Soil Property Effects on Willamette Daisy (*Erigeron decumbens*) within William L. Finley National Wildlife Refuge

by

Emily S. Day

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Natural Resources (Honors Associate)

Presented May 11, 2015
Commencement June 2015
Plant species diversity plays an important role in maintaining ecosystem function and the services that ecosystems provide. Diversity is threatened by habitat loss, invasive species, and global climate change. Willamette Daisy (*Erigeron decumbens*), an endangered species, has been reintroduced at two different sites (Field 29 and 8N) within William L. Finley Wildlife refuge, but it grows better at one site (Field 29) than the other (Field 8N). The purpose of the study was to determine if the differences in growth at the two sites could be caused by differences in soil properties, and determine soil conditions conducive for growth. We grew *E. decumbens* in soil collected from each field in a greenhouse with sterile and unsterilized treatments and performed a soil analysis on samples from each field. Soil sterilization had a significantly negative effect on plant survivorship, suggesting soil biota improves establishment of *E. decumbens*. Field 29 had significantly higher TN, TC, and pH than Field 8N, which may be contributing to the observed differences. Results from this study suggest that these soil properties could benefit *E. decumbens* growth in the wild, and information on soil conditions could improve reintroduction site selection and recovery of this endangered species.

Keywords: Willamette Daisy, *Erigeron decumbens*, soil properties effects, endangered species recovery, reintroduction

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Erin Gray, Committee Member, representing Institute for Applied Ecology

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

______________________________
Emily S. Day, Author
I would like to extend my gratitude first and foremost to Thomas Kaye for mentoring me over the course of my honor thesis. I want to sincerely thank him for the opportunity to pursue this project and all his positive support through analysis, interpretation and the writing process. I want to additionally thank my committee member Jeff Hatten for his tremendous support in soil collection, research, analysis and revision for this project. His knowledge and positive words of encouragement through this process have been invaluable. I would also like to thank my committee member, Erin Gray, for introducing me to this project in the first place and all of her enthusiastic support along the way. I would like to extend me appreciation to Denise Gile-Johnson, who taught me so much about the Willamette Daisy. I would also like to thank Kris Richardson for her continual reassurance and assistance with my soil analysis, Adrian Gallo for his help in collecting soil samples in the field and the rest of the forest soil lab group for all of their support and suggestions. I want to sincerely thank my parents for their continual faith in me and their help in the planting and soil treatment preparation as well as my dear friend Meghan Chuites. Most of all, I want to thank my husband and editor, Oliver Day, for encouraging me to apply to the UHC, for giving me confidence in myself, and for his unwavering support in every way.
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INTRODUCTION

Plant biodiversity plays an important role in maintaining ecosystem function and the services that ecosystems provide (Cleland, 2012; Loreau & Inchausti, 2002). Overwhelming evidence of this relationship has led to an emerging global consensus that conserving high species diversity is essential in maintaining ecosystem function (Chapin, 2004). Plant species diversity is increasingly being threatened by land conversion, habitat fragmentation, invasive species and global climate change (Giam et al., 2010; Tilman & Lehman, 2001) with currently 886 plant species Federally listed as threatened or endangered under the Endangered Species Act (USFWS, 2015). In order to protect and enhance listed species, active conservation and reintroduction is often required for their recovery. Understanding the conditions conducive to their growth and establishment is essential for the rehabilitation of threatened and endangered plant species.

Willamette daisy (*Erigeron decumbens*) is an endangered perennial herb that is endemic to Oregon’s Willamette Valley. Its upland prairie habitat is increasingly being threatened by habitat loss and fragmentation due to urban and agricultural development (USFWS, 2015). Current populations are unable to recover on their own due to their small size, isolation, competition with other species, changes to natural disturbance regime and challenges associated with maintaining genetic diversity (Thorpe & Kaye, 2011). Management practices can also have a negative impact on *E. decumbens* if applied during the wrong season or applied too often. To better understand ways to assist its recovery, the Institute for Applied Ecology (IAE) has conducted research using various management treatments on populations they have introduced throughout the Willamette Valley. Two of these research sites are located within William L. Finley National Wildlife Refuge, in the Willamette Valley in Benton County, Oregon. At these sites, various treatments are being applied to manage the surrounding prairie community that will maintain and enhance *E. decumbens*. These treatments include mowing, prescribed fire, grass-specific herbicide, glyphosate, and carbon addition. Specific threats to be managed include encroachment of woody species and invasion by non-native grasses and forbs (Giles-Johnson, 2012).
Despite management treatments, initial results showed little effect on the survival of the *E. decumbens* at either site. However, one reintroduction site (Field 29) was observed to support larger, healthier looking individuals than another (Field 8N) even though both sites are within approximately 1.35 miles of each other and out-planted propagules came from the same seed source. This indicated that the *E. decumbens* daisy is able to withstand the management treatments applied without causing harm to its current populations. The treatments were also successful in decreasing invasive species and controlling the surrounding plant communities (Giles-Johnson, 2012). While these results have been important in understanding useful techniques for managing habitat in which *E. decumbens* populations have been established, little is known about what may be causing the observed difference in size of individuals in Field 29 when compared to Field 8N. Various abiotic and biotic factors may be affecting plant size, such as differences in topography, soil properties and the surrounding plant community (Huston & McBride, 2002).

