

**ABOVE- AND BELOWGROUND IMPACTS OF OFF-ROAD  
VEHICLES NEGATIVELY AFFECT ESTABLISHMENT OF A  
DOMINANT FOREST TREE**

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**ABSTRACT**

**ABOVE- AND BELOWGROUND IMPACTS OF OFF-ROAD  
VEHICLES NEGATIVELY AFFECT ESTABLISHMENT OF A  
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**KARLA J. KENNEDY**

Off-road vehicles (ORVs) are known to dramatically alter the system in which they operate. Unlike most ORV studies that focus on shrubs, we examined the above- and belowground impacts of ORVs in a system dominated by a large tree, ponderosa pine (*Pinus ponderosa* Englem). Using historical aerial photographs, we verified that our treatment and control sites did not differ significantly prior to the introduction of ORV recreation. We addressed the hypothesis that ORVs would negatively impact factors that relate to the establishment of this species. Four patterns emerged: In our control sites 1) The number of juvenile and first-year trees was greater in control sites, 2) litter cover and depth was greater in control sites, 3) soil bulk density was lower and soil moisture, water infiltration, and root biomass were greater, and 4) ectomycorrhizal colonization, abundance, inoculum potential and community were all greater in our control sites. These factors all have the potential to restrict ponderosa pine establishment, thus altering the population structure of the forest. Without intervention, we predict this altered age structure of the remaining trees will not be able to sustain the stand; that the population will decline.

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**CHAPTER 1**  
**ABOVE- AND BELOWGROUND IMPACTS OF OFF-ROAD VEHICLES**  
**NEGATIVELY AFFECT ESTABLISHMENT OF A DOMINANT**  
**FOREST TREE**

Abstract – Off-road vehicles (ORVs) are known to dramatically alter the system in which they operate, but little is known about their impact on trees, their mycorrhizal associations, and the coarse cinder soils associated with recent volcanic activity. Using historical aerial photographs of the ponderosa pine (*Pinus ponderosa Englem*) forest near Sunset Crater National Park in Arizona, USA we verified that our treatment and control sites did not differ significantly prior to the introduction of ORVs at the Cinder Hills Recreation Area. We addressed the hypothesis that ORVs would negatively impact factors that relate to the establishment of this dominant tree that defines a major vegetation type in the West. Four patterns emerged: In our control sites 1) The number of juvenile and first-year trees was 3-fold and 22-fold greater, respectively, in control sites, 2) litter cover and depth were 6-fold and 10-fold greater, respectively, in control sites, 3) soil bulk density was 30% lower, soil moisture was 30% greater, water infiltration was 6-fold greater and root biomass was 4-fold greater, and 4) ectomycorrhizal colonization was 4-fold greater, ectomycorrhizal abundance was 11-fold greater, fungal inoculum potential was 2-fold greater as measured by ectomycorrhizal colonization in a bioassay experiment, and fungal diversity was 2-fold greater. In combination, these factors reflect a forest in decline that due to the altered soil structure and lack of regeneration in a long-lived tree would require many years to restore. The potential conservation consequences of having a major ORV recreation site on a

relatively rare soil type, which is also adjacent to a National Monument need to be considered in the long-term management of this facility.

**Keywords:** *Pinus ponderosa*, disturbance, off-road vehicles, ORV, soil compaction, ectomycorrhizae

## **Introduction**

Off-road vehicle (ORV) use is an increasingly popular form of recreation in our forests and public lands that results in significant disturbance of the soil and vegetation. The Wilderness Society ([www.wilderness.org](http://www.wilderness.org)) conservatively estimates the number of ORVs in the United States to be 11 million, while the U.S. Forest Service estimates ORV ownership to be around 36 million. The Bureau of Land Management states that 93% of our 262 million acres of public land are open to ORV use. Of our 155 National Forests encompassing 191 million acres, all but two (collectively totaling 207,200 acres) are open to off-road driving. Similar to natural disturbance, ORVs reduce vegetation cover (Webb 1983, Tonnesen and Ebersole 1997, Kozlowski 1999), but the mechanisms are different, and much less studied. Wide-spread soil compaction is the most common result of ORV use (Webb 1983, Wilshire 1983, Milchunas et al. 2000). Soil compaction is known to increase bulk density, decrease soil porosity, and decrease water infiltration, in turn causing decreased water and nutrient availability to plants, as well as increased seedling mortality (Kozlowski 1999). The radicles of germinating seedlings cannot penetrate the compacted soil, resulting in seedling mortality rates of up to 60% (Pomeroy 1949). This



reduction of germinants has been shown to reduce mature tree density by 41% when compared to adjacent, non-compacted stands (Wert and Thomas 1981).

ORVs could potentially have indirect effects on a dominant tree by reducing associated vegetation that facilitates seedling establishment. Nurse plants have been shown to be vital to the establishment and survival of pines in hot, dry climates (Callaway 1995, Stulz 2004). By removing the ameliorating effects of nurse plants, ORV activity could reduce favorable germination and establishment sites for species dependent on these associations. Additionally, ORV movement disrupts the plant litter layer (Waltert et al. 2002). Litter has been shown to slow water loss (Pearson 1942), decrease erosion (Wilshire 1983), provide nutrients to vegetation through decomposition (Enoki and Kawaguchi 2000), and increase recruitment and establishment of seedlings (Wilshire 1983). For example, due to increased soil moisture, seedling emergence was greater in areas occupied by pine litter than in areas free of tree litter (Williams et al. 1990). The presence of litter can also increase mycorrhizal colonization of fine roots (Waltert et al. 2002) by increasing nutrient translocation from litter to seedlings, which increases seedling biomass (Perez-Moreno and Read 2000).

In studying regeneration and forest health it is also important to study the mycorrhizal associations between plant roots and fungi that are frequently mutualistic. Plants benefit from an increase in nutrient and water uptake and protection from pathogens, while the fungus receives photosynthate from the plant (Smith and Read 1997). Combined with the presence of tree litter, this mutualism is especially important in hot, dry environments where water limits seedling recruitment (Wilshire 1983, Gehring and Whitham 1994, Swaty et al. 1998). Potential changes in ectomycorrhizal

fungus community composition are also important to measure because different species of EM fungi vary in their mutualistic capabilities and EM diversity can contribute positively to nutrient uptake by seedlings (Baxter and Dighton 2001). The presence of a litter layer also has been shown to increase EM diversity and change community composition (Brearley et al. 2003).

Several studies have examined the effects of compaction on the arbuscular mycorrhizal fungi that associate with agricultural plant species because soil compaction frequently results from the use of harvesting machinery. Generally, compaction reduces arbuscular mycorrhizal colonization and these changes interact synergistically with changes in litter because both litter and mycorrhizae can be crucial for seedling establishment (Pearson 1942, Steinfield et al. 2003).

While these studies suggest that soil compaction resulting from ORV use will negatively affect plants and their associated mycorrhizal fungi, few studies have examined this issue directly (but see Waltert et al. 2002). Although the Cinder Hills Recreation Area substrate is dominated by coarse cinders that would seem to be highly resistant to compaction, no studies have examined how ORVs might affect these relatively rare cinder soils associated with recent volcanic activity. Furthermore, we are aware of no studies that have examined the effects of ORVs on the colonization and community dynamics of the ectomycorrhizal fungi associated with a dominant tree such as ponderosa pine. Thus, our studies of the mycorrhizae of a dominant tree on a relatively rare soil type at a large ORV recreation site adjacent to a major U.S. National Monument provided a unique opportunity to integrate several understudied factors and issues at the same time.

Here, we examine the above factors on the Cinder Hills Recreation Area (54 km<sup>2</sup>) that has been open to ORV use since 1979. We sought to answer the question: Do ORVs impact the dominant vegetation in which they operate and are these impacts related to changes in the biotic and abiotic properties of the soil environment that contribute to seedling establishment? We focused on young ponderosa pine trees <20 years old as these trees have established since the designation of this area for ORV use. Specifically, we addressed four hypotheses: The movement of ORVs through the area will result in: 1) a reduction in the abundance of juvenile ponderosa pine (both first-year seedlings and juvenile trees <20 years old). 2) reduced litter cover and depth 3) altered soil properties including increased soil compaction as measured by bulk density, increased water infiltration time and decreased soil moisture and fine root biomass, 4) reduced EM colonization and diversity and altered EM community composition. Studies of ORV impacts commonly focus on shrubs or grasslands that suffer clearly visible impacts due to direct physical damage by ORVs. This study is unusual in that it focuses on an ecosystem dominated by large trees where impacts can be less obvious because mature trees are not susceptible to direct physical damage. However, less obvious impacts of ORV use may affect the establishment success of juvenile trees and the long-term sustainability of the ecosystem. In addition, this is the first study to directly measure the impacts of ORV use on the colonization and community composition of the EM fungi that are frequently critical for seedling establishment (but see Waltert et al. 2002).

## **Methods**

**Study site** - We conducted our study at the Cinder Hills Recreation Area about 30km NE of Flagstaff, Arizona, which borders Sunset Crater National Monument which

is approximately one-fourth the size of the recreation area. The ORV area is characterized by a ponderosa pine overstory and an understory dominated by the shrub, *Fallugia paradoxa*. The soils are derived from basaltic ash, cinder, and lava flows from the eruption of Sunset Crater approximately 1,000 years ago. The soils at our sites are classified as Typic ustorthents. These soils have low nutrient status and a low water-holding capacity (Cobb et al. 1997). Three sites inside the Cinder Hills Recreation Area were chosen and designated as “ORV-present”, and 3 sites free of ORV tracks outside the Cinder Hills Recreation Area served as controls. Each site was on level ground. All measurements were taken within these sites, with one exception, noted below.

