

Root responses to nutrient patches in grassland and forest

Sarah E. L. Moar and Scott D. Wilson*

*Department of Biology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada; *Author for correspondence (e-mail: scott.Wilson@uregina.ca; phone: +306-585-4287; fax: +306-585-4894)*

Received 12 January 2005; accepted in revised form 20 September 2005

Key words: Forest, Grassland, Heterogeneity, Nitrogen, Resources

Abstract

Differences between growth forms in root responses to experimentally created heterogeneity have been documented in many greenhouse and plot studies, but not in natural vegetation. Here we examined the response of roots to experimental nutrient patches in undisturbed grassland and forest at the northern edge of the North American Great Plains. Forest vegetation increases the spatial heterogeneity of soil resources, and we tested for differences between forest and grassland roots in response to patches. Ten minirhizotrons (clear tubes, 5 cm diameter, 180 cm long) were installed in both grassland and forest 3 years before the experiment. Minirhizotrons ran horizontally 10 cm beneath the soil surface. Patches of available nitrogen (N) were created over the tubes, using three concentrations (0, 3, 15 g N m⁻² yr⁻¹) and two patch sizes (1 and 10 cm²). Root images were collected beneath patches over the course of a growing season. Root length was significantly greater in grassland than forest at the start and end of the growing season, but did not respond to N patches. Root production was also significantly greater in grassland than forest, and was significantly greater (about 20%) in high-N patches than in unfertilized patches. This increase, however, did not differ between vegetation types. Turnover did not vary with any treatment, and patch size had no effect on any response variable. Overall, differences caused by experimental patches were much smaller than differences between habitats, and did not vary between habitats. Realistic levels of experimentally imposed heterogeneity in established vegetation may not be much greater than background levels, and field vegetation has extant root systems which respond to patches via uptake instead of growth. Both mechanisms should contribute to less root proliferation in field experiments than in greenhouse experiments.

Introduction

Greenhouse (Hutchings et al. 2003; Hodge 2004) and field studies (Pregitzer et al. 1993; Bilbrough and Caldwell 1997; Smilauerova 2001) show that roots respond to patchiness, and have found differences among species in root responses to heterogeneity (Fitter et al. 2000). There are, however, no field experiments that compare the root dynamics of contrasting vegetation types, such as forest and grassland, in response to heterogeneity.

Differences between species that have been found in greenhouse and plot experiments may be greater than those in natural vegetation for two reasons. First, roots in greenhouses and plots fill uncolonized soil, whereas perennial vegetation experiences rapid nutrient uptake by neighbors and microbes (Jackson et al. 1989). This was exemplified by the single species that showed root proliferation in response to experimental patches in a Utah field planting: proliferation was diminished by neighbor roots (Bilbrough and Caldwell 1995). Second,

greenhouse and plot studies have experimentally set high contrast (Day et al. 2003), whereas natural heterogeneity is relatively modest (Kleb and Wilson 1997; Farley and Fitter 1999). Low contrast in the field might induce little root response (Hutchings et al. 2000). Overall, the proliferation of roots in response to nutrient patches beneath long-established vegetation is expected to be less dramatic than in greenhouse and plot experiments.

In the field, contrasting growth forms, such as trees and grasses, differ in their effects on soil resource heterogeneity. Soils dominated by woody plants are characterized by greater spatial heterogeneity than soils supporting grasses (Schlesinger et al. 1996; Kleb and Wilson 1997). Differences in foraging scale should allow large woody plants to benefit from increased heterogeneity relative to small plants such as grasses (Schlesinger et al. 1990; Grime 1994; Wilson 2000).

We tested for differences between grassland and forest roots in their responses to nutrient heterogeneity. Grasses and trees might differ in their responses to nutrient heterogeneity because of fundamental differences in their root architecture. Temperate grassland has a 20-fold greater length of fine roots than temperate deciduous forest (Jackson et al. 1997). Thus *in situ* grasses might have little growth response to a new nutrient patch because an abundance of fine roots enhances their ability intercept any temporary increase in nutrient availability without growing more roots. Grass and tree roots also differ in the scale of their patchiness, with grass roots being distributed on smaller scales than tree roots (Pärtel and Wilson 2002). This difference might confer an ability for grasses and trees to exploit relatively small and large patches, respectively.