Our study focused on the potential influence that physical, chemical and biological soil properties have on the growth of *E. decumbens* at each site as the reason for the observed difference. Soil properties can have a considerable effect on rare plant growth and productivity (Fiedler, 1991). In order to evaluate if the observed difference was influenced by soil properties, we grew *E. decumbens* in sterilized and unsterilized soil collected from each site to compare the survivorship, elliptical crown area, total number of leaves, vigor and the carbon (TC), nitrogen (TN) and ratio of carbon and nitrogen (C:N) of the soils. Results from a pilot study (Thomas Kaye, 2006) showed *E. decumbens* performed better in unsterilized soil rather than sterilized soil, suggesting that there are potential biological components that influence their growth. We also compared differences in soil core samples taken from Field 29 and 8N to compare their particle size distribution, organic matter content, soil pH, TC, TN and C:N.

We hypothesized that healthier and more productive plants would grow in the unsterilized soil from Field 29 after growing 19 weeks in the greenhouse. These plants were expected to have a higher survival rate as well as greater size and vigor than the plants in the unsterilized soil from Field 8N because plants at Field 29 were observed to
be larger. Plants grown in sterilized soil from Field 29 sterilized were predicted to perform poorly compared to those grown on live soil because of an absence of potential beneficial soil biota that may be present in the soil while the sterilized treatment of Field 8N was expected to perform better due to the elimination of soil pathogens that could be contributing to less productive individuals observed in the Field 8N. For the soil core samples, we hypothesized that soil from Field 29 would have significantly different physical and chemical proprieties to Field 8N including particle size distribution, organic matter, pH, TC, TN and C:N. If soil properties were found to be significantly different between fields than this would indicate that these soil factors are important to the success of *E. decumbens* and are conducive to its growth.

By understanding the effects of physical, chemical and biological soil properties on *E. decumbens*, results could contribute to improving management and reintroduction techniques to aid in species recovery. Understanding what types of soils in which the daisy can successfully grow could be beneficial in determining new site locations for reintroduction. If there were no differences found between soil types and growth observed in treatments, this would indicate that the difference in *E. decumbens* growth between Field 29 and Field 8N could be due to other abiotic or biotic site-specific factors such as associated vegetation at the site or topography. This information will also be useful to the restoration of this species by pointing to new directions for continued research.

**METHODS**

**Study Area**

Soil samples and soils for greenhouse treatments were collected in March 2014. Research for the greenhouse study and soil analysis was conducted from March to August 2014. Soil was collect from upland prairie habitat within William L. Finely Wildlife National Refuge (map in Appendix) from experimental Fields 29 and 8N located in western Oregon within the Willamette Valley. Field 29 can be found at approximately 44° 24’ 43 N, 123° 19’ 56 W just south Woodpecker Loop and Field 8N at approximately
44° 23’ 47 N, 123° 18’ 50 W at the southeast base of Pigeon Butte (Figure 1). These fields are within the Corvallis West Recovery Zone, which are part of a series of zones designated in the Recovery Plan for Prairie Species of Western Oregon and Southwestern Washington (USFWS, 2010; map in Appendix). Field 29 and 8N were chosen for reintroduction of the *E. decumbens* based on their ability to provide suitable upland prairie habitat, having minimal exotic species cover and easy access. Treatment plots in both fields consist of 3 blocks, each containing six 6 meter by 6 meter treatment plots with a designated management treatment including mowing, prescribed fire, grass-specific herbicide, glyphosate, and carbon addition. Our study was based on the *E. decumbens* that were out-planted within the treatment plots of these fields in 2007, 2008 and 2011.

Figure 1. Aerial map of William L. Finely Wildlife National Refuge indicating the location of field 29 (in blue) and 8N (in red), located approximately 1.35 miles apart (Google Earth, 2015.)

The soils of Field 29 were mapped as the Santiam soil series a fine, mixed, superactive, mesic Aquultic Haploxeralf (NRCS, 2015). This soil series is very deep, moderately well-drained silty loam and formed in silty glaciolacustrine deposits over clayey old alluvium located on high terraces or foot slopes. The soils of Field 8N were
mapped as the Dixonville-Gellatly complex which are fine, mixed, superactive, mesic Pachic Argixerolls and Pachic Ultic Argixerolls. These soil series’ consists of very deep and well drained silty clay loam formed in clayey colluvium and residuum from basalt located on hills. Both sites have an average annual precipitation of 45 to 50 inches and an average temperature of 52 degrees F (NRCS, 2015).