To determine if tree cover differed between ORV and Control sites prior to ORV use, black and white historic photographs of the study sites were obtained from the U.S. Forest Service (scale 1:24,000). These photographs covered approximately 50 km<sup>2</sup> and were taken in flight rows over the entire study area. Photographs of our study sites were compared for percent tree cover using ArcGIS Desktop Version 9, ArcView License. While physically standing in each of our sites, the photographs were analyzed visually to determine what values of gray represented a tree. Using ArcGIS, we then determined what percent of each photograph was consisted of those values of gray, or “trees.” These data were compared using a one-way ANOVA.

**Seedling recruitment and establishment** - To determine if juvenile ponderosa pines were impacted by ORV use, we made two comparisons between the ORV and control sites: 1) stand size structure to determine if the frequency of juvenile and adult trees was similar at the two site types, 2) direct counts of juvenile trees (~ 20 years of age or less) in sites of both types. In addition we made direct counts of newly germinated

seedlings in one ORV site in areas of high and low track density to examine the impacts of ORV use on the youngest age class of ponderosa pine. Because of patchy recruitment, these measurements were possible only at one site. To compare the total numbers of trees in the ORV and Control areas, 25m<sup>2</sup> plots were delineated in one pair of our study sites. All trees encountered were measured for diameter at breast height to the nearest cm. In the case of trees less than 1.3m in height, basal trunk diameter to the nearest cm was measured. These data were analyzed using a t-test.

To investigate how ORVs impact juvenile pine establishment, we examined younger trees that have established in the Cinder Hills Recreation Area since its designation as an ORV area in 1979. Establishment was determined by running nine 50m transects (3 in each study plot) in each site. Counts of seedlings <20 years old were obtained. These trees were aged by counting bud scars. The difference in the number of seedlings found in the ORV area and in the control area was analyzed using a chi-squared test.

Finally, to determine how ORVs affected newly germinated ponderosa pine seedlings, we quantified seedling recruitment during 2002. We did this by laying four 25x2m transects in areas of high track density (“ORV-present”) and in areas of little to no tracks (“control”). These transects were run outside our regular study sites on a north-facing slope to take advantage of this recruitment event. We counted the first-year seedlings that fell along transects and noted whether they were associated with pine needle litter. The differences in the number and conditions of the seedlings were analyzed using a chi-squared test.

**Litter** - To ascertain if ORVs affected litter cover, we surveyed litter cover and depth using transects associated with 12 trees in each site. Trees within an ORV-Control pair were matched for diameter at breast height (dbh), which ranged from 25-95cm. Under each tree we ran a radial transect, one at each of the 4 cardinal directions. To describe the litter environment of focal trees and the area surrounding them, transect length was 2X the radius of the crown of the focal tree. At every 0.2m the type of ground cover was noted as shrub, herbaceous, litter or bare ground. Also, each transect length was divided by 8, and the litter depth was measured at these 8 points to the nearest 0.1cm. The transect data were pooled for each tree, and percent litter cover and average litter depth were compared using a one-way ANOVA.

**Soil properties** - To test the hypothesis that ORV use altered soil properties, we compared ORV and control sites for soil moisture and bulk density and water infiltration time. To determine how ORVs affect water infiltration times between the sites, we measured the time it took 4L of water to be absorbed by the soil. We did this by inserting a metal cylinder 2cm into the soil. Into the cylinder, we poured 4L of water, and recorded the time it took for the water to be fully absorbed by the soil. This was repeated 15 times in litter and 15 times in bare ground at each site. We analyzed these data using a Mann-Whitney U test.

Changes in soil infiltration rate due to ORV use could contribute to increased water runoff which results in lower soil moisture. To address this hypothesis, we measured gravimetric soil moisture for ten samples per site for each of the 6 sites, for a total of 60 samples. 250cm<sup>3</sup> of soil was collected at the drip line of randomly selected mature trees. Soils were weighed, dried at 105 °C, and then weighed again. Water

content of the original sample was calculated, and the data were compared using one-way ANOVA.

To determine if ORVs alter the bulk density of the soil, cores were taken to a depth of 30cm (core diameter: 6cm, core volume:  $0.00085\text{m}^3$ ) at the drip line of 6 adult ponderosa pine trees per site, for a total of 18 trees in the Control area and 18 trees in the ORV area. The soil was dried and weighed as described above. Density in  $\text{Mg}/\text{m}^3$  soil was calculated and compared using a one-way ANOVA.

**Root biomass** – To establish if ORVs affected the amount of pine root biomass in the soil, cores were taken to a depth of 30cm (diameter and volume described above) at the edge of the drip line of mature ponderosa pine trees between 45 and 70cm DBH. The soil was separated manually from the fine ( $<2\text{mm}$ ) ponderosa pine roots and classified as live or dead. Root length was measured, and the roots were then dried at  $60^\circ\text{C}$  for 24 hours and weighed. The resulting data will then be on a per-tree basis, rather than on a landscape level. This metric was chosen to control for the variations in root biomass due to differences in tree densities at ORV and control sites. The amount of live and dead pine root was compared on a per-tree basis between the ORV area and control using one-way ANOVA.

**Mycorrhizae** - To examine if ORVs affected the level of colonization of ponderosa roots by ectomycorrhizal fungi, we collected roots from randomly selected mature trees in September 2003. We chose 10 mature trees with dbh 10-15cm at random in each of our 3 paired sites, for a total of 60 trees. A small root sample ( $\sim 200\text{cm}$ ) was dug from beneath the drip line on the north side of each tree. Roots were carefully washed and scored for presence or absence of ectomycorrhizae using a dissecting

microscope and the methods of Gehring and Whitham (1991). Mycorrhizal colonization was calculated as the percent of all short roots that were colonized by live ectomycorrhizae (EM). We analyzed colonization data using a one-way ANOVA. In 2004, mature trees (dbh > 50cm) were sampled in the same manner as the 2003 collection. Larger mature trees have a more open understory in contrast to smaller mature trees with branches that extend from the trunk close to the ground, which could block ORV impacts from this area of the understory of the tree. We selected larger mature trees in 2004 to ensure that shallow root systems in the understory of trees in the ORV sites were potentially exposed to ORV effects. Six trees per site were sampled for a total of 18 trees in the control area and 18 trees in the ORV area.

Because EM fungi are species rich (>5000 species) and different EM species can convey different benefits to their host tree, we analyzed the fungal community using a combination of morphological and molecular techniques for the 2004 samples. Living EM root tips were classified based on morphology according to Horton and Bruns (1998) and representatives of each morphotype from each tree were saved and frozen. The DNA from two to three root tips per morphotype per tree was extracted and the internal transcribed spacer (ITS) region of the fungal genome, located between the 18S and 28S rRNA, was amplified using polymerase chain reaction (PCR) with the ITS1F and ITS4 primer pair (Gardes and Bruns 1993). Restriction-length polymorphism (RFLP) data were obtained following the methods of Gehring et al. (1998). The amplified ITS region was characterized using restriction enzyme digestion with *Hinf*I and *Mbo*I, which has been used successfully to discriminate among fungal species in *Pinus edulis* (Gehring et al. 1998, Haskins and Gehring 2004). Digital images of agarose gels were recorded and



analyzed using a Kodak EDAS 290 gel documentation system and accompanying software (Eastman Kodak Company, Rochester, New York, USA). RFLP patterns were compared to those generated from fungal sporocarps and other EM collections. RFLP type richness and Shannon's diversity were compared between ORV and Control sites using t-tests. Community composition was compared between site types using the multiple response permutation procedure in PRORD followed by indicator species analysis (McCune and Mefford 1999).

Because measures of mycorrhizal colonization do not directly assess EM abundance, we combined our data on root biomass and ectomycorrhizal colonization to estimate the number of EM root tips present at a given area of soil in ORV and control sites using the following equation from Haskins and Gehring (2004):

$$\begin{aligned} \text{EM tips/m}^2 \text{ soil} &= (\text{number. EM tips/root length}) \\ & * (\text{root length/oven-dried root mass}) \\ & * (\text{oven-dried root mass/soil area}) \end{aligned}$$

These data were square-root transformed to achieve equal variance prior to analysis using a one-way ANOVA.

**Ectomycorrhizal inoculum bioassay** – Adult trees can provide EM inoculum to establishing seedlings and this may be particularly important in sites where only one member of the plant community forms EM associations, the situation at our study sites. To elucidate how the available EM inoculum at ORV and control sites was realized by germinating seedlings, a soil core was taken to the depth of 30cm adjacent to where the 2004 adult root sample was taken. These cores were transferred intact into pots (706 cm<sup>3</sup>) in the field and taken to the greenhouse. Ten ponderosa seeds were planted 1cm

below the soil surface in each pot. A 2cm layer of small plastic beads was placed on top of the soil to prevent fungal inoculum from splashing between pots. Pots were left in a natural light regime at temperatures of 25°C daytime and 22°C nighttime. Pots were watered every day until germination. If more than one seed germinated in a pot, seedlings were clipped at the base to maintain only one seedling per pot. After germinating, seedlings were watered every other day post-germination until plants reach 4 months of age under a natural light regime beginning in September 2004. Seedlings were harvested in January and the following response variables were measured: height of shoot (from root collar to tip of longest needle), needle length, shoot fresh and dry weight, and root fresh and dry weight. The root systems were scanned using WIN-RHIZO (Regent Instruments, Inc.) to determine total root length. The entire root system of each seedling was scored for percent colonization by EM fungi and root tips were saved for molecular analysis as described above. All data were compared using a MANOVA with ORV and control sites as treatment groups and seedling height, root biomass: shoot biomass, root length and percent colonization by ectomycorrhizal fungi as response variables.

