Creating A Growing Matrix To Support Nitrogen Fixing Plants Using Kimberlite Tailings From The De Beers Victor Diamond Mine

A thesis submitted in partial fulfillment of the requirements for the Honour's degree of Bachelor of Science in Environmental Earth Science

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Abstract

The De Beers Canada Victor Mine is an open pit diamond mine in the Hudson Bay Lowland. Once mining is complete and closure occurs, the area must be restored and re-vegetated to pre-mining conditions. Waste rock from the mine includes; peat overburden, silt overburden, limestone mine rock, fine processed kimberlite (FPK) and coarse processed kimberlite (CPK) and. In a factorial growth chamber experiment, five different soil mixtures from these mixtures were amended with varying levels of waste rock to test the ability to support the growth of two nitrogen-fixing plant species: native green alder (*Alnus crispa*), an actinorhizal shrub and a non-native legume, white clover (*Trifolium repens*). Two different phosphorus fertilization treatments (0.2% and 1.0%) were evaluated to supplement nutrients. The controlled vermiculite soil mixture showed the largest biomass production. However the locally available 100% silt amendment performed the best for nitrogen-fixing plants in this study. Increasing FPK and lowering CPK reduced the growth and nodulation of species. Inoculation of a litter tea proved to be successful for promotion of nitrogen fixation in both species. Phosphorus fertilization didn’t show significance in the promotion of plant development, and the high phosphorus treatment killed nearly all of the green alder.

Introduction

The processes by which communities of organisms originated are not usually our first thought when observing a natural ecosystem. Landscapes and ecosystems are constantly changing. At some point, they began on barren land through primary succession and underwent transitions over ecological time in structure, species composition and function. However, primary succession can be a very slow process (Keeton and Gould 1986), with several limiting factors, including a lack of soil development, low levels of nutrients, pH imbalances, poor water-holding capacity and a harsh microclimate for plant development. Mining sites are examples of severely disturbed areas or new deposits that must be reclaimed to rebuild and establish an ecosystem. They often rely on techniques to speed-up primary succession.

Low nitrogen accessibility in the soil can limit plant growth. It is a key factor that limits plants during primary succession (Walker & Del Moral 2003). Nitrogen is usually taken up by plants as ammonium (NH_4^+) or nitrate (NO_3^-), and less commonly as amino acids and specialized proteins. These compounds are taken up either directly by the roots or by mycorrhizae associated with the roots (Andrews et al. 2011). Nitrogen-fixing plants have the ability to access atmospheric N_2 under low soil nutrient circumstances (Andrews et al. 2011). They convert it to ammonium (NH_4^+), which is biologically available for plants. As such, N-fixing plants play an important role in rebuilding nitrogen stocks in damaged ecosystems. Legumes and actinorhizal shrubs are both groups of N-fixing plants that may be the piece of the puzzle for rebuilding nutrients and sustaining plant growth during primary succession. They can be efficient tools in ecosystem restoration (Norris et al., 1994).

Legumes are a first group that fixes nitrogen under conditions of limiting nutrients. These plants fix the nitrogen in small growth casings, called nodules, within the root's system. Bacteria in these nodules of the genus *Rhizobium* convert N_2 gas to produce NH_4^+, which is absorbed into the plant to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components, vital for life (Lindemann & Glover 2003). Many agricultural species are nitrogen-fixing legumes. For example, soybeans (*Glycine max*) are a highly regarded crop by many farmers. Besides producing food and oils, soybeans fix nitrogen and aid in soil management. Soybeans represent 77% of the crop legumes that fixes nitrogen; they annually fix 16.4 Tg of nitrogen globally (Herridge et al. 2008). Soybeans can possess several hundred nodules per plant and fix around 280 kg of nitrogen per hectare (Lindemann & Glover 2003). Clovers (*Trifolium*) are also advantageous legume for grassland management, helping them to be less dependent on inorganic fertilizers (van Eekeren et al 2009).