Greenhouse Study

Seeds and Cold Stratification

*E. decumbens* seeds used for the greenhouse study came from the USDA Plant Materials Center, Corvallis, Oregon seeds and the Corvallis West source Allen and Allen to maximize genetic diversity. We used the IAE protocol for germinating the seeds and propagating the plants (Kaye & Kuykendall, 2001). To ensure a representative population size based on previously observed low germination and seedling establishment rates (Kaye & Kuykendall, 2001), a total of 2,500 seeds were placed in cold stratification. Seeds were divided into 10 germination boxes with 250 seeds per box. Germination boxes were sterilized with bleach water and lined with one layer of felt and one layer of blotter paper. *E. decumbens* seeds were then placed on the paper spaced 0.5 cm apart and deionized water was added to the felt and blotter paper to keep the seeds moist. The seed trays were then stratified at a temperature of 6 degrees C for 18 weeks in darkness in a germination chamber at the OSU Seed Lab. During that time, seeds were checked 1-2 times weekly for moisture and fungal growth. Moist conditions were maintained by misting deionized water over the seeds as needed. During week 10 of stratification, a solution of 3% hydrogen peroxide spray was used to kill fungal growth in 2 trays. The spray successfully killed the fungus without damaging the seeds. After 18 weeks, the germination boxes were taken out of cold stratification and placed in the OSU West greenhouse. They were put under artificial sunlight under Sun System 3 – 400 HPS light bulbs for 14 hours and kept at 21 degrees C during the day and 13 degrees C at night in an OSU West greenhouse for one week to germinate. The germination rate was approximately 50 percent.
**Treatments**

The soil treatments were (1) field-collected soil from Field 29, (2) sterilized field-collected soil from Field 29, (3) field-collected soil from Field 8N, (4) sterilized field-collected soil from Field 8N, (5) a control treatment of potting soil, and (6) a control treatment of sterilized potting soil. Soil in treatments 1 through 4 contained a 3:1 ratio of 25% of the soil from their assigned site and 75% SB40 Sunshine Growers potting soil in order to allow for adequate aeration and soil structure for root growth and to avoid soil compaction within the containers (Montagu et. al., 2001). For the sterilized soil treatments 2, 4 and 6, both the field soil and the potting soil that made up the 3:1 ratio mixes were sterilized to kill all microbial activity to compare plant growth response to live, unsterilized soil. This procedure involved placing the soils in a steam-pressurized autoclave held at a temperature of 132 degrees C for 2 hours. Each 3:1 ratio treatment was thoroughly homogenized before filling individual pots.

**Planting**

Seedlings were planted in individual Ray Leach Cone-tainers™ that have a 3.8 cm diameter, a depth of 14 cm and a that were filled with the 6 different soil treatments. To ensure sterility of Field 29, Field 8N and potting soil sterile treatments, 5 of the germination boxes were designated for sterilized treatments and 5 were designated for unsterilized treatments. Tweezers used for planting seedlings were also sterilized in bleach water when planting each treatments type, both sterile and unsterile, to avoid cross contamination of soil sources. Each treatment was grouped into trays that held 98 pots for a total of 12 trays and placed in sterilized containers to avoid cross contamination and to allow us to water the seedlings in a group from below. Seedlings were given 14 hours of artificial sunlight under Sun System 3 – 400 HPS light bulbs and kept at 21 degrees C during the day and 13 degrees C at night in an OSU West greenhouse. Containers were moved at random between two greenhouse benches every three days in order to account for variation in greenhouse conditions such as light and air circulation. Plants were watered as needed to keep soil treatments from drying out and allowed to grow for 6 weeks. Plants were then randomized across treatments by filling one tray at a time and
leaving an additional space between each pot within the trays. Trays were placed back into the watering tubs and allowed to grow for 13 more weeks under the same light and temperature until plants appeared to be on the. Plants were watered once per week and tubs were moved at random between two greenhouse benches every three days. Data was then collected after plants had been growing a total of 19 weeks on 1) the number of planted seedlings that survived, 2) elliptical crown area (mm^2) measured by the widest and perpendicular diameters of each plant multiplied by π, 3) number of leaves, and 4) proportion of living leaves to dead leaves. The proportion of living leaves was used as measure of plant vigor.

**Soil Sampling**

Ten soil core samples (0-15cm depth) were taken at random around the edge of each field site using a push probe soil sampler. The first sample was taken at the origin, T-post that designates the 3 treatment blocks within each field. Samples of the 6 greenhouse treatments were also taken. All samples were dried at 40 degrees C for 48 hours. They were then sieved through a 2 mm sieve and homogenized. Samples for carbon and nitrogen were ground to a fine powder using a mortar and pestle and then homogenized.

