.

Partitioning of biomass between shoots and roots from the first to second harvests was described using the allometric growth coefficient ( $k$ ) according to the following equation:

$$\ln S = \ln b + k \ln R$$

where  $S$  is shoot biomass,  $R$  is root biomass, and  $b$  is a constant (Troughton, 1956). Data were  $\ln$  transformed for analysis to improve homogeneity of variance. Relative root and shoot growth are equal if  $\ln k = 0$ . When  $\ln k$  is  $>0$ , relative shoot growth is greater than relative root growth. Conversely, if  $\ln k$  is  $<0$ , then relative root growth is greater than relative shoot growth.

Data were analyzed twice with a randomized complete block ANOVA design with each of the three greenhouse sections as a replication. Species and environmental treatments were randomized within each replication. The data were analyzed as a general linear model (Proc GLM, SAS Institute, 2002). In the first analysis, the model included species and treatment (control, drought, and low N) as main effects and a species by treatment interaction. In the second analysis, the model included plant functional group (grass, legume, forb) and treatment as main effects and the functional group by treatment interaction. When effects were significant at  $P = 0.05$  the least significant difference (lsd) was calculated for main effects and significant interactions.

## RESULTS

Drought and nitrogen stress significantly reduced shoot biomass compared with controls by 25 and 32%, respectively ( $P < 0.01$ ). Grass, legume and forb aboveground biomass in the control treatment were not significantly different ( $P > 0.05$ ). Drought stress reduced forb and legume aboveground growth by 31 and 32%, respectively, whereas, grass biomass was reduced by 20% ( $P < 0.01$ ). Low N caused a greater than 50% reduction in grass and forb aboveground biomass ( $P < 0.01$ ), but only an 18% reduction in legumes ( $P < 0.05$ ).

Grasses, on average, had significantly ( $P < 0.05$ ) greater root biomass than forbs or legumes in the control and low N treatments (Fig. 1), and greater root biomass than legumes but not forbs under drought. Forb root biomass was also significantly greater than that of legumes in the control treatment ( $P < 0.05$ ), but that was primarily due to the very large root biomass of chicory (*Cichorium intybus* L.) which had the largest root system of any species in both the control and drought treatments (Fig. 2).

Averaged across species, drought stress had no significant effect on root biomass for any of the functional groups (Fig. 1). However, among the individual species, drought stress increased root biomass for alfalfa (*Medicago sativa* L.), plantain (*Plantago lanceolata*), and small burnet (*Sanguisorba minor*). Perennial ryegrass and timothy showed significant decreases in root biomass in response to drought (Fig. 2). Low N significantly reduced root biomass of grasses but not of legumes and forbs. Forbs showed a trend toward reduced root biomass in response to N stress but this was due to a significant 55% reduction in chicory with no significant change in other forb species. Among the grasses, perennial ryegrass (*Lolium perenne* L.), prairie grass (*Bromus willdenowii*), reed canarygrass (*Phalaris arundinacea* L.) and timothy (*Phleum pratense* L.) showed reduced root biomass in response to N stress.

There were significant interactions between treatments and functional groups for allometric growth coefficients ( $P < 0.05$ ). Legumes had a significantly higher  $\ln k$ , and, thus, greater allocation to shoot growth, than forbs and grasses, irrespective of treatment (Fig. 1). In general, stress treatments had little effect on root/shoot partitioning in grasses compared with their relatively large effects on legume and forb species. Nitrogen stress had no effect on the allometric growth coefficient for legumes and grasses but significantly increased partitioning to roots for forbs (Fig. 1). Among individual species, only 'ARS-2620' birdsfoot trefoil, increased partitioning to shoots under N stress, whereas, N stress caused a decrease in partitioning to shoots for yarrow (*Achillea millefolium* L.), gray goldenrod (*Solidago nemoralis*), and plantain.

