

Won't you be my neighbor: neighborhood effect influences mycorrhizal and endophyte colonization

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Independent Research

2013

ABSTRACT

Abiotic and biotic factors have been known to affect plant communities. How these factors affect root-colonizing fungus such as arbuscular mycorrhizae (AM) and dark septate endophytes (DSE) fungal communities is still novel to ecology. Nitrogen addition has been shown to either increase or decrease AM colonization. Furthermore, AMF and DSE colonization has shown to be altered by host-neighbor interactions. The purpose of this study was to further understand (1) the effects that nitrogen addition has on AM and DSE colonization in *Festuca thurberi* and *helianthella quinquenervis*, (2) the effects of removing a *F. thurberi* has on AM and DSE colonization with *H. quinquenervis*, and if (3) *F. thurberi*'s fungal community has any influence on the fungal community of *H. quinquenervis*. I conducted the research in a sub-alpine meadow outside of Gothic, Colorado USA. A full factorial between nitrogen additions crossed with *F. thurberi* removal was applied randomly to 36 plots. Soil samples were taken from under *F. thurberi*, and *H. quinquenervis* plants near and far from *F. thurberi*. Results indicated nitrogen had no significant effect except with *Festuca thurberi* as there was an increase in AMF colonization and decrease in DSE colonization. I found no significant effects from *F. thurberi* removal on AMF or DSE with *H. quinquenervis*. I did find significant results indicating that *F. thurberi*'s fungal community influenced the fungal community of *H. quinquenervis* as AM colonization decreased while DSE colonization increased in *H. quinquenervis* near *F. thurberi*, and AM colonization increased while DSE colonization decreased in *H. quinquenervis* far from *F. thurberi*. I can conclude from this study that neighbor effect influences AM and DSE colonization more than nitrogen and further research is suggested to understand the impact of neighboring fungal communities as within plant communities as it could be applied towards maintenance and restoration.

INTRODUCTION

Symbioses between plants roots and root-colonizing fungi are known to form ubiquitous mutualisms within terrestrial plant communities (Peay *et al.* 2008). Two frequently occurring root

colonizing fungi are arbuscular mycorrhizae (AM fungi) and dark septate endophytes (DSE). It is observed these root-fungal associations have significant impacts on shaping plant communities as they play a major role in nutrient transfer to the plants (Hartnett *et al.* 1999).

AM fungi belong to the phylum Glomeromycota and form associations with roughly 80% of land plants by penetrating structures called hyphae through the cortical cells of roots (Tawaraya 2003). AM fungi form extensive deep underground networks within plant communities which increases plant root surface area (Johnson 2003). By increasing the surface area of the roots, plants colonized by AM fungi are able to uptake crucial nutrients from the soil, such as nitrogen and phosphorus more than non AM-colonized plants (Smith and Read 1997). Being able to access soil nutrients, especially phosphorus, directly increases plant growth (Bever *et al.* 2001).

Dark septate endophytes belong to the phylum Ascomycota. Knowledge in regards to DSE fungi functionality in plant communities is limited compared to AM fungi (Knapp *et al.* 2012). Dark septate endophytes have melanized hyphae which also colonize root cells; however, differs from AM fungi as they form septated intracellular structures called microsclerotia (Jumpponen and Trappe 1998). It is observed that DSE fungi seem to form mutualistic relationships with plants similar to AM fungi and has been hypothesized that DSE fungi associations increase in plant communities as abiotic stress increases in the environment (Knapp *et al.* 2012). Furthermore, prior experiments indicate that fungi with melanized cell walls have higher tolerance to drought stress and heat (Knapp *et al.* 2012).

Due to the influences that AM fungi and possibly DSE fungi have on shaping plant community structure, it is important to consider any factors that may affect these fungal communities, such as abiotic and biotic factors. Nitrogen, being an essential abiotic factor to AM fungi, may adversely impact the abundance of the fungi dependent upon the amount of nitrogen available (Treseder 2004). As the amount of nitrogen availability increases with plants, more photosynthate will be directed to stems and leaves, and less towards roots and AM fungi (Marschner *et al.* 2006). Prior studies on this matter show that

nitrogen enrichment can decrease AM fungi in some grass species in soils containing high levels of phosphorus but increases AM fungi colonization in soils that are phosphorus deficient (Johnson et al. 2003).