For the core samples, deionized water was mixed with each soil sample using the 2:1 ratio method (Thomas, 1996) and used to measure pH using a Metler Toledo FiveEasy™ pH meter under the standard operating procedure. A two point (4.01 and 7.0) calibration was used and was checked every 10th sample using both buffer solutions. Percent organic matter content for each sample was measured and calculated using a standard loss on ignition (Nelson and Sommers, 1996) produced by heating 2-gram samples in a muffled furnace for 2 hours at 550 degrees C and weighing them after they had been cooled. Particle Size Analysis (PSA) was determined using laser diffraction on 0.5 gram samples. Four of the core samples from each field were chosen at random and used for the PSA. Preparation for PSA required removal of the organic matter by adding 30% hydrogen peroxide and heating in a hot water bath at 70 degrees C until activity ceased. Samples were then run through a Mastersizer 2000 grain size analyzer using laser
diffraction to obtain the relative distribution of particle sizes within a range of size classes based on the given volume of the sample. Specific surface area (m$^2$/g) of the field cores samples were also determined using the Mastersizer, calculated by dividing the total area of the particles by the total weight under the assumption the particles are non-porous and spherical in shape.

A Flash 1112 dry combustion elemental analyzer was used to determine the total percent carbon (TC) and nitrogen (TN) content and carbon to nitrogen mol ratio (C:N) of all soil samples including greenhouse treatments. Based on the organic matter content, each sample was weighed in a tin capsule and flash combusted at 900 to 1000 degrees Celsius. The accuracy of the calibration curve was judged using the R$^2$ of a linear fit of standard calibrates and an R$^2$ 0.99 was achieved for all TC and TN runs. Biological elements of the soil were not tested due to limited resources.

**Statistical Analysis**

**Greenhouse Study**

We used logistic regression to determine the survivorship of seedlings between treatment types. This method compared the odds ratio of success and failure of the main explanatory variables, soil source and sterilization, to determine if the proportion of plants that remained alive after 19 weeks of growth differed between groups. Logistic regression was chosen because the individual plants were the samples and their survival or death were Poisson distributed. Seedlings that did not survive during the 19-week growth period were counted as dead. Confidence intervals (95%) were derived using the normal approximation of the binomial distribution.

Analysis of variance (ANOVA) was used to test for effects of 1) the source of the soil and 2) whether or not the soil was sterilized. Elliptical crown area (mm$^2$), total leaf number and proportion of live leaves (vigor) were compared between treatments. The threshold for statistical significance (alpha) was a 0.05 level of probability. A General Linear Model was also used due to the variable number of individual plants across treatment groups. Both crown area and leaf number were log transformed in order to follow a normal distribution and meet the assumptions of analysis of variance. Figures
were created from back-transformed data of the upper and lower limit of standard error.

**Soil Analysis**

A series of t-test procedures was run on the soil core samples to determine if there were significant differences \( p < 0.05 \) between the means of Field 29 and Field 8N soil properties. These properties included total carbon, total nitrogen, carbon and nitrogen ratio (molar), pH, particle size distribution \((d_{10}, d_{50}, d_{90})\) and organic matter content (loi; loss on ignition). Total carbon (TC), total nitrogen (TN), carbon and nitrogen ratio (C:N; molar) were measured in soil treatments in the greenhouse study procedure to compare their content between treatment types using ANOVA (t-test). A Tukey HSD test was used to determine the treatment groups differed from one another and categorized them using means with homogeneous subsets for TC, TN and C:N. All assumptions of normality were met under these analyses.

**RESULTS**

**Greenhouse Study**

**Survivorship**

Seedling survival was higher for unsterilized soil than sterilized treatments regardless of field soil location \( p < 0.0001; \) Figure 2). The odds of plants surviving on unsterilized treatments were about 17 times that of plants grown in sterilized treatments \( \text{odds ratio} = 0.06 \). For Field 29 soils, the odds of plants surviving on unsterilized soil were about 20 times that of plants grown in sterilized soil \( \text{odds ratio} = 0.05 \). For Field 8N soils, the odds of plants surviving on unsterilized soil were about 25 times that of plants grown in sterilized soil \( \text{odds ratio} = 0.04 \). For 100% potting soil, the odds of plant surviving on unsterilized soil were 13 times that of plants grown in sterilized soil \( \text{odds ratio} = 0.08 \). Mean survivorship ranged from 79% to 85% in unsterilized treatments and 16% to 28% in sterilized treatments. Plant mortality occurred primarily during the
seedling establishment phase. Once seedlings were established, plants grew at similar rates in all treatments until 19 weeks of growth was reached.

![Figure 2](image-url)

Figure 2. Survivorship of the six treatment groups showing low survival of seedlings in sterilized treatment and high survival in unsterilized treatments regardless of soil source.