Drought stress caused a significant reduction in  $\ln k$  for legumes and forbs but not for grasses ( $P < 0.01$ ), and in general relative partitioning to roots increased more under

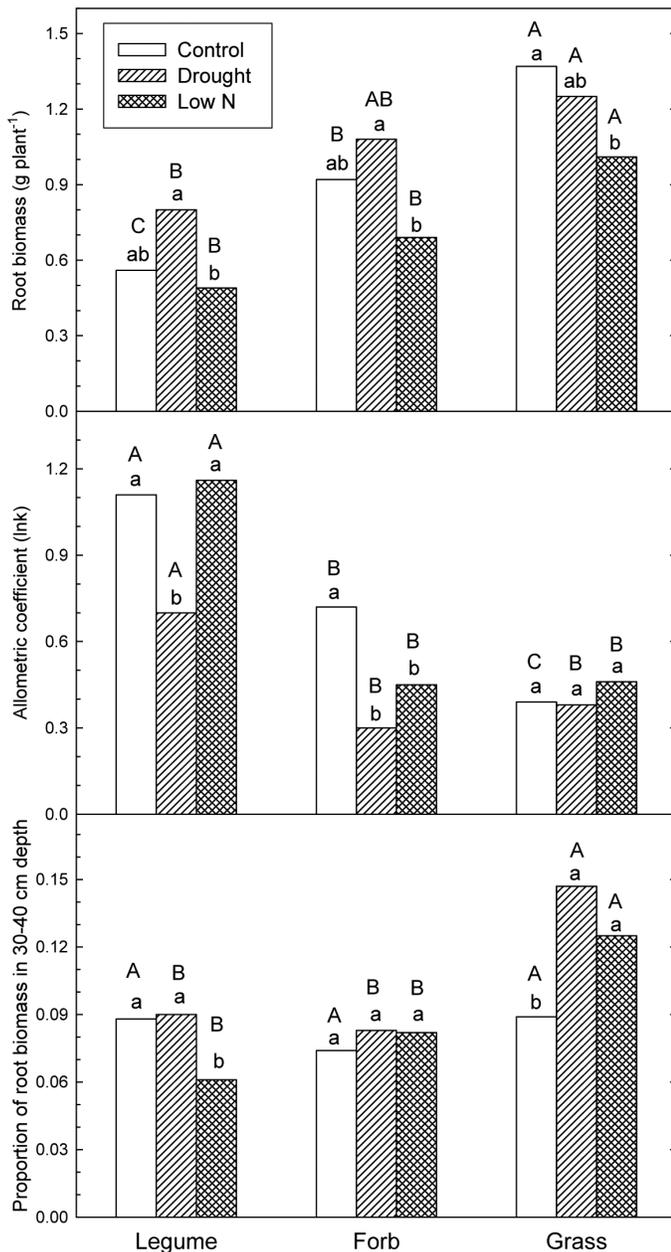


Figure 1. Effects of drought and low N on (A) root biomass, (B) biomass allocation between shoots and roots (ln transformed allometric coefficient, lnk), and (C) the proportion of roots in the 30- to 40-cm soil layer for three functional groups, legumes, forbs, and grasses after 4 wk of treatment. Uppercase letters indicate significant differences at  $P = 0.05$  among functional groups within a given treatment. Lowercase letters indicate significant differences among treatments within a functional group. Following ln transformation of k, relative root and shoot growth are equal if  $\ln k = 0$ , and relative shoot growth is greater than relative root growth if  $\ln k > 0$ .

drought than under low N. Drought stress increased partitioning to roots for seven species including alfalfa, 'Norcen' birdsfoot trefoil, red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), gray goldenrod, and plantain. Even though lnk decreased under drought stress for many species, only one species, chicory, showed greater partitioning to roots than shoots under drought (Fig. 3).