Actinorhizal shrubs are another group of plants that fix nitrogen and are among the best plants to establish in nitrogen poor habitats (Bargali 2011; Seeds & Bishop 2009). They include numerous genera, and in northern Ontario include members of Myricaceae (*Myrica*), Betulaceae (*Alnus*) and Elaeagnaceae (*Shepherdia* and *Elaeagnus)* (Huguet et al 2001). These shrubs form a symbiosis with nitrogen-fixing filamentous actinobacteria, *Frankia,* within nodules among the roots (Bargali 2011; Seeds & Bishop 2009). However, actinorhizal shrubs also require mycorrhizal associations. Mycorrhizae are a symbiotic relationship between a fungus and the roots of a vascular plant (Kirk et al. 2001). The presence of mycorrhiza can help the plants grow in two ways: 1) directly by increasing plant growth through the uptake of other nutrients such as phosphorus; or 2) indirectly by improving soil conditions, such as adding aggregates and improving stability to the soil (Tisdall & Oades 1982; Abbott et al. 1992). A mycorrhizal infection of the actinorhizal host plant increases the uptake of phosphate, the nodulation and consequently nitrogen-fixation, resulting in overall enhancement of plant growth (Gardner 1986). Actinorhizal plants add nitrogen to the surrounding soil by leaf litter and by the dead tissues of the roots (Vogel & Dawson 1985). Most actinorhizal plants are claimed to be primary colonizing species that establish in young soils where available nitrogen is scarce, as in disturbed landscapes from mining sites (Schwintzer & Tjepkema 1990). Actinorhizal shrubs thereby play a critical role by enriching the soil and enabling the establishment of other species to begin ecological succession (Schwintzer & Tjepkema 1990) (Bargali 2011).

Nitrogen fixation by both legumes and actionorhizal shrubs are heavily dependent on the development of symbiotic relationships with bacteria and fungi. Many of the bacteria are host-specific. They may be present in the soil microflora. However, inoculation helps provide essential sources of bacteria and fungi to enhance germination, root development, and the start of nodule formation (Barea et al. 2002). It makes a significant difference when the appropriate rhizobia and mycorrhizae fungi are added to the soil. Hutton et al. (1997) found that inoculating soils greatly increased the growth potential of species in the mycorrhizal family Epacridaceae in a highly disturbed mined area in Australia

Chemical nutrients can affect the growth, productivity and the ability to nodulate in nitrogen fixing plants. Fertilizers such as nitrogen, and phosphorus may enhance or reduce the nitrogen-fixing plant's ability to establish on barren lands. For instance, the addition of a nitrogen fertilizer to the soil of a N-fixing plant reduces the ability to form nodules and also decreases the nitrogen fixing capability (Burgess & Peterson 1987). Phosphorus fertilizers seem to have the opposite effect on nitrogen fixing plants. They enhance the formation of nodules in infertile soils, but reduce the formation of mycorrhiza (Koo et al.1996 ; Seilers & McCormick 1982). Koo et al. 1996 did a 10 week experiment on red alder, *Alnus rubra*, and found that using nitrogen fertilizers resulted in a negative effect that decreased the amount of nitrogen being fixed by the plant, causing a lower number of nodules that were observed to have damaged *Frankia* vesicles (Koo et al.1996). However, phosphorus fertilizers actually enhanced the amounts of nitrogen that was fixed by the red alder (Koo et al.1996). The N/P ratio of a plant is very particular. As the nitrogen fixing plant species matures, the plant increases the amount of nitrogen in its biomass, requiring more phosphorus. The increased demand of phosphorus to the plant depletes the amount of available phosphorus in the soil. Therefore, it is beneficial for N-fixing plants to develop in high phosphorus soils (Koo et al.1996).

The pH of the soil can also affect N-fixing plants and limit the total biomass yield. A study by Neeraj et al. (2009) tested biomass productivity of the nitrogen-fixing legume, mung bean (*Vigna radiate*) across pH ranges from 5.5 to 9.0. The optimal for growth for the mung bean was observed at pH 7.2, and one species showed optimal growth at pH 8.0 (Neeraj et al. 2009). These pH limitations vary amongst *Rhizobium* species.