**Crown Area**

The effect of soil sterilization depended on the source of the soil on which the plants were grown (i.e., there was a significant interaction between soil sterilization and soil source, p = 0.015), with sterilization resulting in larger crown area in soils from Field 29, but smaller crown area in soils from Field 8N and potting soil only. Plants grown in unsterilized potting soil had the largest crown area (median = 26,017 mm$^2$) and were significantly larger than plants grown in unsterilized soil from Field 29 (p = 0.015; median = 19,464 mm$^2$), but were not different in crown area than plants grown in the Field 8N unsterilized treatment (p > 0.05; median = 24,165 mm$^2$). Plants grown in unsterilized soil from Field 29 had the smallest crown area, but in sterilized from Field 29, crown area increased significantly (p < 0.05; unsterile median = 19,464 mm$^2$; sterile median = 21,547 mm$^2$). Sterilization had an opposite effect with soils from Field 8N, significantly decreasing crown area when sterilized vs. unsterilized (p < 0.05; sterile median = 21,609 mm$^2$; unsterile median = 24,165 mm$^2$).
Plant Vigor

Soil source and sterilization treatments both had significant effects on the number of leaves produced per plant (p = 0.032 and p < 0.0001, respectively), and there was no significant interaction between these factors (p = 0.792). Total number of leaves per plant showed no difference across Field 29 and 8N treatments (median = 13 to 15), while potting soil tended to have a higher number of total leaves on average (median = 16). Soil sterilization significantly reduced leaf number from a median of 15.8 to 11.4.

The proportion of living leaves out of total number of leaves was used as a measure of plant vigor with higher percentages of living leaves representing healthier plants. Although there was no significant difference in vigor of plants grown in sterilized and unsterilized soil across treatments (p = 0.53), there was a significant interaction between sterilization and soil source (p < 0.01; Figure 4). The unsterilized potting soil treatment showed lower vigor when compared to Fields 29 and 8N (p < 0.0001; 70% living leaves). There was no significant difference in plant vigor between Field 29 (83% living leaves) and Field 8N (80% living leaves). For sterilized treatments, Field 29 had higher vigor (86% living leaves) than Field 8N (73% living leaves) and plants grown in potting soil (p < 0.0001; 78% living leaves). Plants grown in soil from Field 29 and

Figure. 3. Crown area comparison (mm$^2$) between treatment groups of sterilized and unsterilized Field 29, 8N and potting soil (PS).
grown in potting soil showed a slight increase in vigor when sterilized and Field 8N showed a decrease in plant vigor when sterilized.

![Live leaves (%) vs Soil Treatment](image)

Figure 4. Proportion of living leaves shown as a percentage between soil treatments as a measure of plant vigor.

**Soil Analysis**

**Field Core Samples**

Soils from Field 29 had a higher TN concentration than soils from Field 8N (p < 0.0001; Table 1). This was also true for TC (p < 0.0008). There was no significant difference between the C:N of the two fields (p > 0.05). For particle size distribution, Field 8N had a significantly larger d50 and d90 values (p < 0.04) relative to Field 29 indicating coarser texture (Table 1). Conversely, Field 8N had a significantly smaller d10, than Field 29, suggesting that is finer in texture. The behavior of soil particles is strongly dependent on their particle specific surface area and Field 8N had significantly higher specific surface (S.S. area) area (p = 0.043). The pH of Field 29 soils has a significantly higher pH (p <0.0001) than Field 8N. There was no difference found in the amount of organic matter (O.M.) between fields.
Table 1. Comparison of 9 soil properties from core samples taken from Field 29 and 8N. Numbers represent the average of each soil property with its associated standard error (±). P-values represent the results from a t-test between the two fields (N=10).

<table>
<thead>
<tr>
<th>Site</th>
<th>TC (g 100g⁻¹)</th>
<th>TN (g 100g⁻¹)</th>
<th>C:N (mol)</th>
<th>O.M. (g g⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field 29</td>
<td>2.45±0.07</td>
<td>0.20±0.01</td>
<td>14.2±0.21</td>
<td>0.102±0.002</td>
<td>5.08±0.04</td>
</tr>
<tr>
<td>Field 8N</td>
<td>2.08±0.06</td>
<td>0.17±0.01</td>
<td>14.6±0.30</td>
<td>0.097±0.01</td>
<td>4.82±0.03</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0008</td>
<td>0.0001</td>
<td>0.2916</td>
<td>0.4383</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>d10 (µm)</th>
<th>d50 (µm)</th>
<th>d90 (µm)</th>
<th>S.S. area m²/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field 29</td>
<td>2.0±0.05</td>
<td>8.817±0.27</td>
<td>114.6±13.43</td>
<td>1.22</td>
</tr>
<tr>
<td>Field 8N</td>
<td>1.77±0.02</td>
<td>10.36±0.29</td>
<td>163.8±12.95</td>
<td>1.33</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0033</td>
<td>0.0076</td>
<td>0.0387</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Greenhouse Soil Treatments