Averaged across species, the proportion of roots found in the 30- to 40-cm layer (deep roots) in the control treatment was 0.085 and did not differ among forbs, grasses, and legumes (Fig. 1). Drought stress had no effect on the proportion of deep roots for forbs and legumes, but significantly increased the proportion of deep roots for grasses to 0.147 ( $P < 0.01$ ). All grass species had a tendency to increase the proportion of deep roots under drought stress, but the increase was significant only for orchardgrass (*Dactylis glomerata* L.), perennial ryegrass, smooth brome (*Bromus inermis* Leyss.), timothy, and Virginia wildrye (*Elymus virginicus* L.) (Fig. 4). Nitrogen stress increased rooting depth of grasses, had no effect on forbs, and decreased rooting depth of legumes (Fig. 1). As with drought stress, all grasses showed a tendency toward a greater proportion of deep roots in the low N treatment, but differences were significant for only two entries, orchardgrass and 'Jessup MaxQ' tall fescue (Fig. 4). Although low N did not have an overall significant effect among forbs, yarrow showed a significant increase in rooting depth in response to low N. Even though legumes as a group had significantly fewer deep roots under N stress, Norcen birdsfoot trefoil was the only individual legume species to show a significant decrease.

## DISCUSSION

Among common plants of temperate pasture communities, we found that grasses as a group had the largest root systems compared to legumes and forbs. Chicory was one exceptional forb that had the largest root system of any species as long as it was not deprived of N. Legumes in general had the highest allometric coefficient, indicating that they allocated more resources to shoot growth, but were also most plastic in their allometric response to drought. Grasses as a group were generally most plastic in shifting the distribution of their root system to deeper depths in response to drought and low N.

Caldwell et al. (1981) suggested that relatively more allocation to shoots combined with curtailed root growth following defoliation results in a more rapid approach to the preclipping balance between root and shoot systems and improves grazing tolerance and forage production. Morgan et al. (2001) observed that plants increased allocation to shoot growth at the expense of root growth during the first 10 d following defoliation as shoots were reestablished, and then stabilized allocation between shoot and root growth during the next 10 d of the regrowth cycle. With the exception of drought stressed chicory, the allometric coefficient indicated greater partitioning to shoot than to root growth during the 28-d regrowth period for all species and treatments, suggesting that canopy reestablishment following defoliation was the primary priority.

Of equal importance to the balance between allocation to shoot and root growth is the plasticity of this balance, which may allow plants to optimize resource allocation

to maximize growth under limiting conditions. Interestingly, we found that just legumes and forbs were plastic in balancing their shoot and root growth in response to drought and only forbs were plastic to low N. The unresponsiveness to low N is not surprising for legumes because of their N fixation capabilities. The limited responsiveness of grasses in readjusting allocation patterns between roots and shoots was potentially related to their generally greater allocation to root biomass under all conditions.

The effects of drought and N stress on total plant biomass were much different from their effects on allocation between above and belowground tissues. Drought stress reduced plant biomass by 16 to 18% with no difference between grasses, legumes, and forbs. In contrast, the low N treatment reduced grass and forb biomass by 44 and 48%, respectively, whereas, legume biomass was reduced by only 17%. Thus, the N fixation capacity of legumes reduced, but did not totally eliminate the growth reduction caused by the low N treatment.

Because nutrients were added with the irrigation water the possibility must be considered that the drought treatment imposed both water and N limitations on plant growth. During the 28-d regrowth period the drought treatment received 54% of the N applied to the control, whereas, the low N treatment received 1%. An examination of legumes, where N stress was minimal, revealed that root biomass was greater under drought than under low N, and allocation to roots and the proportion of roots in the 30 to 40 cm soil depth increased (Fig. 1). This suggests that drought had a qualitatively different effect on legume roots than did low N. Similar qualitative differences between drought and low N treatments were observed for forbs and grasses, although differences between the two treatments were typically not as great as for legumes. This could indicate that forbs and grasses were experiencing a degree of N stress under drought. In addition to the lower amount of applied N, drought can also reduce N availability by reducing the transport of nitrate through the soil to the root surface (Abreu et al., 1993). Therefore, any N stress experienced under the drought treatment could have been due to reduced N application or to reduced transport to the root surface.