For biotic factors, host and neighbor plants may play an important part in structuring fungal communities even more so than abiotic. Local plant neighborhoods have been known for quite some time to affect above and below ground composition within the community (Hausmann *et al.* 2009). A past study on grass species *Nassella pulcra*, indicates when there is a neighbor plant of the same type, the proportion of AM fungal root length colonization is significantly higher than compared to when *N. pulcra* had a neighbor plant that was an annual, which showed a significant decrease in AM fungi root length colonization (Hausmann *et al.* 2009).

The purpose for this study was to gain a further understanding of how abiotic and biotic factors affect AM and DSE fungi colonization in host plants *Festuca thurberi* and *Helianthella quinquenervis*. The questions I sought out to answer were (1) what effects does nitrogen enrichment have on AM and DSE fungal colonization in *Festuca thurberi* and *Helianthella quinquenervis*, (2) what effect does nitrogen enrichment and removal of a dominant, highly AM fungal colonized plant species, such as *F. thurberi* have upon the root-colonization of sub-dominant species *H. quinquenervis*, and (3) does *Festuca thurberi*'s fungal community influence the fungal community of *Helianthella quinquenervis*?

METHODS

Field site and Sampling

I conducted my research in a sub-alpine meadow (2926m) outside Gothic, Colorado USA (fig. 4). At this site, the amount of anthropogenic nitrogen is low, which established baseline data for my research. The vegetation in this meadow contains dominant plant species *F. thurberi*, which is a grass known to have high AM and DSE fungi colonization. Furthermore, *Helianthella quinquenervis* is a sub-dominant

species found in this meadow. The amount of AM or DSE fungi colonization in *H. quinquenervis* was unknown prior to experiment.

The area I worked in was 20 m square, containing 36 randomly-distributed plots. Each plot was 1.5 x 1.5 m, with all sampling conducted in the central 1 x 1 m. The two treatments applied to the plots were *F. thurberi* removal (dominant species) and nitrogen addition. Nitrogen was in the form of inorganic ammonium nitrate (10 g inorganic N m⁻² y⁻¹), and organic urea (10 g organic N m⁻² y⁻¹) fast-release fertilizer pellets. There was a full factorial setup of ammonium nitrate, urea, and no treatment (control) crossed with *F. thurberi* removal, and no *F. thurberi* removal (control). Nitrogen treatments were first added in 2012 and were reapplied in June, 2013.

To obtain AM and DSE fungi, I collected root samples using a soil corer within each plot. I took core samples from under *F. thurberi* and *H. quinquenervis*. Two root samples were taken from each plant species. To test neighbor influence of *F. thurberi*, I collected two root samples of *H. quinquenervis* that were less than 10 cm from *F. thurberi* (near), and two root samples which were greater than 20 cm from any *F. thurberi* (far). Neighbor samples were conducted in both *F. thurberi* removal plots, as well as control plots.

Lab Analysis

To analyze the amount of AM and DSE fungal root colonization composition, I followed the methods described in an article written by Molina *et al.* from 1978. Using distilled water, I washed the soil off the roots and transferred the root fragments to individual tissue cassettes which I submerged in a beaker containing distilled water, overnight. Next, I placed the root samples in 500 ml of 10% KOH solution and let them steep for three days. After steeping, I poured the KOH solution off and then rinsed the cassettes in water for five minutes. After the rinsing, I added 500 ml of 2% HCl to the cassettes and let the solution sit for 30 minutes before draining. After pouring off the 2% HCL solution, I added 500 ml of 0.05% of trypan blue to the beakers, covered them with parafilm, and let them steep overnight. After

steeping in the trypan blue solution, I placed the cassettes in 1:1 acidified glycerol solution. The container was covered with parafilm and was stored in a refrigeration unit for two days before examination. After two days, the stained root fragments were mounted on microscope slides to count and determine AM fungi and DSE composition. For AM fungi, the presence of vesicles, arbuscules, and hyphae were noted, and stained blue. For DSE, presence of septate hyphae and microsclerotia were counted for composition, and were brown in color.