TC, TN and C:N were determined for the soil treatments used in the greenhouse study. Soil treatments of pure potting soil had significantly higher TN (Table 2) content than Field 29 and 8N regardless of sterile and unsterile treatment types (p < 0.0001). No significant difference in TN content was found between Field 29 and Field 8N (p > 0.05). The potting soil treatments had a significantly higher TC (Table 3) content than all other treatments (p < 0.0001). Sterile potting soil contained more TC than its unsterile treatment (p < 0.004). Field 8N treatments had more TC than Field 29 (p < 0.0001). The sterilized Field 29 treatments had significantly higher TC than its unsterilized treatment (p < 0.0001) while no difference was found in TC content between Field 8N treatments (p > 0.05). The C:N (Table 4) of both potting soil treatments was the highest of all treatments (p < 0.0001). When field 29 treatment was sterilize, its C:N significantly increased when compared to it unsterile treatment (p < 0.01). No difference was found between the Field 29 sterile treatment or the sterile and unsterile treatments of Field 8N.
Table 2. Comparison of TN between the six treatments types categorized by subsets indicating 2 homogeneous groups with a $p > 0.05$. Field 29 and 8N treatments consisted of 75% potting soil. Numbers represent the average of each treatment with its associated standard error (±).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TN (g100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Subset 1</strong></td>
</tr>
<tr>
<td>Field 29 unsterile</td>
<td>0.333±0.02</td>
</tr>
<tr>
<td>Field 29 sterile</td>
<td>0.400±0.02</td>
</tr>
<tr>
<td>Field 8N unsterile</td>
<td>0.370±0.02</td>
</tr>
<tr>
<td>Field 8N sterile</td>
<td>0.370±0.02</td>
</tr>
<tr>
<td>P.S. unsterile</td>
<td>0.820±0.02</td>
</tr>
<tr>
<td>P.S. sterile</td>
<td>0.783±0.02</td>
</tr>
<tr>
<td><strong>p -value</strong></td>
<td>0.097</td>
</tr>
</tbody>
</table>

Table 3. Comparison of TC between the six treatments types categorized by subsets indicating 5 homogeneous groups with a $p > 0.05$. Numbers represent the average of each treatment with its associated standard error (±).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC (g100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Subset 1</strong></td>
</tr>
<tr>
<td>Field 29 unsterile</td>
<td>10.18±0.18</td>
</tr>
<tr>
<td>Field 29 sterile</td>
<td>14.87±0.18</td>
</tr>
<tr>
<td>Field 8N unsterile</td>
<td>13.64±0.18</td>
</tr>
<tr>
<td>Field 8N sterile</td>
<td>13.43±0.18</td>
</tr>
<tr>
<td>P.S. unsterile</td>
<td>38.54±0.18</td>
</tr>
<tr>
<td>P.S. sterile</td>
<td>39.70±0.18</td>
</tr>
<tr>
<td><strong>p -value</strong></td>
<td>1.000</td>
</tr>
</tbody>
</table>

Table 4. Comparison of C:N between the six treatments types categorized by subsets indicating 3 homogeneous relationships with a $p > 0.05$. Numbers represent the average of each treatment with its associated standard error (±).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C:N (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Subset 1</strong></td>
</tr>
<tr>
<td>Field 29 unsterile</td>
<td>35.53±1.32</td>
</tr>
<tr>
<td>Field 29 sterile</td>
<td>43.13±1.32</td>
</tr>
<tr>
<td>Field 8N unsterile</td>
<td>43.47±1.32</td>
</tr>
<tr>
<td>Field 8N sterile</td>
<td>42.47±1.32</td>
</tr>
<tr>
<td>P.S. unsterile</td>
<td>55.03±1.32</td>
</tr>
<tr>
<td>P.S. sterile</td>
<td>59.53±1.32</td>
</tr>
<tr>
<td><strong>p -value</strong></td>
<td>1.000</td>
</tr>
</tbody>
</table>
DISCUSSION

Greenhouse Study

Our results from the greenhouse study indicated that soil sterilization significantly reduced the survival of *E. decumbens*, regardless of the soil source. The odds of a seedling surviving in unsterilized treatments of Field 29, Field 8N and potting soil were significantly higher than seedlings grown in sterile soils. Our results also indicated that seedlings that passed the initial growth stage in sterilized treatments grew at comparable rates to plants grown in unsterilized treatments during the 19-week growth period. This suggests that the biota present in the soil may be critical for seedling establishment (Bowman and Panton, 1993) and is conducive to the survival of *E. decumbens* at its early life stage, regardless of its source. The total number of leaves was also significantly higher in the unsterilized treatments when compared to sterile treatments of both fields. This suggests that soil organisms can have a net positive effect on plant growth and productivity (Kulmatiski et. al., 2014) of *E. decumbens*.