Few studies are available for comparing the allocation responses of individual species included in this study, especially

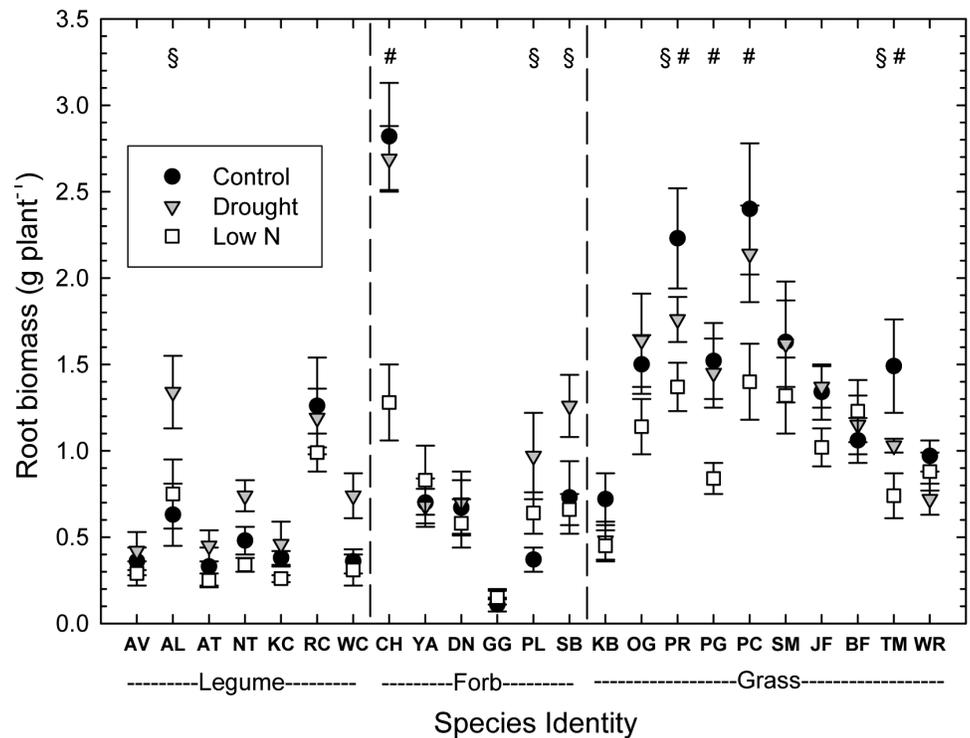


Figure 2. Total root biomass for 23 perennial forages harvested 35 d after transplanting into 50 cm deep PVC pots. Species abbreviations: American vetch, AV; alfalfa, AL; 'ARS-2620' birdsfoot trefoil, AT; 'Norcen' birdsfoot trefoil, NT; kura clover, KC; red clover, RC; white clover, WC; chicory, CH; yarrow, YA; dandelion, DN; gray goldenrod, GG; plantain, PL; small burnet, SB; Kentucky bluegrass, KB; orchardgrass, OG; perennial ryegrass, PR; prairie grass, PG; reed canary grass, PC; smooth brome, SM; 'Jessup MaxQ' tall fescue, JF; 'Barolex' tall fescue, BF; timothy, TM; Virginia wild rye, WR. Error bars indicate  $\pm 1$  SE. § indicates significant difference between drought and control plants at  $P = 0.05$ . # indicates significant difference between low N and control plants at  $P = 0.05$ .

following defoliation. In one study without defoliation, Shipley and Meziane (2002) compared allocation responses to nutrient stress for 22 herbaceous species including smooth brome, chicory, timothy, plantain, and Kentucky bluegrass (*Poa pratensis* L.). In that study, four of the five species (timothy was the exception) showed increased allocation to roots under low compared with high N. In the current study, only plantain experienced a similar increase in allocation to roots under N stress. The allometric coefficient was unaffected by N stress for the other species. Our results were similar to a study by Muller et al. (2000) that included smooth brome, perennial ryegrass, Kentucky bluegrass, and white clover, also without defoliation. They also found that none of these species altered their allocation to roots in response to low N. This suggests that the current results are broadly consistent with previous studies, although numerous factors including the presence or absence of defoliation, other environmental and cultural differences among experiments and even cultivar differences within species could explain differences in the response of individual species to the imposed stress.