Data Analysis

To assess the effects of abiotic and biotic factors on AM and DSE fungal colonization between *F. thurberi* and *H. quinquenervis*, I used ANOVA tests using JMP version 10 (SAS Institute Inc., Cary, NC). To meet requirements for ANOVA, I had to transform the data for the proportion of DSE colonization. To assess the effects of the different nitrogen treatments on the proportion of DSE and AM fungi colonization in *F. thurberi*, I performed a Tukey post hoc test.

RESULTS

1. Effects of nitrogen enrichment on AM and DSE fungal colonization in *F. thurberi* and *H. quinquenervis*.

Results indicate for *H. quinquenervis* there was no significant effect amongst nitrogen treatments on the proportion of DSE colonization ($p=0.5009$, $F=0.6966$) (fig. 1) or on the proportion of AM colonization ($p=0.7354$, $F=0.7354$) (fig. 1). However, I did find significant results for *F. thurberi* with ammonium nitrate treatment with mean DSE colonization of 43 % ($p=.0229$, $F=4.476$) (fig. 1) when compared to an increase of mean AM colonization at 57 % ($p=.0127$, $F=5.3057$) (fig. 1).

2. Effects of nitrogen enrichment and dominant species removal of *F. thurberi* on fungal communities of *H. quinquenervis*.

I found no significant effects between the nitrogen treatments and removal of *F. thurberi* on *H. quinquenervis* with DSE colonization ($p=0.8071$, $F=0.4570$) (fig. 2) or with AM colonization ($p=0.9320$, $F=0.2632$) (fig. 2).

3. *Festuca thurberi*'s fungal community influence on the fungal community of *H. quinquenervis*.

I found significant results with *H. quinquenervis* plants that were near *F. thurberi* as the mean colonization of DSE fungi was 58 % and mean AM colonization of 48% ($p<0.001$, $F=0.3582$) (fig. 2), compared to *H. quinquenervis* plants that were far away having a higher mean colonization of AM fungi of 65% and a decrease in mean DSE colonization of 33% ($p<0.001$, $F=45.5005$) (fig. 2).

DISCUSSION

Nitrogen enrichment had no effect on DSE and AM fungal colonization within the roots of *H. quinquenervis* but ammonium nitrate addition increased AM fungal colonization and decreased DSE colonization in *F. thurberi*. One explanation for the results observed in *H. quinquenervis* could be that there was not enough time allocated for the nitrogen treatments to significantly impact DSE and AM fungal colonization. A past study evaluating the effects of nitrogen enrichment on California shrublands, did not show any significant effect on AM colonization until three years, in which a decline in colonization was observed (Egerton-Warburton and Allen 2000, Siguenza 2000, Egerton-Warburton et al. 2001). I predicted to see a decrease in both AM and DSE colonization in both species of plants because of the low nitrogen availability within these soils. In work done by Johnson *et al.* (2003), their group found in soils of low nitrogen availability, a decrease in AM fungal colonization.

The increased AM fungi colonization with ammonium nitrate addition in *F. thurberi* may have occurred if nitrogen was the limiting nutrient for *F. thurberi*, or if the levels of phosphorus in the soil were deficient. This finding is consistent with prior studies indicating that AM fungal colonization is mediated

by nitrogen and phosphorus availability in the soil (Treseder and Allen 2002). Previous greenhouse trials have shown an increase in AM colonization with addition of ammonium nitrate when there is phosphorus deficiency (Smith *et al.* 1986).

Removal of *F. thurberi* had no effect on *H. quinquenervis* AM or DSE fungal colonization. *Festuca thurberi* was removed in 2012, thus ecologically one year of removal may not have been enough time to have an influence on fungal colonization. Another explanation for this finding is legacy effect. Studies have shown that soil legacies can occur from previous effects on soil biota that will remain to affect future plant and soil microbial growth within the community (Kostenko *et al.* 2012). Despite the removal of *F. thurberi*, its underground fungal community may still be mediating the fungal colonization within the community.