Our results did not support our hypothesis that the Field 29 unsterilized treatment would have a greater survival rate, total number of leaves, crown size and vigor than the Field 8N unsterilized treatment in the greenhouse study. There was no difference found between Field 29 and Field 8N with regards to survivorship or total number of leaves. The significant difference found between crown area and vigor of the two fields were also the opposite of what we predicted to occur. The unsterilized treatment of Field 29 had a significantly smaller crown area and similar vigor to the unsterilized treatment from Field 8N.

Our results also did not support our hypothesis that the Field 29 sterilized treatment would perform poorly when compared to the Field 8N sterilized treatment due to beneficial biota in Field 29 soil. When sterilized, Field 29 showed a significant increase in crown area and while Field 8N showed a significantly decreased in both crown area and vigor relative to their sterilized treatment. This suggests that sterilization had a net positive growth effect on the Field 29 treatment by eliminating pathogens present in the soil while sterilization had a net negative effect on the growth of the Field 8N treatment, suggesting that this treatment had more beneficial soil biota than Field 29.
(Troelstra et. al., 2001). This indicates that at a later life stage, productivity increases when plants are grown in sterilized soil from Field 29 by decreasing the negative effect of soil biota on plant growth. It is unlikely this difference is because of chemical and physical soil properties as both fields had a similar N content when sterilized while texture was also likely similar due to the 3:1 potting mixture.

Attempting to draw direct correlations between observed growth effects of soil experiments in an atypical environment (the greenhouse) and what has been observed in the field may be dangerous due to the dynamic nature of the outdoor environment (Troelstra et. al., 2001). Therefore growth (survivorship, crown area, total leaf number and vigor) observed in the greenhouse environment might be an unreliable indicator for growth differences observed in the field of *E. decumbens* individuals. The dilution of the field soil 3:1 treatments to minimize compaction in individual pots likely influenced the growth effects due to changes in texture, TC, TN and C:N relative to plants grown in the field. These changes were observed by comparing treatments to the core samples from both fields. Field 29 cores had significantly higher TN and TC than Field 8N while no significant difference was found between TN of both sterile and unsterile field treatments used in the greenhouse treatments. The C:N also significantly increased in the sterile Field 29 treatments relative to its unsterile treatment. These changes to TC, TN, C:N in treatments relative to the field core samples suggest that greenhouse observations may be insufficient in drawing conclusive relationships between plants grown in the greenhouse study and plant growth observed in the field (Wassom et. al., 2003).

**Soil Analysis**

Our results from the soil core analysis supported our hypothesis with regards to observing a significant difference in physical and chemical soil properties in Field 29 relative to Field 8N. Field 29 soils had a significantly higher TC and TN content than Field 8N soils, suggesting that Field 29 has a higher rate of productivity due to greater TC and TN accumulation (Fornara & Tilman, 2008). However, there was no difference found between them with regards to C:N and organic matter content. This lack of difference indicates the source of the organic matter is consistent with grassland
ecosystems and shows that both soils hold similar organic carbon stabilization mechanisms (Wedin, 1996).

Field 29 soil had a significantly higher pH (less acidic) soil than Field 8N, which suggests that plants in Field 29 have more plant available nutrients and a higher rate of productivity (Kemmitt et. al., 2006). Soil pH plays an important role in controlling the exchange of cations and hydrogen atoms in the soil. A higher pH in Field 29 indicates the cation exchange capacity of the soil could be greater, which would increase its ability to exchange positively charged cations that plants utilize (Helling et. al., 1964). This may be the reason Field 29 has a higher N content over Field 8N, having the ability to absorb more ammonium (NH4+) cations for plant use (Oldham, 2010). Acidity may also have an effect on the microbial activity by reducing nitrate-nitrogen release when pH is below 5.0 as it is in Field 8N (Lucas & Davis, 1961). More acidic soils can significantly decrease productivity while having little affect on the TN and TC in the soil (Kemmitt et. al., 2006).

Our results from the particle size analysis indicated that Field 8N has larger d50 and d90 meaning Field 8N has larger median and 90th percentile particle size than Field 29. However, Field 8N had a significantly smaller d10, which indicates that 10% of its mineral particles are much smaller than the particles in the d10 size class of Field 29. These results are somewhat contradictory because the d50 and d90 values of Field 8N indicate it has coarser texture while its d10 value indicates that is has finer texture soils. Field 8N has greater specific surface area, which suggests that its particles should behave like finer textured soil. Because Field 8N has a greater surface area, this would indicate that Field 8N has a higher nutrient storage capacity than Field 29, however the availability of these nutrients may be limited by a more acidic pH. Field 8N may have a lower pH due its subtle difference in parent material that is derived from basaltic colluvial material from the nearby Pigeon Butte (Yu et. al., 2013). A greater surface area would also indicate greater water holding capacity, holding onto water at a potential that is more negative than the wilting point for plants. Because the surface area of Field 29 soil particles are smaller, this would help to increase plant available water, holding water at potential that is less negative than soils that have more surface area. Infiltration,
aeration, and internal drainage would also be improved (Brady & Weil, 2010). These factors could have an influence on the growth of *E. decumbens* by allowing plants to have better access to water in Field 29, especially during the summer months and may be contributing to the increase in productivity when comparing the two fields. Field 29 and 8N also have different drainage classes with Field 29 classified as moderately well drained and Field 8N as well drained. This suggests that water drains more slowly in Field 29 and that the water table may be higher than Field 8N. This may be increasing the amount of water availability during drier months, improving plant productivity (Schoeneberger, 2002).