**Shrub association and removal experiment** – To determine if juvenile ponderosa pines were found more commonly in association with other vegetation, 3 random 50m<sup>2</sup> plots were delineated in two control sites. This comparison was made only in two control sites due to the low numbers of juvenile ponderosa pines in the third control site and the lack of shrubs and other potential nurse plants in the ORV sites. In these plots, two measurements were taken. First, three 50m transects were run in each plot and the type of vegetation was noted every 50cm to determine available habitat for

juvenile ponderosas. Second, all juvenile ponderosas were counted within the entire plot, and the presence of an association noted. Any intersection of the juvenile ponderosa and other vegetation was considered an association (Charters and Whitham 1997).

To determine how the presence or absence of associated vegetation affected juvenile ponderosa pine survival, a shrub removal experiment was undertaken. Eighty juvenile ponderosa in association with the shrub *Fallugia paradoxa* (Apache plume) were selected and paired for size and proximity. Age of the ponderosa pines ranged from approximately 5 to 30 years. Mid-day water potentials were measured on a randomly selected sub-sample of pairs to insure that pre-treatment water stress levels did not differ significantly. Within each pair, one tree was randomly selected to have the shrub completely removed at the base in May 2004. Repeat trimming over the ensuing months removed resprout growth. Post-treatment water potentials were measured on the same sub-sample of trees in August 2004. Mortality of the juvenile ponderosa pines was monitored from May 2004 to March 2005.

## **Results**

**Photo analysis before and after ORV** - Analysis of the vegetation cover in historical photos of our study sites revealed that there were no significant differences between the sites we chose for this study of ORV impacts ( $F=0.947$ ,  $p=0.39$ ) (**Figure 1**). This result allows us to assert that the impacts we observed in this study are a result of ORV use in the sites designated for recreational use.

**Mature, juvenile and seedling ponderosa**– Overall, we found that ORVs are reducing the numbers of ponderosa pines. Our measurements of the number and size class of all tree size classes in our study area confirm that there are fewer mature trees in

the ORV area. We found an average of 16 trees/ha in the ORV area and >5 fold more (i.e., 84 trees/ha) in the control area. Additionally, the size class distribution is broader in the Control area, with more size classes represented than in the ORV area (**Figure 2**) This decline in tree size categories has the potential to dramatically change the foliage height complexity of the landscape of the Cinder Hills Recreation Area. Particularly lacking are the smallest age classes of trees, suggesting a lack of recruitment.

In agreement with the above data, we found that juvenile ponderosa pines (< 20 years old) were almost 3 fold more abundant in control sites than ORV sites (**Figure 3**) ( $\chi^2=22.5$ ,  $p<0.001$ ). With this disparity in numbers of juvenile trees, we predicted that seeds are preferentially germinating in the control area.

In support of this prediction, we found that ponderosa pine seedling recruitment occurred primarily in areas of low track density and in association with litter ( $\chi^2=498.7$ ,  $p<0.001$ ) (**Figure 4**). Of 222 seedlings counted, 200 occurred in such conditions, compared with 22 seedlings in other conditions. This argues that ORV activity limits ponderosa recruitment possibly by affecting the distribution of litter and by physically disturbing seed beds.

**Litter and soil**- In support of our hypothesis, we found a 6-fold increase in litter cover associated with mature trees in the control sites relative to ORV sites ( $F=33.36$ ,  $p<0.001$ ) (**Figure 5a**) and a 10-fold increase in litter depth in ORV sites compared to control sites ( $F=22.81$ ,  $p<0.001$ ) (**Figure 5b**).

In support of our hypothesis that ORVs would result in greater soil compaction and reduced water infiltration, we found that ORV sites had 30% higher soil bulk density than the control sites. The average bulk density in the control area was  $0.6935 \text{ Mg/m}^3$

while it was 0.9679 Mg/m<sup>3</sup> in the ORV area ( $F=8.97$ ,  $p<0.001$ ) (**Table 1**). In agreement with an increase in soil bulk density, we found water infiltration time to be about 7-fold longer in the ORV area ( $F=22.80$ ,  $p<0.001$ ) (**Table 1**). The decrease in water infiltration was also associated with lower soil moisture. We found a 30% decrease in soil moisture in the ORV sites ( $F=4.273$ ,  $p=0.002$ ) (**Table 1**).

**Root biomass and ectomycorrhizae** – Soil core data reveal that control sites had 4-fold greater live ponderosa fine root biomass than ORV sites. In the control area, there was an average of 95.1 g/m<sup>2</sup> of live ponderosa root while there was an average of 23.8 g/m<sup>2</sup> in the ORV area ( $F=3.34$ ,  $p=0.016$ ) (**Table 1**). With these differences in live roots per tree, we predicted that there would also be alterations to the mycorrhizal fungal colonization and community of fine roots.

In our 2003 survey, we found 50% greater EM colonization in the control area compared to the ORV area. Roots taken in the ORV area were 60% colonized while the Control roots were 88% colonized (**Figure 6**) ( $F=8.75$ ,  $p<0.001$ ). Similarly, in our 2004 analysis of older trees, we found nearly 4-fold higher EM colonization in the control sites than the ORV sites (**Figure 7**) ( $F=10.57$ ,  $p<0.001$ ). The average colonization in the Control sites was 59% while it was only 15% in the ORV area. Five trees in the ORV sites had no living EM, while all trees in the control sites had living EM. The decrease in overall colonization between years may be due to the altered sampling methods which more accurately measured ORV effects in 2004.

When we combined our data on root biomass and EM colonization into a single index, we observed that EM abundance was more than 10-fold greater at the control sites

than the ORV sites. EM tips/m<sup>2</sup> soil was about 77,000 in the control area but only 6,000 in the ORV area ( $F=15.24$ ,  $p<0.001$ ) (**Figure 8**).

With reduced mycorrhizal colonization in the ORV area, we predicted that there would be a reduction in mycorrhizal inoculum in ORV soils relative to control soils. In agreement with this prediction, we found a significant difference between seedlings grown in soil from the ORV and control sites (Wilks' Lambda  $F = 5.92$ ,  $p<0.001$ ). However, univariate tests showed that only the percent colonization by mycorrhizal fungi contributed significantly to the overall effect ( $F=14.52$ ,  $p<0.001$ ). On average, mycorrhizal colonization of seedlings grown in soil from the control sites was almost twice that of seedlings grown in soil from the ORV sites (**Table 2**).

EM fungal communities also differed between ORV and control sites. We found a total of 27 unique RFLP types across both site types, 5 were unique to the ORV site and 17 were unique to control sites. ORV sites had significantly lower species richness with an average of 1.85 species per tree compared to 3.33 species per tree in the control sites ( $t=3.91$ ,  $p<0.001$ ) ORV sites also had a lower Shannon's diversity with an average of 0.426 compared to 0.900 in the control site ( $t = 2.64$ ,  $p = 0.007$ ).

Ectomycorrhizal community composition also differed between ORV and control sites ( $A = 0.076$ ,  $p < 0.001$ ). ORV sites had the only RFLP type as a significant indicator, K1 ( $p = 0.002$ ). This RFLP type was abundant on all but 2 trees at the ORV site and appeared only rarely on trees at the control site. On 31% of trees in ORV sites, K1 was the only species observed in our sample. Only 3 species could be identified taxonomically using available data.

**Nurse plant association and shrub removal experiment** – Because many conifers in arid lands require nurse plant associations for establishment, we predicted that the negative impacts of ORVs on shrubs could negatively impact ponderosa pine recruitment. Our transect data revealed that shrub density is significantly lower in the ORV sites than the control sites. Apache plume represents 33% of the available habitat in the control area compared with only 2% of the available habitat in the ORV area ( $\chi^2 = 27.5$ ,  $p < 0.001$ ).

Consistent with the nurse hypothesis, we found that ponderosa pine seedlings were far more likely to be associated with Apache plume than open ground. Transect data revealed that, in the control area, juvenile ponderosa pines were found in association with Apache plume 65% of the time but only 15% in open ground ( $\chi^2 = 39.094$ ,  $p < 0.001$ ). This pattern also held for the ORV area (**Figure 9**).

While our observational studies were consistent with our nurse hypothesis, our experimental studies did not confirm this prediction. All juvenile trees experienced the same level of water stress prior to experimental manipulation as shown by the lack of difference in water potentials ( $t_{24} = 0.44$ ,  $p = 0.66$ ). However, the shrub removal did not have an effect on the mortality of the trees (only one tree died in the study), nor on the post treatment water potentials ( $t_{24} = 0.024$ ,  $p = 0.49$ ) (**Table 3**).

## **Discussion**

The reduction of tree seedlings, juveniles and overall tree density in our impacted sites are evidence that ORV use is inhibiting ponderosa pine establishment. In 26 years of ORV use, this form of recreation has clearly altered the population structure of the dominant tree on the landscape. ORV use has not yet drastically altered the numbers of

mature trees, but our findings argue that by reducing the numbers of juvenile trees and limiting the recruitment of seedlings, ORV use will soon change the distribution of mature trees by limiting or ending successful regeneration. Here we discuss the mechanisms involved in this limitation.

**Litter and soil** - We found that control sites had 6-fold more space covered with litter and litter depth was 10-fold greater than ORV sites. A well established litter layer can be important to both tree seeds and seedlings. Litter is known to reduce the risk of seed predation (Nilson and Hjältén 2003), and to maintain the soil moisture necessary for seedling emergence (Williams et al. 1990). Similarly, a reduction in the litter layer can result in reduced seedling biomass (Brearley et al 2003) and dramatically increased seedling mortality (Williams et al. 1990). Although too much pine litter can impede germination (Pearson 1942), the enormous reduction in litter resulting from ORV use is likely to be a detriment to germination success and the subsequent establishment of juvenile trees in sites heavily used by ORVs.

Our results also demonstrate a surprising result that in coarse cinders are being greatly compacted by ORV activity. Due to compaction, we found that water infiltration time increased 7-fold, soil moisture was reduced by 30% and bulk density was 30% greater in the ORV area relative to the control sites. Increases in soil bulk density often result from soil compaction which decreases soil porosity (Lei 2004). This decrease in soil porosity is known to reduce water infiltration leading to decreased soil moisture, soil erosion and the formation of gullies leading to increased seedling mortality (Kozlowski 1999). While moderate soil compaction can confer benefits to germinating seedlings, severe compaction, such as the levels found in recreational areas, can



contribute to early seedling mortality by reducing the ability of seedlings to reach the reduced levels of soil water (Kozlowski 2002).