The most significant findings overall in this study was the influence of *F. thurberi*'s fungal community on *H. quinquenervis*'s fungal community. *Helianthella* plants that were near (<10 cm) *F. thurberi* had a high DSE colonization and low AM fungi colonization when compared to *H. quinquenervis* plants that were far (>20 cm) from *F. thurberi*, where we observed an increase AM fungi colonization and decrease in DSE colonization. Overall, *F. Thurberi* had a higher colonization of DSE versus AM fungal colonization with a DSE of 68% to AM of 54% colonization ratio (fig. 3). The AM and DSE communities of *H. quinquenervis* were significantly altered when *F. thurberi* was its neighbor plant. Given these results, we can conclude that neighbor effect has more of an impact than nitrogen on fungal communities by either increasing or decreasing the amount of colonization within roots and because of this finding, further research is suggested to fully understand the impact on neighboring fungal communities, and their influence within plant communities as it could facilitate maintenance and restoration practices.

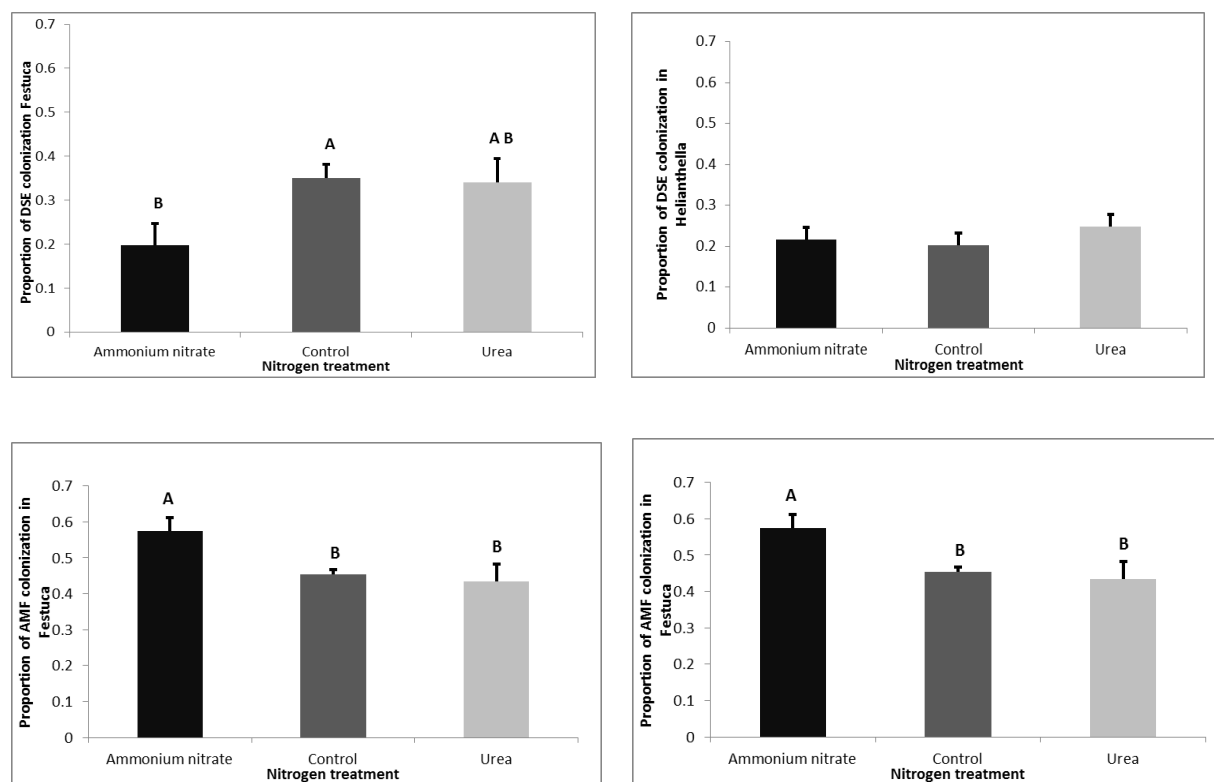


Fig. 1 Mean proportion of AM and DSE fungal colonization between nitrogen treatments. The vertical lines above the bars represent the mean standard error. Singles letters that are different above the bars are significantly different from one another. Letters that are combined show no significant difference. (Tukey Kramer test, $P < 0.05$).