The Web Soil Survey (NRCS, 2015) texture classification of these fields with Field 8N listed as silty clay loam and Field 29 as silt loam. This indicates that Field 8N has more clay while Field 29 has more silt than clay. This is consistent with greater surface area observed in Field 8N. They both hold different soil orders with Field 8N as a Mollisol and Field 29 as an Alfisol. Mollisols are typically associated with more organic matter however, the lack of difference in organic matter can be attributed to Field 8N having a deeper A horizon (30 cm) than Field 29 (15 cm).

**Other Potential Effects on Growth**

Other site-specific factors besides the soil properties may also contribute to the observed growth difference between sites (Huston & McBride, 2002). Field 8N also has a greater slope, which could lead to higher rates of water run-off with the combination of lowering infiltration rates having a higher clay content. Animal and insect herbivory may be a factor affecting the observed growth of differences. Differences in plant community composition may also be a factor. Field 29 is primarily perennial bunchgrasses including *Elymus glaucus* and *Festuca roemeri* along with a number of invasive grasses and forbs such as the *Anthoxanthum aristatum* and *Aira caryophyllea* with very low shrub cover of the invasive *Rubus armeniacus*. Field 8N has a very different plant community and cover that may be inhibiting the growth of *E. decumbens*. The field is dominated by *Lupinus arbustus*, which can grow up to 70 cm in height and hold a wide stature (USDA, NRCS, 2015) along with some native perennial bunch grasses. It also has a high cover of
invasive forbs such as *Hypochaeris radicata*. Both *Lupinus arbustus* and *Hypochaeris radicata* grow in well-drained soils, which explains their presence at Field 8N and absence at Field 29. Both species may play a role in inhibiting the growth and productivity of *E. decumbens* in this field. This inhibition may be occurring because more competitive species tend to outcompete rarer, less competitive plants for resources (Gillespie & Allen, 2004; Guo, 2007).

**Conclusion / Suggestions**

Our comparison of sterilized to unsterilized treatments emphasizes the importance of soil biota and organisms in the establishment and growth of *E. decumbens*. Soil microbes can play an important role as drivers of plant productivity, making up 5-20% of all nitrogen fixed in grassland systems (Van Der Heijden et. al., 2008). The net positive effect of soil biota may then be out weighed by the net negative effect of soil pathogens at a later life stage where pathogens inhibit productivity. This was seen with an increased crown area in Field 29 and a decrease in crown area and vigor in Field 8N when these soils were sterilized. However, our greenhouse results may be an unreliable indicator of the dynamic growth effect seen in the field due to the changes in TC, TN and C:N in treatments when mixed with potting soil and when applying sterilization. Growth patterns observed in the greenhouse study accentuate the complexity of plant-soil biota interactions and the difficulty of determining the net positive verses net negative effect on plant growth and how this translates to the dynamic field environment. Understanding the specific relationship between soil organisms and *E. decumbens* would be useful knowledge to advance the future recovery of this rare species.

The differences found between soil properties of Field 29 and Field 8N may be associated with the observed growth patterns of *E. decumbens* seen in the field. The significant differences in texture, pH, TC, and TN found in the soils of Field 29 suggest that these soil attributes may be conducive for *E. decumbens* plant growth and are contributing to healthier, more productive individuals observed in Field 29. This also suggests that these soil properties in general are favorable for *E. decumbens* growth and may offer further support in their recovery. These results provide valuable insights when
choosing sites that hold similar soil properties for future restoration and reintroduction projects.

Future research could be directed towards studying other site-specific factors that may also influence growth of this species, such as the direct impacts of specific soil biota, topographic position, and plant community have on the growth of *E. decumbens* in both fields. Future studies could also benefit by incorporating biomass as a measure of vigor and productivity as an additional way of comparing the treatment groups. These suggestions for the future study of *E. decumbens* would likely enhance our understanding of how to assist its recovery by developing better management and restoration techniques that are conducive to its growth and productivity.
REFERENCES


APPENDIX

Map of William L. Finley National Wildlife Refuge (USFWS, n.d.)
Map of the Recovery Zones of *E. decumbens* in Western Oregon and Southwestern Washington including Corvallis West Recovery Zone in which field 29 and 8N are located (USFWS, 2010).