**Mycorrhizae** - ORV use resulted in a 35% reduction in EM colonization of roots of mature ponderosa pine, and a 50% decrease in the fungal community diversity. This result contrasts with the findings of Waltert et al. (2002) who found the negative effects of recreational activities, including ORV use, to be restricted to a decreased ergosterol content of juvenile tree roots. The reduced abundance and altered species composition of EM fungi on adult trees may detrimentally affect their performance. EM fungi play important roles in nutrient and water uptake and protection from pathogens (Smith and Read 1997). Reduced abundance of these fungi may further increase the susceptibility of mature ponderosa pines to stresses such as disease and drought. There is a small but growing body of literature indicating that soil compaction negatively impacts EM fungi. However there is a relatively large base of knowledge regarding the negative effects of soil compaction on arbuscular mycorrhizal (AM) fungi, especially as it relates to decreased benefits conferred to the host plant such as a decrease in nutrient uptake and decreased root biomass (Nadian et al. 1996, Entry et al. 2002)

The detrimental effects of ORVs on the EM of mature trees also may be important to seedlings that rely on adult trees roots as an important source of EM fungal inoculum (Dickie et al. 2002). The results of our bioassay support this hypothesis. We observed a 20% reduction in ectomycorrhizal colonization of seedlings grown in soil from the ORV sites compared to controls sites. The four-fold increase in live ponderosa root biomass and the 13-fold increase in EM abundance in control sites relative to ORV sites also supports this hypothesis. The benefits of mycorrhizae to seedlings have been shown in

many systems, and having a fungal symbiont increases conifer seedling survival in dry, disturbed soils (Steinfeld et al. 2003, Amaranthus and Perry 1987). In a similar bioassay study, Haskins and Gehring (2005) showed that soils from areas of low pine density contribute correspondingly low levels of appropriate fungal inoculum for seedlings, resulting in a low number of seedlings colonized by mycorrhizal fungi. Additionally, these areas of low pine density were colonized by a less rich fungal community compared to pines surrounded by conspecifics. This alteration of fungal community composition could have detrimental effects to seedlings establishing in the area as seedlings tend to rely on mature trees as a source of inoculum (Haskings and Gerhing 2005). Additionally, an increase in ectomycorrhizal community richness is correlated to increased plant performance (Baxter and Dighton 2001). Therefore, as ORVs reduce the amount of mycorrhizal inoculum and species richness in the soils, we would expect to see a decrease in seedling success.

**Nurse plant relationships** – Our observations and experiments present mixed support for ponderosa pine having a nurse plant relationship with the shrub, Apache plume. First, our observational studies showed a greater than expected association of ponderosa pine with Apache plume, which suggest a nurse plant relationship (Callaway 1995). However, studies in near-by sites in the extremely dry year of 2002 showed that juvenile ponderosa pine experienced 3-fold greater mortality when not in association with a nurse plant (Ecton 2002, unpublished data). Finally, we found no effect on juvenile ponderosa pines when their associated shrub was removed. In contrast, a nurse removal by Stulz (2004) at nearby sites it was found that juvenile pinyon pines required a shrub nurse in these stressful cinder soils. Future work in this area could include an

investigation into the physical protection that these shrubs offer to juvenile ponderosa pines from being run over by ORVs.

**Long-term impacts of unmanaged recreation** - The impacts of ORV use that we have described are likely to contribute to poor establishment success and high mortality of juvenile ponderosa pine. When environmental conditions are favorable, ponderosa recruitment is episodic, with large seed crops occurring every three years on average and germination events at most twice per decade (Bailey and Covington 2002). Given other considerations such as increased fire intervals and grazing impacts, decades have passed with no ponderosa recruitment. Our data argue that ORVs also may limit recruitment by removing above- and belowground refugia for germination. By reducing the litter cover, ORVs could increase the chances for seed predation (Nilson and Hjältén 2003), and decrease the soil moisture necessary for seedling emergence (Williams et al. 1990). Reductions in litter depth may also contribute to poor seedling performance indirectly by reducing EM fungal diversity as has been observed in tropical systems (Brearley et al. 2003). Reductions in EM inoculum potential could result in low levels of colonization by these important pine mutualists. World-wide, 97% of land plants are mycorrhizal obligates (Smith and Read 1997). This study adds support to the current base of literature that acknowledges that soil compaction reduces mycorrhizal colonization, but is the first to show that ORVs can reduce mycorrhizal colonization and reduce fungal community richness. The life span of fungal inoculum in the soil is widely variable (Haskins and Gehring 2005). Without a host for the mycorrhizal fungi to colonize, these inoculum sources will die, further suppressing the ability of these plants to re-colonize areas disturbed by recreation.

According to the model proposed by Bailey and Covington (2002), an understory free of grasses and deep litter that inhibit seedlings is vital to the regeneration of ponderosa pine. It could be argued that ORVs promote these clearings and provide opportunities for ponderosa pine regeneration. Generally, fire is the natural process that facilitates juvenile establishment and restoration treatments use manual thinning and low-intensity burns to achieve the reduction of grasses that can out-compete ponderosa seedlings, while maintaining large, old-growth trees as a seed source. Our data suggest that ORVs do not mimic the results of prescribed fire for two reasons. First, while ORVs reduce ground cover, a potential inhibitor to recruitment, they also change belowground factors that limit successful germination and establishment. Second, as shown by our stand density data, ORVs reduce the amount of juvenile trees necessary to maintain a self-sustaining population. This loss in juvenile trees will result in a future reduction of the trees necessary to provide an adequate seed source to maintain the stand. Williams and Johnson (1990) show that an age distribution that lacks juveniles, similar to the ponderosa pine distribution in the ORV area, represents a population that is in decline, and not self-sustaining. With a population that cannot replace itself, vegetative interspaces are likely to grow, which reduces favorable habitat for mycorrhizal inoculum spores to thrive, further exacerbating the ORV impacts (Marshall 2000).

Unmanaged recreation has been identified by the Forest Service Chief Dale Bosworth as one of the Four Threats facing the future health of the national forests and grasslands. In his statement, he acknowledged that it takes a small amount of ORV use to make a lasting change on the landscape and it is these lasting impacts that limit other's ability to enjoy these public lands ([www.fs.fed.us](http://www.fs.fed.us)). Our data suggests that while ORV

use has been detrimental to the area, mitigation may not be difficult. Our study shows that pine litter is crucial to germinating seeds. By restricting ORVs from areas of high impact natural litter islands would be able form at the base of trees, creating habitat for seedlings. In addition to helping seedlings, this additional litter would have the potential to increase mycorrhizal colonization of juvenile and mature trees, which would feed back to increase the inoculum available to germinating seeds. By reducing or removing ORVs from high-impact areas, natural regeneration could begin to create the young age class that is missing in ORV impacted areas.