In summary, woody plants increase soil resource heterogeneity, but it is not known whether they respond to increased patchiness differently from grasses. We tested whether root production and turnover in response to nutrient patch size and concentration differed between grassland and forest.

Methods

We worked at White Butte Recreation Area (50°28' N, 104°22' W), 18 km east of Regina, Saskatchewan, near the northern edge of the Great

Plains. We used two communities, mixed-grass prairie dominated by native *Agropyron* spp., *Bouteloua gracilis* (HBK) Lag., *Carex* spp., *Koeleria macrantha* (Ledeb.) J. A. Schultes f., *Poa* spp., *Stipa comata* Trin. and Rupr. and *Selaginella densa* Rydb, and aspen forest dominated by *Populus tremuloides* Michx. and an understory of *Symphoricarpos occidentalis* L. The climate is continental with mean daily temperatures of -17 °C in January and 19 °C in July. Mean annual precipitation is 384 mm (Environment Canada, unpublished data). The parent soils for both grassland and forest vegetation at White Butte are regosols on silty sand (Anonymous 1992), and have not been disturbed by humans. The area may have been grazed by cattle in the past, but no cattle grazing occurred for at least 12 years before our study. There are no apparent differences in topography or soil texture between forest and grassland. Grasslands in this region are characterized by relatively low levels of soil resource availability and heterogeneity, high species diversity (Kleb and Wilson 1997) and intense belowground competition (Wilson 1993).

We followed root dynamics using minirhizotrons, clear tubes (5 cm diameter, 180 cm long), installed horizontally 10 cm below the soil surface in 1999 (Pärtel and Wilson 2002). We installed the tubes horizontally in order to examine horizontal heterogeneity in root production and because most roots occur at this depth (Wilson and Kleb 1996). Tubes allowed access by a digital camera (Bartz Technology, Santa Barbara, California). Ten tubes were installed in each of grassland and forest, along a 500-m long front of invading aspen. Grassland tubes were 10–30 m outside the forest; forest tubes were 10–20 m inside the forest. The open end of each tube entered a galvanized steel box (155 cm long, 17 cm wide, 23 cm deep) that allowed access to the tubes.

We examined the response of roots to patches of available nitrogen (N). N is the limiting nutrient in these temperate grasslands (Tilman 1987), and the rate of grassland invasion by trees is correlated with deposition rates of anthropogenic available N (Köchy and Wilson 2001). N was applied at one of three concentrations (0, 3 and 15 g m⁻² yr⁻¹), chosen to reflect levels at which temperate grasslands respond to nutrient additions (Tilman 1987).

N was added to patches of two sizes (1×1 and 1×10 cm), selected to reflect the size of patches

affected by root-induced heterogeneity (Kleb and Wilson 1997, Pärtel and Wilson 2002). In summary, treatments imposed on each tube comprised two factors, concentration (three levels) and size (two levels) for a total of six treatment combinations. Treatment combinations were arrayed randomly along each tube, with the same randomization pattern used for all tubes. Treatment areas were separated by 15 cm.

Patches were created in May 2002 by injecting 0.5 ml of NH_4NO_3 solution into the soil over the tubes. Field trials using colored water and a minirhizotron indicated that this volume produced a patch about 1 cm in diameter. Ten centimeter long patches comprised 10 adjacent 1×1 cm patches. Nitrogen was added five times with 5 days between each addition to ensure that the patch persisted in time. A field study suggested that patches in forest soil persist < 4 weeks (Farley and Fitter 1999). Patches without N received distilled water. A template ensured consistency in injection sites over time.