For root distribution data from pot experiments to be useful, they must represent as closely as possible results from field plantings. Results from greenhouse experiments can

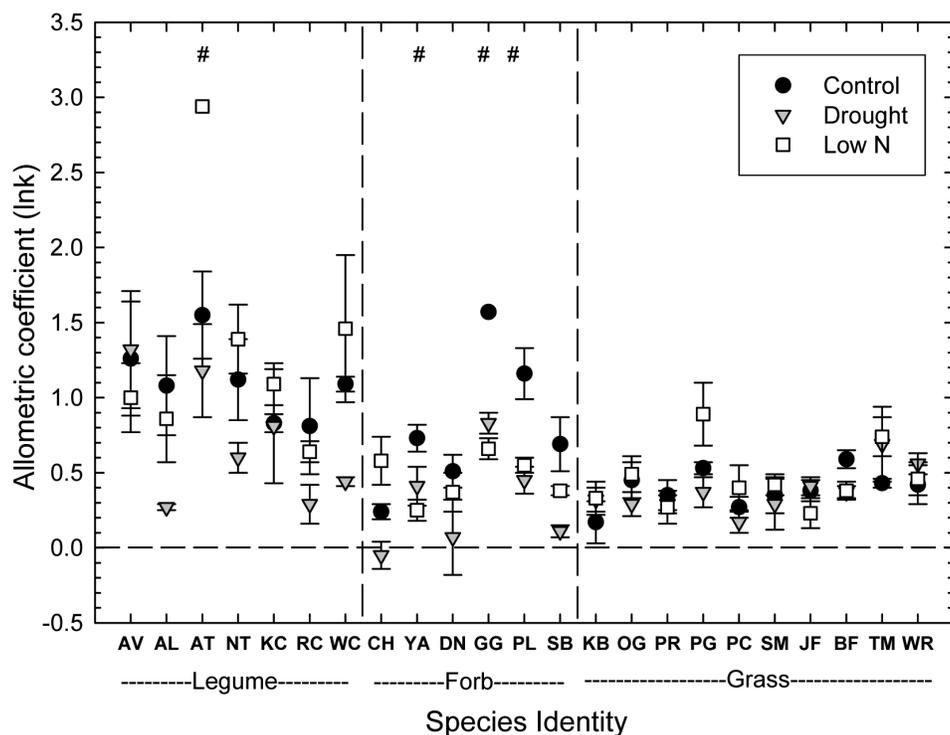


Figure 3. Allometric partitioning coefficient (lnk) describing relative partitioning between root and shoot growth of perennial forages receiving adequate water and nutrients (control) or subjected to drought or nitrogen stress. When lnk is > 0 then relative shoot growth is greater than relative root growth. Conversely, if lnk is < 0 then relative root growth is greater than relative shoot growth. Species abbreviations: American vetch, AV; alfalfa, AL; 'ARS-2620' birdsfoot trefoil, AT; 'Norcen' birdsfoot trefoil, NT; kura clover, KC; red clover, RC; white clover, WC; chicory, CH; yarrow, YA; dandelion, DN; gray goldenrod, GG; plantain, PL; small burnet, SB; Kentucky bluegrass, KB; orchardgrass, OG; perennial ryegrass, PR; prairie grass, PG; reed canary grass, PC; smooth brome, SM; 'Jessup MaxQ' tall fescue, JF; 'Barolex' tall fescue, BF; timothy, TM; Virginia wild rye, WR. Error bars indicate  $\pm 1$  SE. § indicates significant difference between drought and control plants at  $P = 0.05$ . # indicates significant difference between low N and control plants at  $P = 0.05$ .

vary depending on pot size and shape (Campbell et al., 1985) and evidence exists that plants can sense the volume of available rooting space and alter root mass in response to differences in rooting volume (Hess and de Kroon, 2007). Thus, it was important to determine if rooting patterns in the current experiment were consistent with results from the field. However, this is not a trivial task since field results can also vary depending on soil characteristics, climate, and nutrient distribution. In addition, no field studies have examined all species included in this greenhouse experiment.