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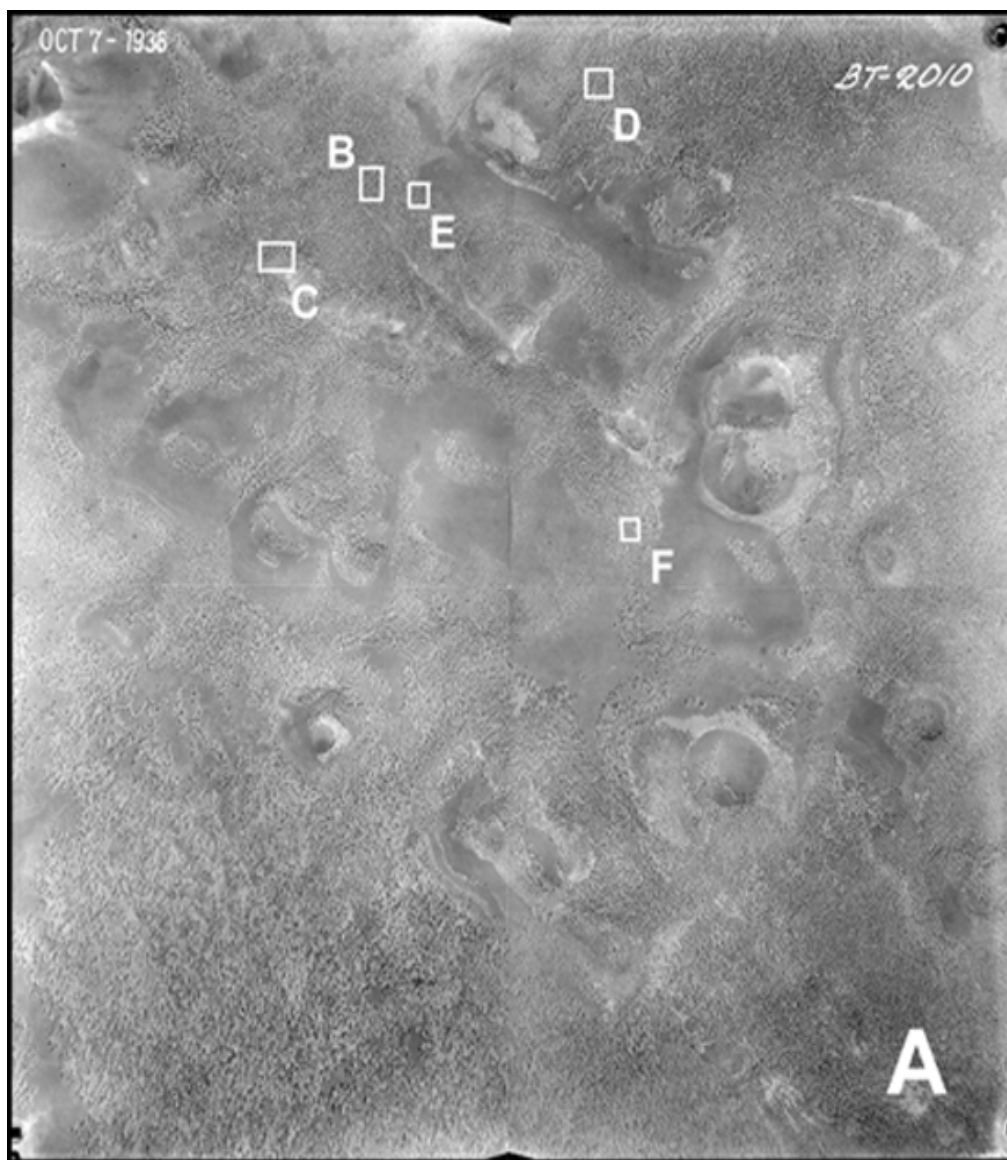
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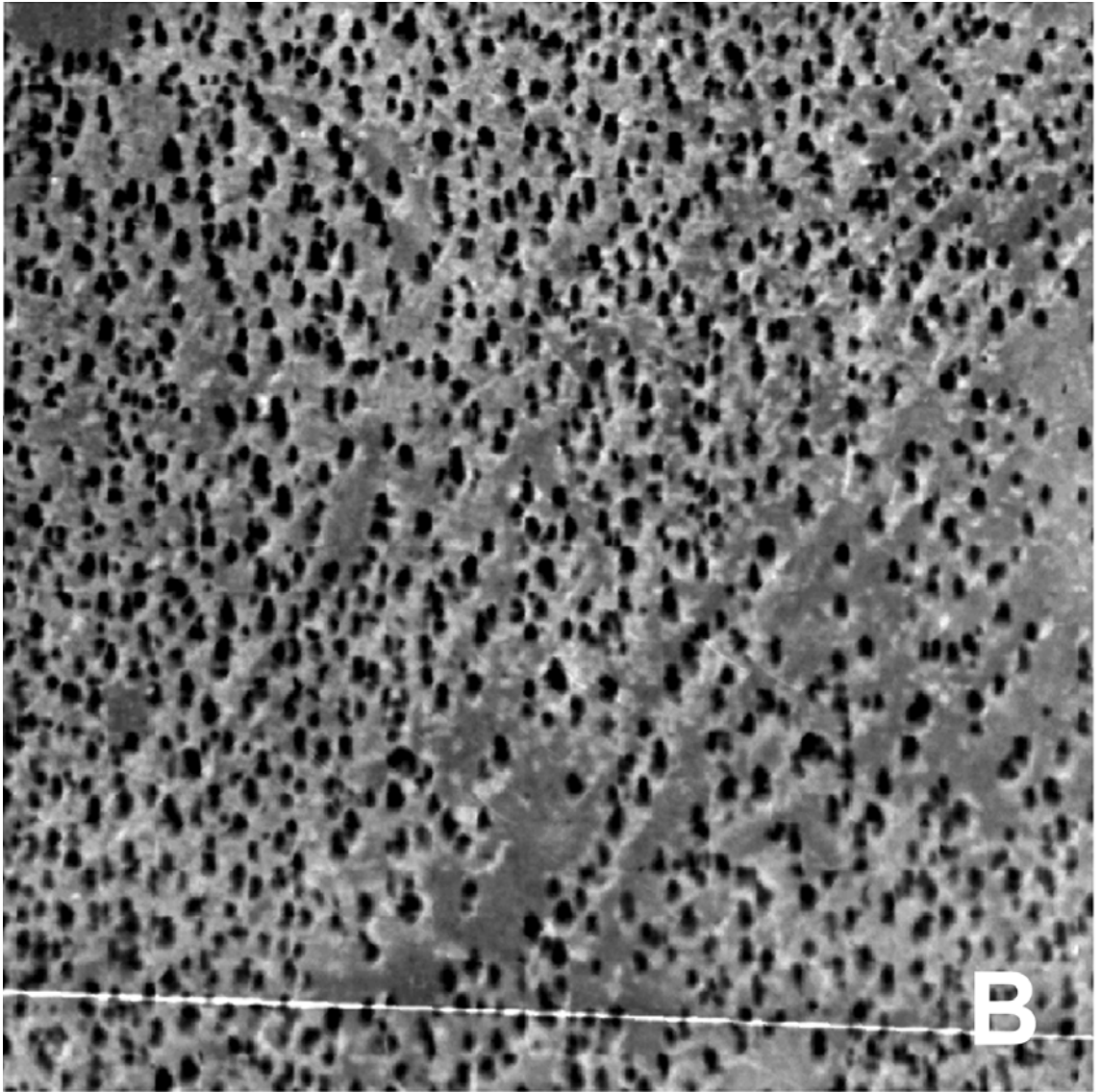
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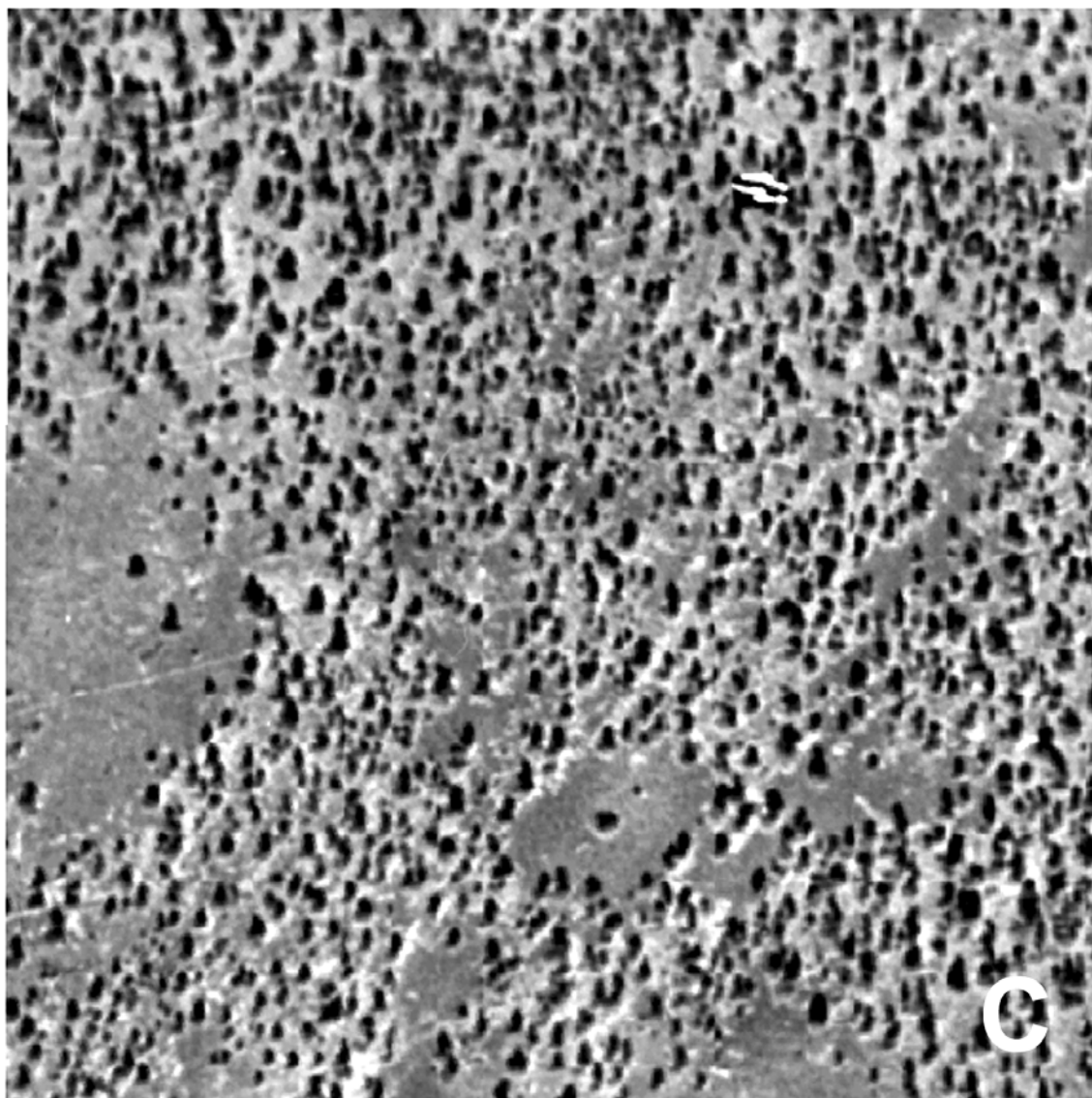
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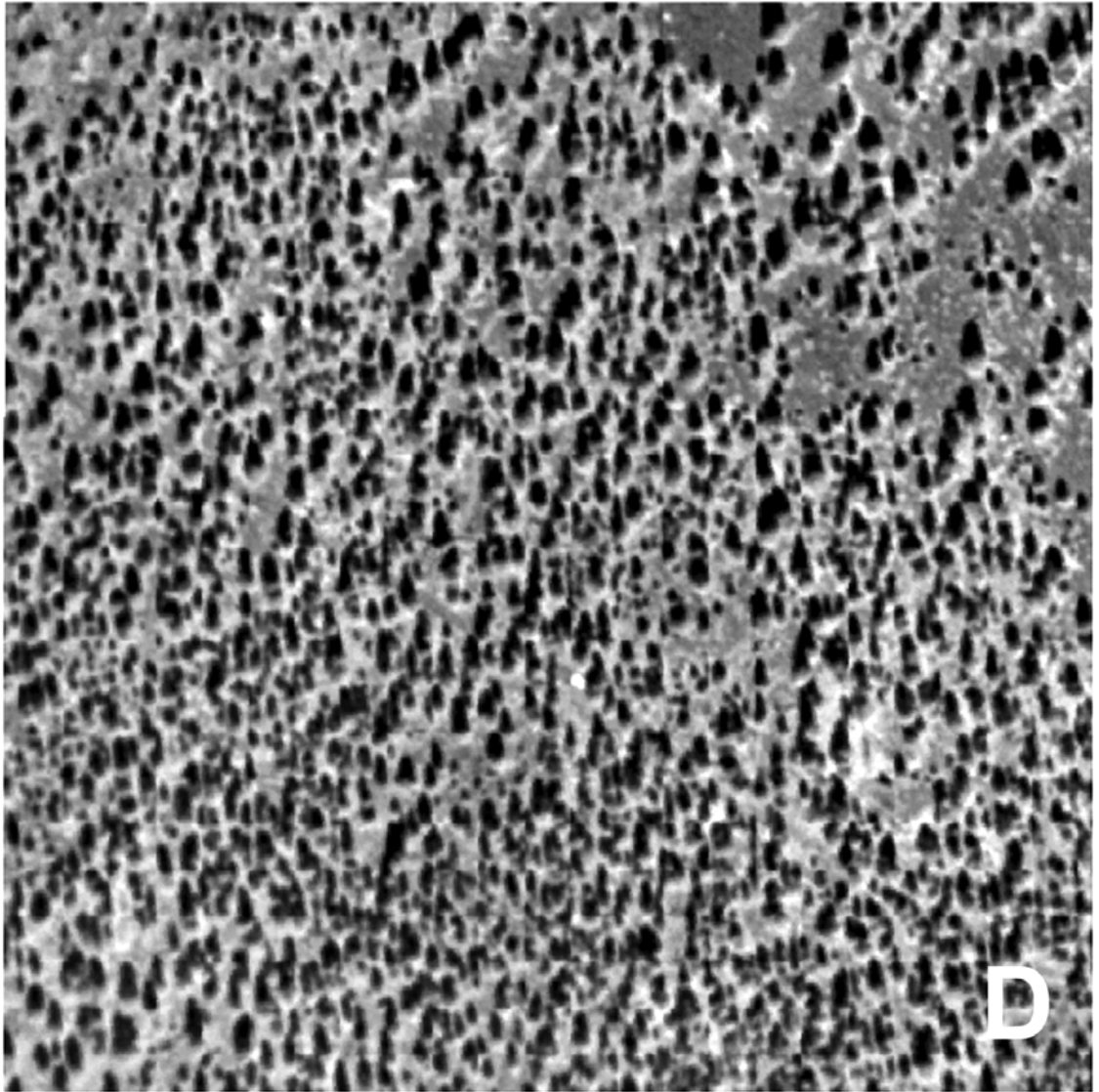
**Figure 1** – The 1936 aerial photograph that encompasses all of our field sites. Sunset Crater is in the upper left corner of the photograph. Boxes B-F represent the approximate locations of our field sites, and are not to scale. **B-D**) are zoomed-in photographs of our 3 control sites. **E-F**) are zoomed-in photographs of our 3 ORV sites.