Images (13.5×18 mm) were collected directly beneath each patch (i.e. from the upper surface of the tube) every 2 weeks until late August. This encompasses the period of root production (Pärtel and Wilson 2002). Consecutive images were taken from exactly the same location, allowing us to follow root production and death. We took one image under the center of each small patch and two under each large patch. Data from the two images under each large patch were averaged for all analyses. Seven patches were excluded from the analysis due to procedural errors, and four because they contained no roots throughout the study, leaving 109 patches for analysis.

We calculated total root length at the end of the growing season, root production, and root turnover for each patch. We considered all white and brown roots to be alive, and all black, disintegrated or disappeared roots to be dead. Root production was defined as the sum of length of all new roots expressed as a proportion of initial root length; windows without roots at the start of the experiment were excluded from this analysis. Root turnover was defined as the ratio between the sum of all root length that died and all root length produced, and was examined because of the finding that greenhouse-grown tree seedlings show differences in root demography but not morphology (Espeleta and Donovan 2002). Proportional

data were arcsine-square root transformed and other data were square root transformed to meet the assumptions of analysis of variance.

Split-plot analysis of variance (SuperANOVA, Abacus Concepts, Berkley, CA) was used to test for effects of patch size, N concentration, and habitat on total root length, production and turnover. Habitat was the main-plot effect, and concentration and patch size were split-plot effects. Repeated measures ANOVA examined variation in root length over the course of the experiment.

Results

Root length was significantly greater in grassland than forest, both at the start ($F = 6.62$, $p < 0.05$; one-way ANOVA across all other factors) and the end of the growing season (Table 1, Figure 1). Root length at the end of the growing season did not vary significantly with N concentration or patch size (Table 1). Root length at the start of measurement, immediately after N addition, also did not vary with patch concentration or size (Table 1). Repeated measures ANOVA revealed similar results with regards to the experimental factors (data not shown).

As in the case of root length, root production was about twice as high in grassland as in forest (Figure 2). Production was significantly greater in grassland, but did not vary significantly with patch size or N addition (Table 1). The effect of N, however, had a relatively high F -value (Table 1) so we repeated the analysis with the middle N level

Table 1. ANOVA results (F -values) for the effects of habitat (grassland and forest), concentration (0, 3 and 15 g N m^{-2}), and patch size (1 and 10 cm^2) on final root length, production, and turnover.

Source	df	Final length	Production	Turnover
Tube	9	1.42	3.15	2.94
Habitat	1	22.98*	13.82*	0.10
Tube \times habitat	8	1.36	1.86	3.01*
Concentration	2	1.96	2.57	1.14
Concentration \times habitat	2	0.12	0.19	1.37
Size	1	0.48	0.34	0.30
Size \times habitat	1	0.26	0.13	0.77
Size \times concentration \times habitat	4	0.58	0.46	2.09
Residual	80			

$n = 10$; * $p < 0.05$.

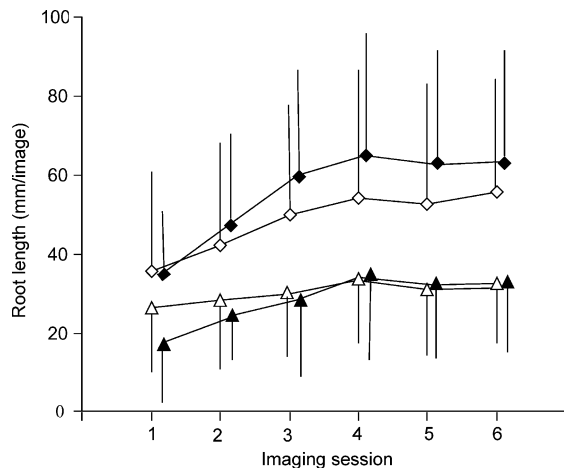


Figure 1. Root length (± 1 SD) in grassland (diamonds) and forest (triangles) in unfertilized patches (open symbols) and patches receiving $15 \text{ g N m}^{-2} \text{ yr}^{-1}$ (filled symbols). Means are averages across patch sizes. Imaging sessions occurred every 2 weeks in June–August 2002. Root length and production were significantly greater in grassland, and root production was significantly higher in fertilized patches.