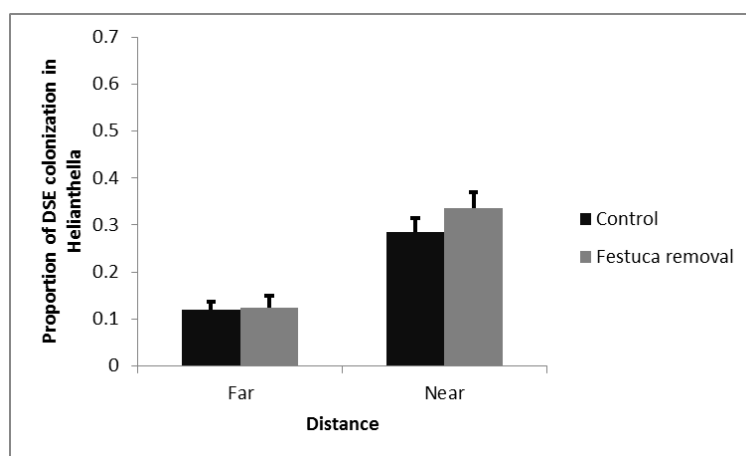
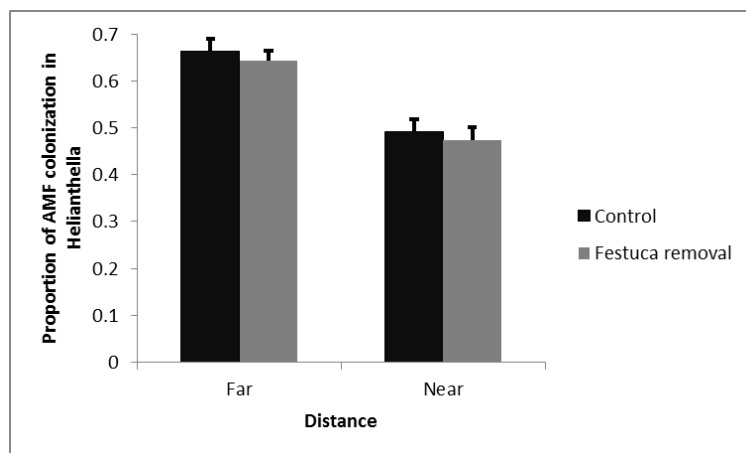


Fig. 2 Mean proportion of AMF and DSE colonization in *Helianthella* far and near *Festuca* and *Festuca* removal.

The vertical lines above the bars represent the mean standard error.

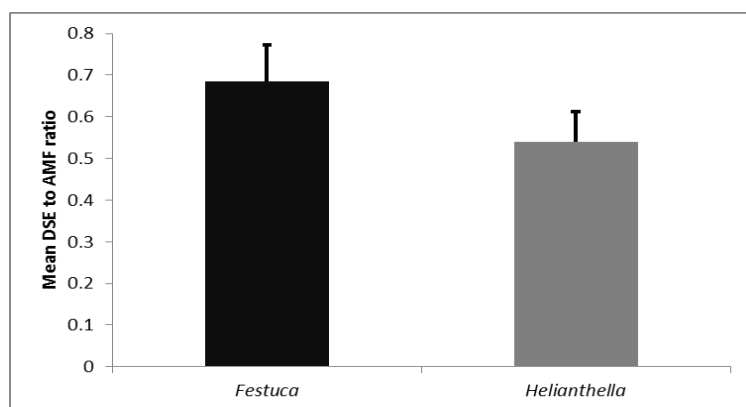


Fig. 3 Mean DSE to AMF colonization ratio. The vertical lines above the bars represent the mean standard error.