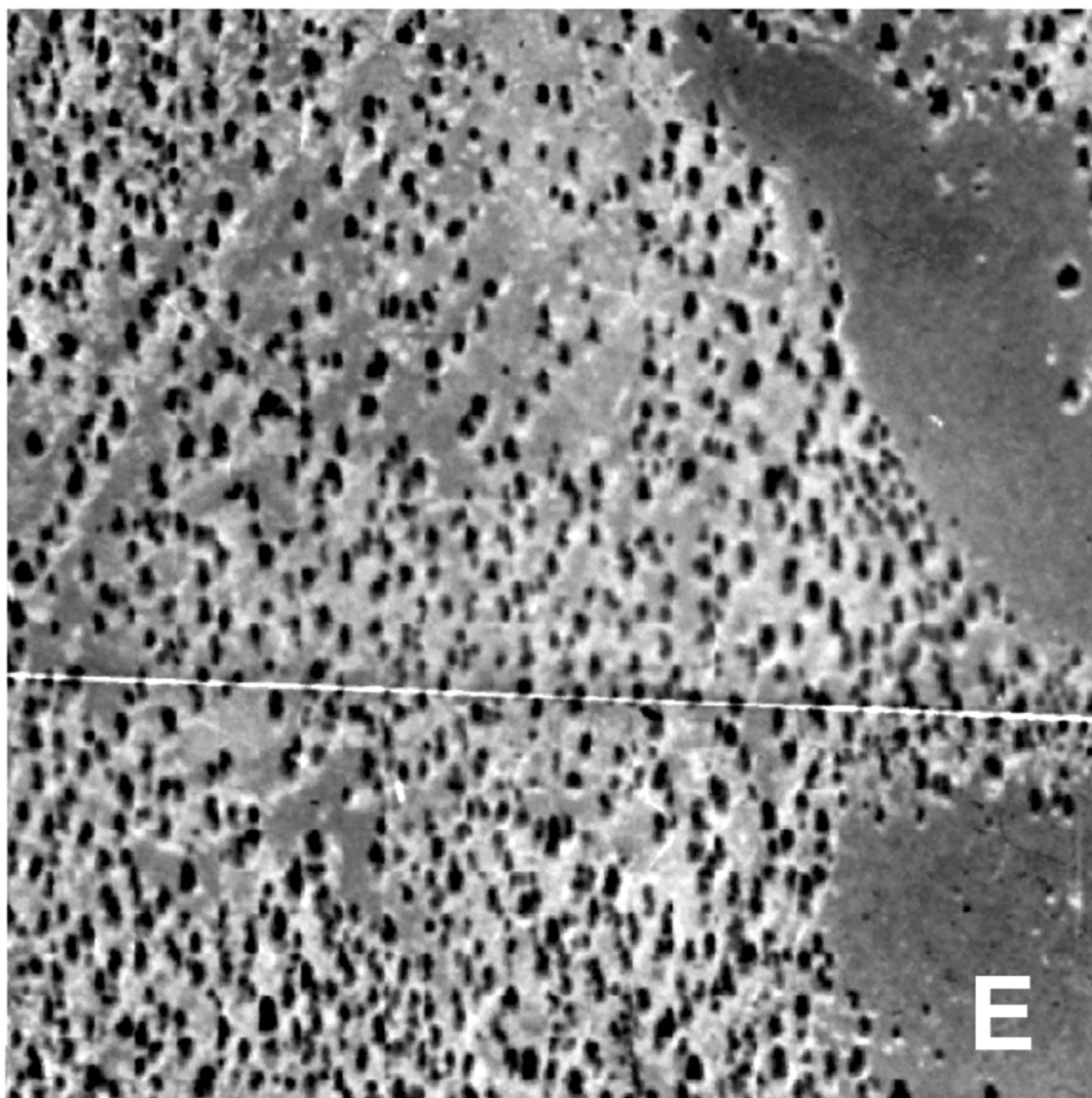
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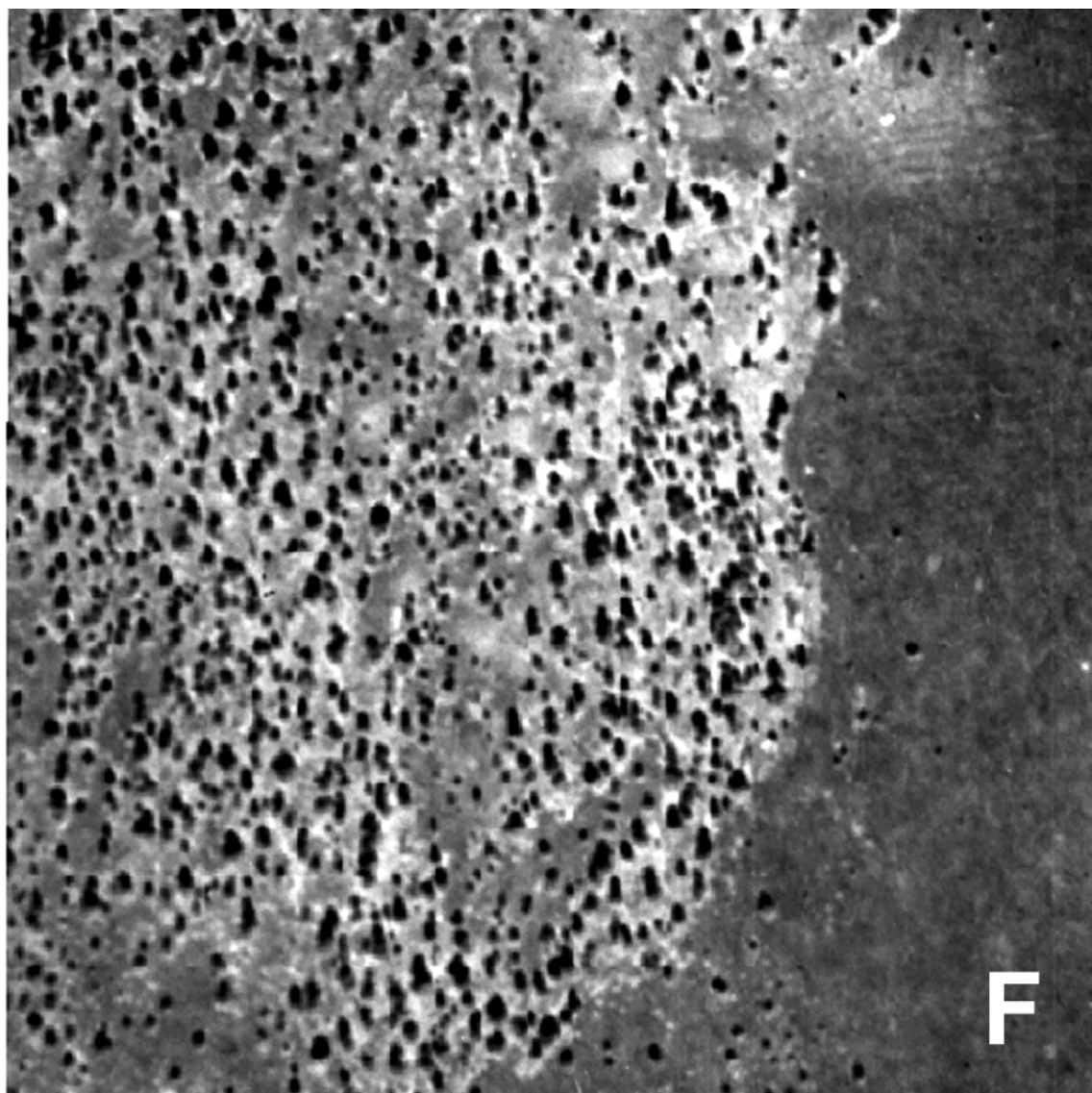