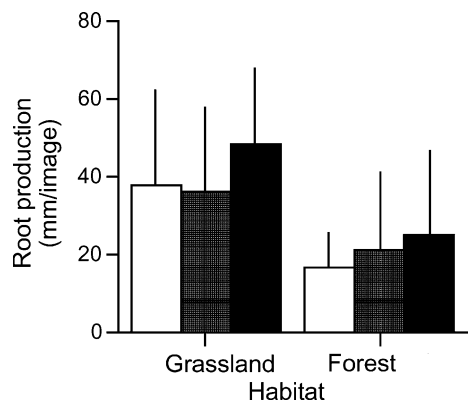


Figure 2. Root production ($+1$ SD) in grassland and forest in patches receiving no additional N (open bars), $3 \text{ g N m}^{-2} \text{ yr}^{-1}$ (shaded bars), and $15 \text{ g N m}^{-2} \text{ yr}^{-1}$ (solid bars), averaged across patch sizes. Root production was significantly greater in patches receiving high rates of N than in those without added N when the middle N level was excluded from the analysis.

excluded in order to increase the contrast between fertilized and unfertilized patches. In this case, root production was significantly higher in fertilized patches than in unfertilized patches ($F=4.13$, Figure 2). As before, root production was significantly higher in grassland than forest, but there was no interaction between habitat and concentration, suggesting that the magnitude of response was similar between habitats.

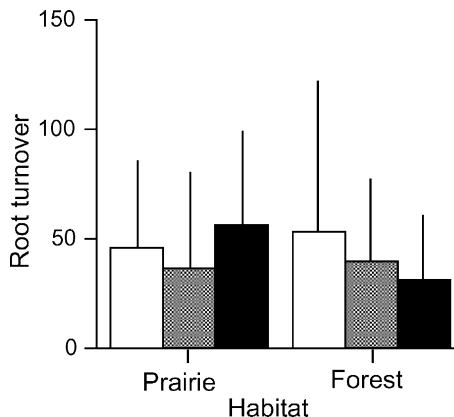


Figure 3. Root turnover ($+1$ SD) in grassland and forest in patches receiving no additional N (open bars), $3 \text{ g N m}^{-2} \text{ yr}^{-1}$ (shaded bars), and $15 \text{ g N m}^{-2} \text{ yr}^{-1}$ (solid bars), averaged across patch sizes. Turnover did not vary significantly with any factor (Table 1).

Root turnover did not vary significantly with any factor or interaction (Figure 3, Table 1).

Discussion

Root length was much higher in grassland than forest in accordance with other reports of differences between grasslands and forests, both at our study site (Pärtel and Wilson 2002) and globally (Jackson et al. 1997). This difference suggests that the two vegetation types have the potential to differ in their root responses to nutrient heterogeneity.

Root production was also higher in grassland than forest. Production was significantly higher in N-enriched patches, as often occurs in greenhouse studies (Hutchings et al. 2000; Hodge 2004). Interestingly, production was significantly higher in patches only in a comparison of unfertilized patches and those receiving the highest rate of N addition. Roots may respond to small patches of nutrients only when there is a substantial difference between patch concentration and background levels.

In other studies, N addition in grasslands comparable to ours results in complete turnover of grassland species composition (Wilson and Tilman 2002) and increases in biomass (Peltzer et al. 1998). The difference between our small experimental patches ($1 \times 10 \text{ cm}$) with small responses to nutrient addition and large plots (e.g., $5 \times 5 \text{ m}$,

Wilson and Tilman 2002) with large responses suggests that rates of nutrient addition that alter the composition and productivity of large plots do not cause root proliferation at small scales. Changes in biomass in large plots may be partly caused by species turnover, which is probably not a factor in ephemeral patches in perennial vegetation.

Most importantly, we found no differences between grassland and forest in root response to nutrient patches. It is possible that differences between grasses and trees exist in other attributes, such as uptake kinetics (Ivans et al. 2003). A field experiment showed that grass roots were able to take up ammonium within 1 day of a water pulse, but that new root growth did not become apparent until 3 days after the pulse (Cui and Caldwell 1997), suggesting that uptake can change without proliferation. On the other hand, root length: mass ratios in the tussock grass *A. desertorum* were increased by small N patches (Bilbrough and Caldwell 1995). Trees and grasses may also differ in responses other than root production and turnover.