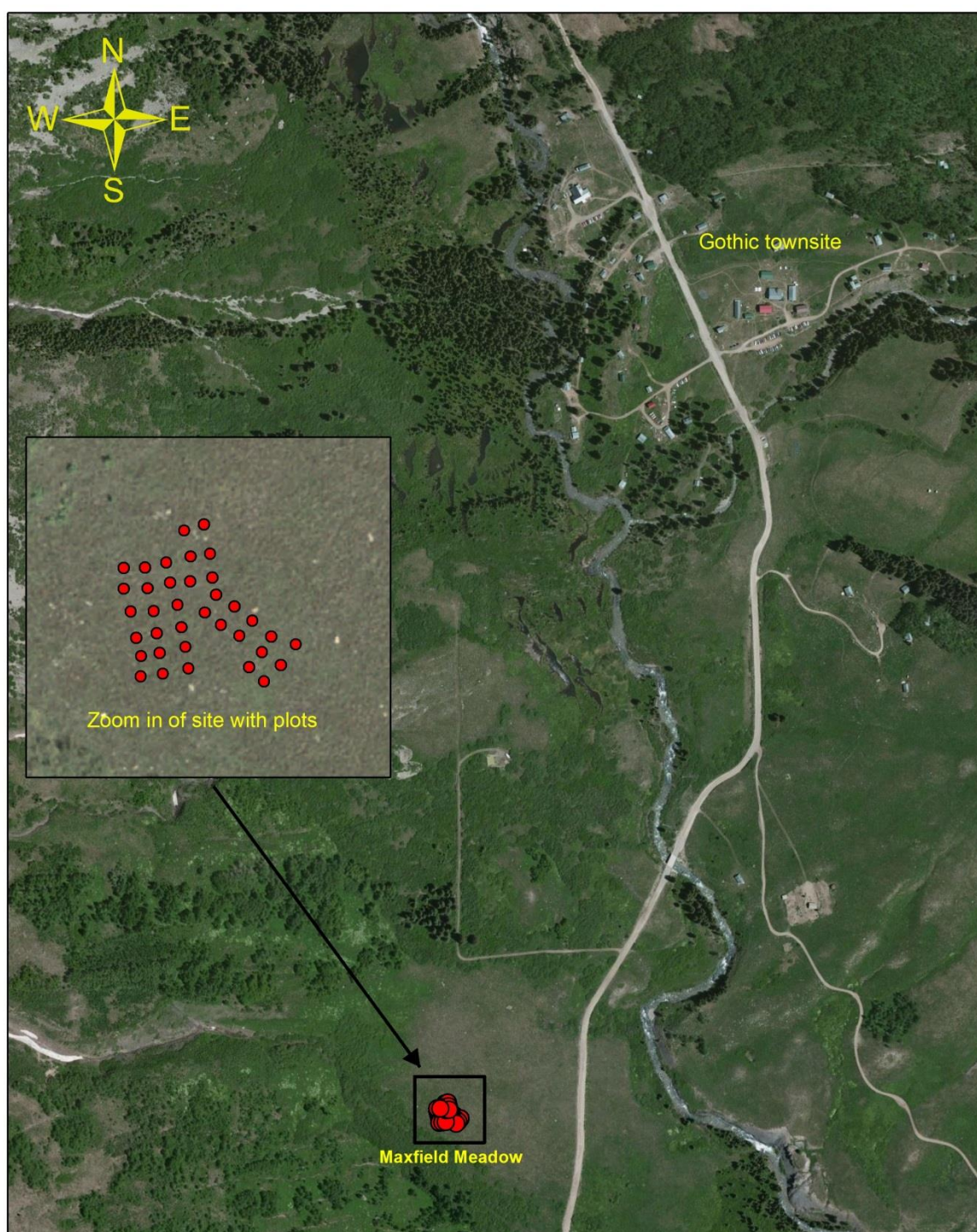


Fig. 4 Map of research site 1 km outside of Gothic, Colorado USA.

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