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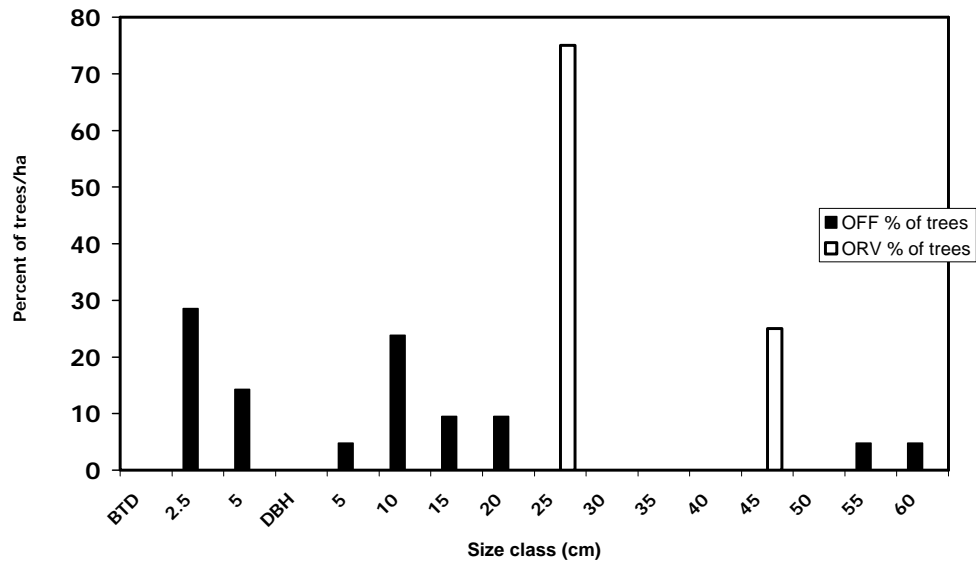


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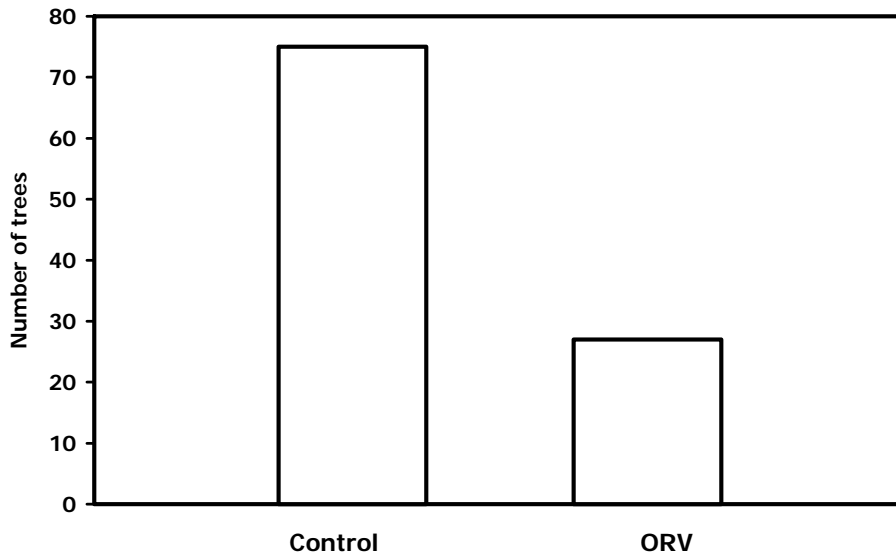




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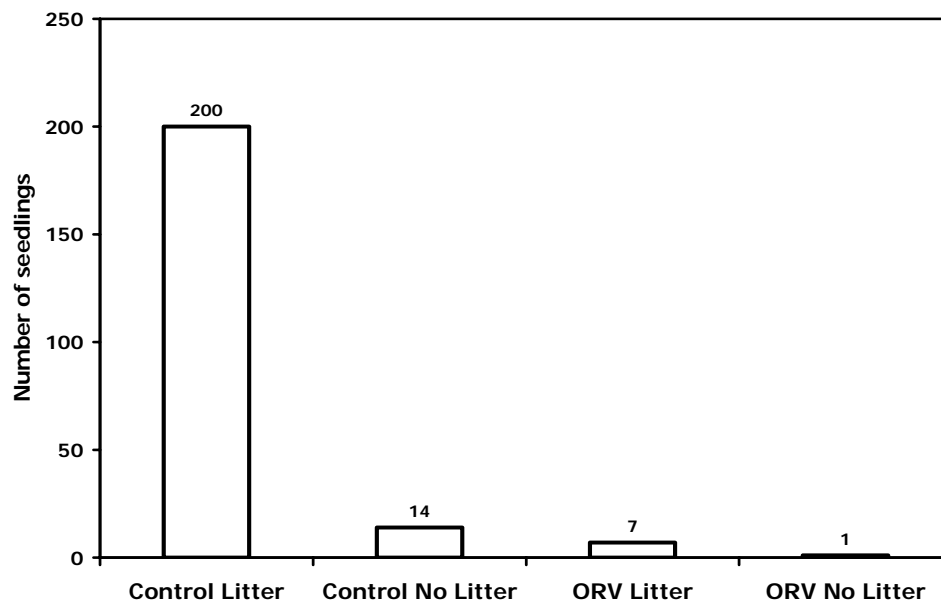


**Figure 2** – Counts of the percent of trees per hectare and their size class found in one pair of our study sites.



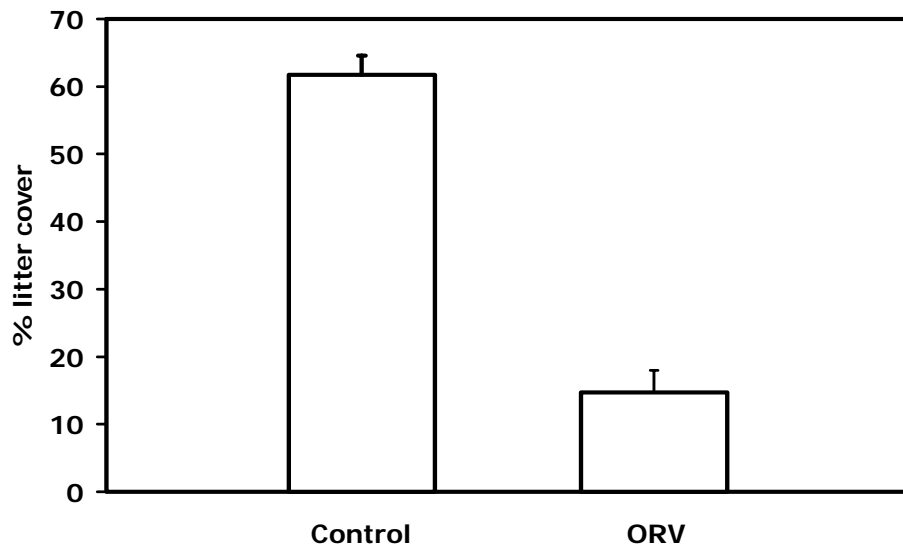
**Figure 3** – Counts of juvenile trees (<20 yrs old) in control and ORV sites. There was 3-fold greater juvenile trees in the control sites ( $X^2 = 22.5$ ,  $p < 0.001$ ).



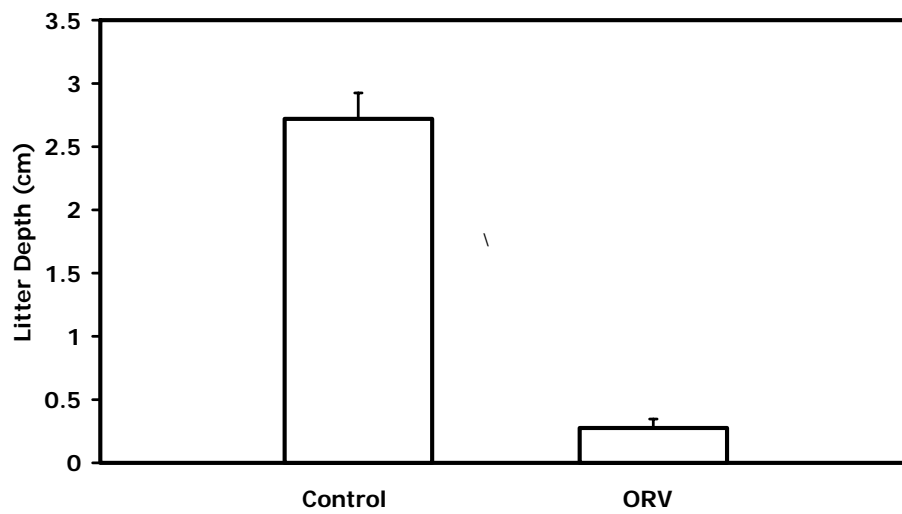


**Figure 4** – Counts of first year germinants with notation of the habitat condition in which they were found. First-year recruitment of seedlings is greatest in control sites and in association with litter ( $X^2=498.7$ ,  $p<0.001$ ).