Patch size had no effect on root dynamics. Semivariance analysis of root distributions along our tubes suggested that grassland roots have a patch size of 3–4 cm, compared with 8–12 in forest (Pärtel and Wilson 2002), but our experiment suggests that patches at the low (1 cm) and high (10 cm) ends of this range invoke similar growth responses from roots.

Root turnover was similar in grassland and forest. In contrast, root turnover along our tubes 2 years earlier was more than twice as high in forest than grassland, due to higher mortality of roots in forest (Pärtel and Wilson 2002). Differences in root mortality among years may reflect differences in seasonal patterns of precipitation and temperature. Root responses to pulses are also likely to vary over time; we worked in a season with high root production when responses should occur.

The use of pulses should not have diminished our ability to detect root responses. We created patches of nutrient addition using five pulses of NH_4NO_3 over the course of 21 days. This method of delivering N in the form of pulses is consistent with other field studies that found that plant roots respond most strongly to nitrogen given in pulses and least strongly to a continuous nutrient supply

(Bilbrough and Caldwell 1997). Further, the duration of nutrient addition we used was similar to natural pulse duration in forest (Farley and Fitter 1999).

In general our experiment in intact soil and vegetation found weak responses to patches. This contrasts with greenhouse studies (Pärtel and Wilson 2001; Day et al. 2003), and with outdoor plot studies (Pregitzer et al. 1993; Bilbrough and Caldwell 1997; Smilauerova 2001) where patch responses tend to be large and clear. Differences between soils are probably responsible for the differences in results. First, soils in pots and plots are homogenized before use, whereas field soils contain background levels of heterogeneity. Second, soils under intact perennial vegetation are full of roots at the start of the experiment (Figure 1) in contrast to the young roots filling uncolonized soil in greenhouse and plot experiments. Roots and soil microbes quickly take up added N (Jackson et al. 1989; Cui and Caldwell 1997; Bardgett et al. 2003), so nutrient patches may have small effects on root dynamics in intact vegetation. Overall, differences caused by experimental patches were much smaller than differences between habitats, and did not vary between habitats.

Acknowledgements

We thank M. Pärtel for tube installation, F. Gendron, J. Roy and D. Steinaker for field assistance, Saskatchewan Environment for permission to work at White Butte, D. Kolybaba for making the injection guide, M. Oesterheld, D. Steinaker and reviewers for comments on the manuscript, and NSERC for funding.

References

- Anonymous 1992. Soil Landscapes of Canada: Saskatchewan. Centre for Land and Biological Resources Research, Research Branch, Agriculture Canada, Contribution 87–45, Publication 5243/B, Ottawa.
- Bardgett R.D., Streeter T.C. and Bol R. 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* 84: 1277–1287.
- Bilbrough C.J. and Caldwell M.M. 1995. The effects of shading and N status on root proliferation in nutrient patches by the perennial grass *Agropyron desertorum* in the field. *Oecologia* 103: 10–16.