In the current experiment, 18 to 26% of root biomass was located below 30 cm, with grasses having slightly deeper roots than legumes and forbs. In field studies where roots were measured to similar depths, roots below 30 cm contributed as little as 4% to more than 40% of total root biomass (Sanderson and Jones, 1993; Gentile et al., 2003; Skinner et al., 2004, 2006; Nie et al., 2008). In a global analysis, Jackson et al. (1996) calculated that 17% of temperate grassland root biomass was found at depths >30 cm. Thus, results from this study were solidly within the range of root distributions commonly found in the field.

More field data are needed for comparison against the rooting depths of individual species assessed in our greenhouse study before we can determine if species rankings hold true. In an Australian study, tall fescue had one of the deepest root systems, whereas perennial ryegrass, orchardgrass, and plantain had relatively shallow roots (Nie et al., 2008). In the current study, no significant difference in the percentage of deep roots existed among tall fescue, orchardgrass, and perennial ryegrass which all had some of the deepest root systems in the study. However, plantain had a relatively shallow root system, consistent with the observations of Nie et al. (2008).

Taproot species found among the legumes and forbs are generally thought to have relatively deep root systems. However, this was not the case in the current study. In this study, coarse roots and taproots were generally confined to the top 10 cm of the soil profile with taproots only occasionally growing into the 10- to 20-cm layer. These larger roots accounted for 23 and 27% of total root biomass for legumes and forbs, respectively, but only 5% of grass roots. Concentration of these large, heavy roots in the upper soil layers likely skewed the root distribution of legumes and forbs to shallower depths than would typically be observed for more mature plants in the field. However, when only fine roots were considered, grasses still had the largest root systems with a greater proportion of their root system at depth than legumes or forbs.

Root systems can show a great deal of plasticity in response to environmental stress or in response to localized nutrient conditions. In a field study, Skinner (2008) found under well-watered conditions that three-species mixtures containing chicory, white clover, and either orchardgrass or perennial ryegrass had greater root counts in deep soil layers than did white clover–orchardgrass, or white clover–perennial ryegrass mixtures. However, the vertical root distribution in the grass/legume mixtures in that study exhibited a more plastic response to the imposition of drought, developing deeper root systems than drought-stressed mixtures that included chicory. In the current study, neither chicory nor white clover showed a significant change in the proportion of deep roots in response to drought (Fig. 4). Conversely, the proportion of deep roots more than doubled under

drought stress for both orchardgrass and perennial ryegrass. In the Skinner (2008) study, grass species formed a larger component of the two- than three-species mixtures, and it is likely that the increased plasticity in rooting depth in response to drought in the two-species mixtures was due to the greater responsiveness of orchardgrass and perennial ryegrass to drought than that of either white clover or chicory.

Several field studies have found that increasing the number of species in mixtures increased the proportion of root biomass found in deep soil layers. For example, Skinner et al. (2004) found in clipped small plots that five-species mixtures containing grasses, legumes, and forbs had a greater proportion of their root systems in the 60 to 90 cm soil depth than did two-species grass/legume mixtures. In a grazing study, the proportion of roots at 30 to 60 cm increased from 0.05 for a two-species mixture to 0.14 for a mixture containing 11 species (Skinner et al., 2006). It was not possible in either study to determine if interactions among species had induced the plants to extend their roots into deeper soil layers as has been suggested by Wardle and Peltzer (2003) or if the increased rooting depth in the more complex mixtures was an artifact of the sampling effect (Wardle, 1999), whereby increasing the number of species simply increased the possibility of including species with inherently greater rooting depth. Data presented here allows us to account for inherent species differences in rooting depth and plasticity to address the sampling effect issue.