**A**



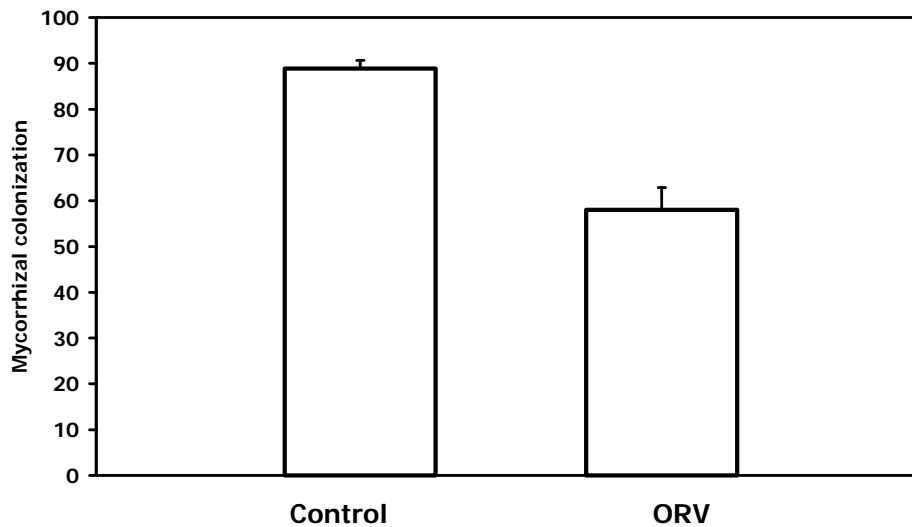
**B**



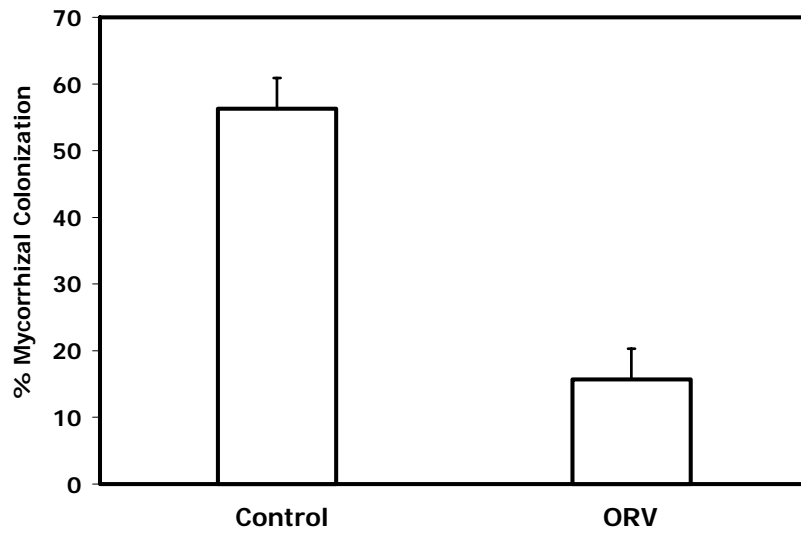
**Figure 5** – Measurement of average per-tree litter cover and depth in ORV and control sites. **A)** Average litter cover per tree was 3-fold greater in the control sites ( $F=33.36$ ,  $p<0.001$ ). **B)** Average litter depth per tree was 10-fold greater in the control sites ( $F=22.81$ ,  $p<0.001$ ).

	Control	ORV
Water Infiltration Time (seconds) ***	36.2 (5.2)	359.5 (102.7)
Bulk Density (Mg/m <sup>3</sup> )**	0.635 (0.0351)	0.968 (0.0606)
Live Root Biomass (g/m <sup>2</sup> )**	95.12 (16.50)	23.82 (7.08)
Soil Moisture (g water/g soil)***	0.0702 (0.0039)	0.0493 (0.0039)

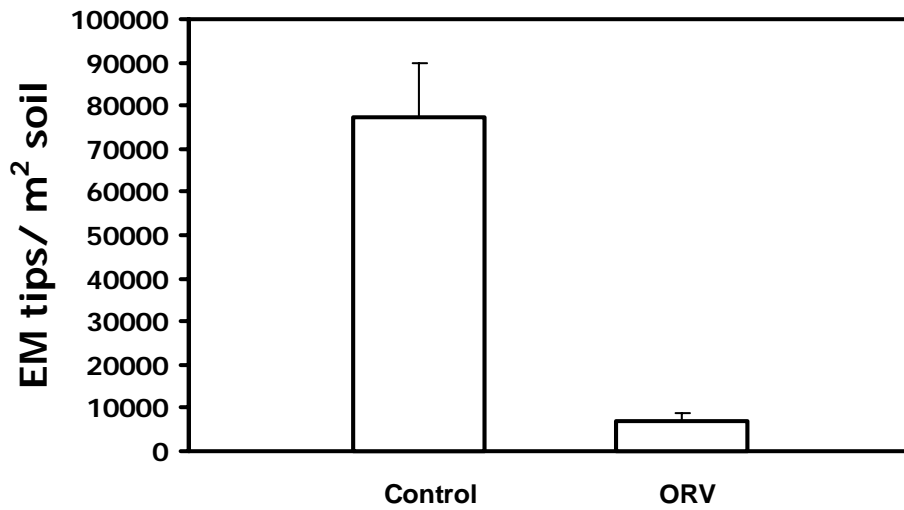
**Table 1** - Soil measurements taken at the drip line of mature trees. Averages with standard errors in parentheses. \*\* Denotes significance at  $p < 0.001$ , \*\*\*  $p < 0.0001$ .



**Figure 6** – Measurement of 2003 mycorrhizal colonization of ponderosa fine roots. Colonization of fine roots was 30% greater in the control sites ( $F=8.75$ ,  $p=0.016$ ).



**Figure 7** – Measurement of 2004 mycorrhizal colonization of ponderosa pine roots. Colonization was almost 4-fold greater in the control sites. ( $F=74.90$   $p<0.001$ ).

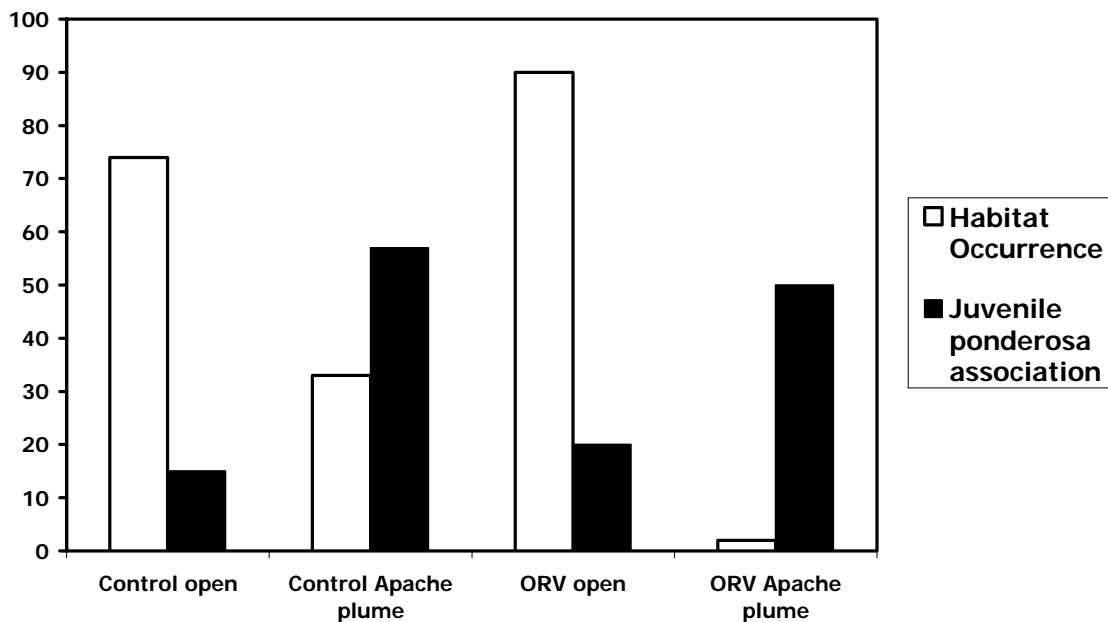


**Figure 8** – Calculation of mycorrhizal abundance in EM tips/m<sup>2</sup> soil. Abundance was 11-fold greater in the control area.  $F=15.24$   $p<0.001$ .

	Control	ORV
Colonization***	58.28% (4.07)	30.17% (4.46)
Height (cm)	9.14 (0.35)	8.52 (0.25)
Root:Shoot	1.49 (0.35)	1.14 (0.057)
Root Length	254 (16.51)	265 (19.14)

**Table 2**

Measurements taken on ponderosa pine seedlings in the mycorrhizal inoculum bioassay. Means with standard errors in parentheses. \*\*\*Denotes significance at  $p < 0.001$ .



**Figure 9** – Measurements of available habitat and juvenile ponderosa association.

Apache plume represented 33% of available habitat for juvenile ponderosa pine in the Control area but only 2% of available habitat in the ORV area ( $\chi^2 = 27.5$ ,  $p < 0.001$ ).

Regardless of availability, juvenile ponderosa were associated with Apache plume about 50% of the time ( $\chi^2 = 39.094$ ,  $p < 0.001$ ).

	Shrub Intact	Shrub Removed
Pre-treatment water potential (MPa)	1.54 (0.085)	1.61 (0.114)
Post-treatment water potential (MPa)	2.00 (0.098)	2.00 (0.098)
% Mortality	0%	0.03%

**Table 3** – Measurements taken on juvenile ponderosa pine in the shrub removal experiment. Means with standard error in parentheses. All data are non-significant.