- Bilbrough C.J. and Caldwell M.M. 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. *Ecology* 78: 231–243.
- Cui M.Y. and Caldwell M.M. 1997. A large ephemeral release of nitrogen upon wetting of dry soil and corresponding root responses in the field. *Plant and Soil* 19: 291–217.
- Day K.J., Hutchings M.J. and John E.A. 2003. The effects of spatial pattern on nutrient supply on yield structure and mortality in plant populations. *Journal of Ecology* 91: 541–553.
- Espeleta J.F. and Donovan L.A. 2002. Fine root demography and morphology in response to soil resources availability among xeric and mesic sandhill tree species. *Functional Ecology* 16: 113–121.
- Farley R.A. and Fitter A.H. 1999. Temporal and spatial variation in soil resources in a deciduous woodland. *Journal of Ecology* 87: 688–696.
- Fitter A.H., Hodge, A. and Robinson D. 2000. Plant response to patchy soils. In: Hutchings M.J., John E.A. and Stewart A.J.A. (eds.), *The Ecological Consequences of Environmental Heterogeneity*, Blackwell Science, Oxford, pp. 71–90.
- Grime J.P. 1994. The role of plasticity in exploiting environmental heterogeneity. In: Caldwell M.M. and Pearcy R.W. (eds.), *Exploitation of Environmental Heterogeneity by Plants*, Academic Press, San Diego, pp. 1–19.
- Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist* 162: 9–24.
- Hutchings M.J., John E.A. and Stewart A.J.A. 2000. *The Ecological Consequences of Environmental Heterogeneity*. Blackwell Science, Oxford.
- Hutchings M.J., John E.A. and Wijesinghe D.K. 2003. Toward understanding the consequences of soil heterogeneity for plant populations and communities. *Ecology* 84: 2322–2334.
- Hutchings M.J., Wijesinghe D.K. and John E.A. 2000. The effects of heterogeneous nutrient supply on plant performance: a survey of responses with special reference to clonal herbs. In: Hutchings M.J., John E.A. and Stewart A.J.A. (eds.), *The Ecological Consequences of Environmental Heterogeneity*, Blackwell Science, Oxford, pp. 91–110.
- Ivans C.Y., Leffler A.J., Spaulding U., Stark J.M., Ryel R.J. and Caldwell M.M. 2003. Root responses and nitrogen acquisition by *Artemisia tridentata* and *Agropyron desertorum* following small summer rainfall events. *Oecologia* 134: 317–324.
- Jackson L.E., Schimel J.P. and Firestone M.K. 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. *Soil Biology and Biochemistry* 21: 409–415.
- Jackson R.B., Mooney H.A. and Schulze E.-D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences USA* 94: 7362–7366.
- Kleb H.R. and Wilson S.D. 1997. Vegetation effects on soil resource heterogeneity in prairie and forest. *American Naturalist* 150: 283–298.
- Köchy M. and Wilson S.D. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. *Journal of Ecology* 89: 807–817.
- Pärtel M. and Wilson S.D. 2001. Root and leaf production mortality and longevity in response to soil heterogeneity. *Functional Ecology* 15: 748–753.
- Pärtel M. and Wilson S.D. 2002. Root dynamics and spatial pattern in prairie and forest. *Ecology* 83: 1199–1203.
- Peltzer D.A., Wilson S.D. and Gerry A.K. 1998. Competition intensity along a productivity gradient in a low-diversity grassland. *American Naturalist* 151: 465–476.
- Pregitzer K.S., Hendrick R.L. and Fogel R. 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytologist* 125: 575–580.
- Schlesinger W.H., Raikes J.A., Hartley A.E. and Cross A.F. 1996. On the spatial pattern of soil nutrients in desert ecosystems. *Ecology* 77: 364–374.
- Schlesinger W.H., Reynolds J.F., Cunningham G.L., Huenneke L.F., Jarrell W.M., Virginia R.A. and Whitford W.G. 1990. Biological feedbacks in global desertification. *Science* 247: 1043–1048.
- Smilauerova M. 2001. Plant root response to heterogeneity of soil resources: Effects of nutrient patches, AM symbiosis, and species composition. *Folia Geobotanica* 36: 337–351.
- Tilman D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs* 57: 189–214.
- Wilson S.D. 1993. Belowground competition in forest and prairie. *Oikos* 68: 146–150.
- Wilson S.D. 2000. Heterogeneity, diversity, and scale in plant communities. In: Hutchings M.J., John E.A. and Stewart A.J.A. (eds.), *Ecological Consequences of Habitat Heterogeneity*, Blackwell Science, Oxford, pp. 53–69.
- Wilson S.D. and Kleb H. 1996. The influence of prairie and forest vegetation on soil moisture and available nitrogen. *American Midland Naturalist* 136: 222–231.
- Wilson S.D. and Tilman D. 2002. Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. *Ecology* 83: 492–504.