All mixtures in the Skinner et al. (2006) study were dominated by orchardgrass and tall fescue (72–80% of total aboveground biomass), which, in the current study, did not significantly differ from each other in terms of allometric coefficient, or proportion of deep roots. Thus, there was no reason to expect, based on the inherent rooting properties found in this study, that rooting depth should have increased as species richness increased. This suggests that interactions among species in the field could have induced changes in rooting patterns resulting in deeper root distribution in the more complex mixtures, similar to the results observed for some species by Wardle and Peltzer (2003). Such shifts in rooting depth and accompanying changes in access to belowground resources could help explain the increase in forage production often observed with increased species richness.

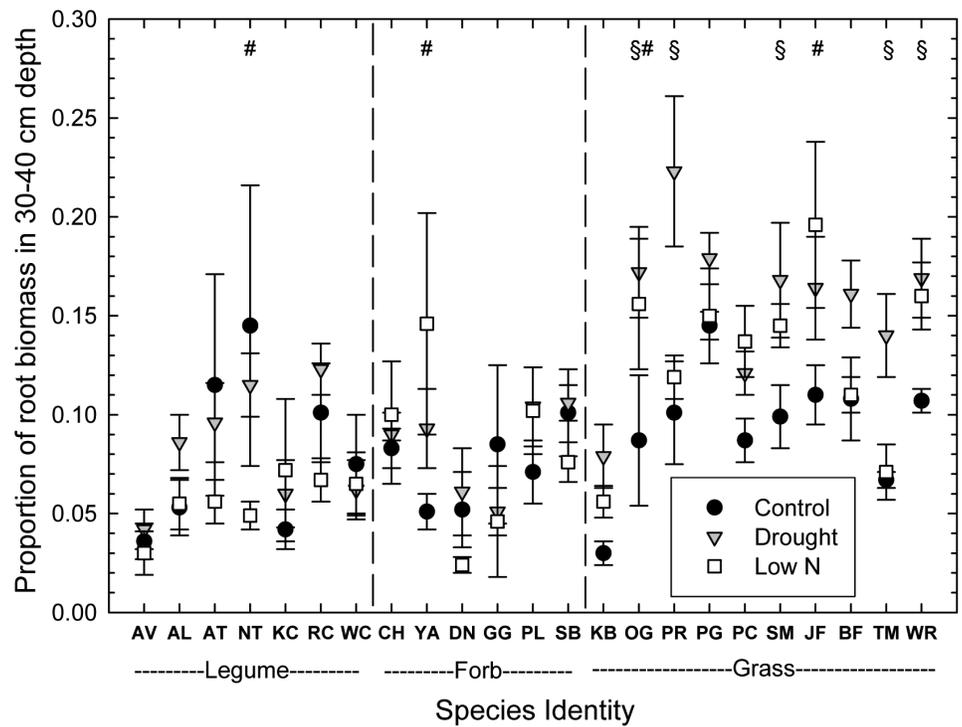


Figure 4. Proportion of root biomass in the 30- to 40-cm depth segment for 23 perennial forages harvested 35 d after transplanting into 50-cm deep PVC pots. Species abbreviations: American vetch, AV; alfalfa, AL; ‘ARS-2620’ birdsfoot trefoil, AT; ‘Norcen’ birdsfoot trefoil, NT; kura clover, KC; red clover, RC; white clover, WC; chicory, CH; yarrow, YA; dandelion, DN; gray goldenrod, GG; plantain, PL; small burnet, SB; Kentucky bluegrass, KB; orchardgrass, OG; perennial ryegrass, PR; prairie grass, PG; reed canary grass, PC; smooth brome, SM; ‘Jessup MaxQ’ tall fescue, JF; ‘Barolex’ tall fescue, BF; timothy, TM; Virginia wild rye, WR. Error bars indicate  $\pm 1$  SE. § indicates significant difference between drought and control plants at  $P = 0.05$ . # indicates significant difference between low N and control plants at  $P = 0.05$ .

In another study that examined four forage mixtures of varying species composition and complexity (Skinner et al., 2004), a grass/legume mixture containing 65% Kentucky bluegrass and 35% white clover had the shallowest root system, consistent with the inherently shallow roots observed in this study for Kentucky bluegrass and to a lesser extent for white clover (Fig. 4). However, a five-species mixture dominated by chicory had a relatively small but deep root system which would not be anticipated based on the current study where chicory had a large root system (Fig. 2) with a high proportion of roots near the soil surface (Fig. 4).

## CONCLUSION

Comparisons of rooting characteristics for 23 temperate pasture species and cultivars suggested that grasses had greater allocation of biomass to roots and greater rooting depth than legumes and forbs. In general drought stress increased allocation to roots and increased the proportion of deep roots, whereas, low N had less effect on either parameter. Although these broad characterizations were generally true, individual species were occasionally found whose responses contradicted the general rule. Information on root production and distribution from perennial forages grown in monoculture in 50-cm deep pots was able to provide insight

**Table 1. List of species, cultivar, common name, and two-letter abbreviations for the 21 species used in this study.**

Species and cultivar	Common name	Abbreviation
Cool-season grasses		
<i>Bromus inermis</i> 'Saratoga'	Smooth brome	SM
<i>Bromus willdenowii</i> 'Lakota'	Prairie grass	PG
<i>Dactylis glomerata</i> 'Pennlate'	Orchardgrass	OG
<i>Elymus virginicus</i> 'Omaha'	Virginia wildrye	WR
<i>Lolium arundinaceum</i> 'Barlox'	Tall fescue (endophyte-free)	BF
<i>Lolium arundinaceum</i> 'Jessup Max Q'	Tall fescue (nontoxic endophyte)	JF
<i>Lolium perenne</i> 'BG-34'	Perennial ryegrass	PR
<i>Phalaris arundinacea</i> 'Palaton'	Reed canarygrass	PC
<i>Phleum pretense</i> 'Climax'	Timothy	TM
<i>Poa pratensis</i> 'Side Kick'	Kentucky bluegrass	KB
Legumes		
<i>Lotus corniculatus</i> 'ARS-2620'	Birdsfoot trefoil (rhizomatous)	AT
<i>Lotus corniculatus</i> 'Norcen'	Birdsfoot trefoil (nonrhizomatous)	NT
<i>Medicago sativa</i> 'Amerigraze'	Alfalfa	AL
<i>Trifolium ambiguum</i> 'Endura'	Kura clover	KC
<i>Trifolium pretense</i> 'Plus'	Red clover	RC
<i>Trifolium repens</i> 'Will'	White clover	WC
<i>Vicia Americana</i>	American vetch	AV
Forbs		
<i>Achillea millefolium</i>	Common yarrow	YA
<i>Cichorium intybus</i> 'Puna'	Chicory	CH
<i>Plantago lanceolata</i> 'Plan 3'	Narrow-leaf plantain	PL
<i>Sanguisorba minor</i>	Small burnet	SB
<i>Solidago nemoralis</i>	Gray goldenrod	GG
<i>Taraxacum officinale</i>	Dandelion	DN

into root distribution results from previously conducted field studies. In one case, proliferation of deep roots in response to drought stress in the field could be explained by the high degree of plasticity in response to drought exhibited by orchardgrass and perennial ryegrass roots. Results from this study also suggest that in some cases increased rooting depth with increasing number of species in forage mixtures probably resulted from interactions among species that caused roots to extend deeper into the soil profile rather than from the presence of more deep-rooted species in the most species-rich mixtures. Thus, this controlled environment study was able to help explain results from field studies where it would have been difficult to obtain a comparable level of information on root growth and allocation processes.

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