Soil texture is another important variable that needs to be considered for water holding characteristics for success of plant survival and growth. In simple terms, the larger the diameter of the particle, the smaller relative surface area it has, so less water can be stored in the soil matrix (Saxton & Rawls 2006). However, smaller diameter particles have larger surface area, which holds on to water more strongly in the soil matrix (Saxton &Rawls 2006). As such, the soil texture will have major effects on the wilting point, the minimal point of soil moisture that the plant requires not to wilt, and field capacity, the maximum amount of water held in a soil. Finer textures like clay (particle size of < 2 μm) have a high wilting point and field capacity, whereas coarser grained particles like silt and sand (particle size; slit- 2-5 μm, sand 5μm-2mm) have a lower wilting point and field capacity (Saxton & Rawls 2006; Dexter 2004). The amount of organic matter should be considered along with the texture of soil to access how well a soil will support plant life (Saxton &Rawls 2006). It affects both the field capacity and the wilting point. So, an increase in the proportion of organic matter in a mineral soil greatly assists the development and growth of plants (Dexter 2004).

The DeBeers Canada Victor Mine is an example of a site requiring ecosystem reclamation and would benefit from the use of nitrogen-fixing species during reclamation. It is an open pit diamond mine in the subarctic Hudson Bay Lowland, around a cluster of kimberlite pipes that infringed on limestone around 170 Ma ago (Hattori, 2008). The bulk waste product of this mine has been projected to include 8.1Mt of fine processed kimberlite (FPK), 10.4 Mt of coarse processed kimberlite (CPK), 26 Mt of limestone, 11.3 Mm^3 of silt overburden and 1.8 Mm^3 of peat (AMEC 2004). DeBeers Canada is required to reclaim the land to a functioning, productive ecosystem after mine closure.

Kimberlites are ultramafic rocks that can contain diamonds. It is a product of altered olivines, with an approximate composition of $H_4 (Mg\ Fe)_3 Si_2 O_9$ (Roberts 1980). Processed kimberlite waste is similar to serpentine soils, which are classed as having very little nutrients with nutrient imbalances. The Ca-Mg ratio is usually below one (Reid & Naeth 2005), so calcium must be amended to processed kimberlite to prevent nutrient imbalances. Kimberlite waste rock is alkaline with a pH above 8.0 (Reid & Naeth 2005; Rouble 2011; Campbell & Bergeron unpublished data). Essential nutrients like nitrogen and phosphorus are almost absent from processed kimberlite or waste materials at Victor

Mine and must be added to any soil mix.

Different mixes of local deposits, including processed kimberlites, waste rock, silt and peat overburdens, are currently being tested to build suitable soils and kick-start the primary succession of this landscape (Bergeron and Campbell, unpublished data; Rouble 2011). In two separate studies, Rouble (2011) and Bergeron (unpublished) created soil mixes from the waste material from the Victor diamond mine to test which would allocate the highest productivity of biomass when growing Kentucky Blue grass (*Poa pratensis*) and White Clover (*Trifolium repens*). The best soil mixture for producing the largest biomass in each plant was a mixture that contained 20% fine processed kimberlite (FPK), 60% coarse processed kimberlite (CPK), and 20% silt, with the addition of 40% peat (Campbell and Bergeron unpublished data; Rouble 2011). Nutrients were added into these soils through mild N, P, K fertilizations.

Nitrogen-fixing plants would provide a good long-term solution to build nutrient stores on soil mixes made from processed kimberlites from the Victor Mine. In this way less fertilizer could be used and a more natural development of primary succession could be established. The question remains as to whether N-fixing plants survive and grow on these soil mixes. In this study, it is hypothesized that 1) Nitrogen-fixing plants can grow in kimberlite based on available mining waste material. 2) Based on previous soil amendment studies, higher CPK and lower FPK are better mixes for N-fixation. 3) Both a legume and an actinorhizal plant can grow and produce nodules in amended soils with a promotion of inoculation and 4) Phosphorus fertilizers help in plant development. The goals for remediation are to use local stockpile waste materials to create sustainable soils with suitable support for nitrogen fixing plants to remediate De Beers Victor diamond mine once mining is complete.

Material & Methods

Mine waste from the DeBeers Victor mine, including fine processed kimberlite (FPK), coarse processed kimberlite (CPK), silt and peat, which were shipped to Sudbury in the summer of 2011. To reduce the moisture within the stocks, and to keep consistency, all material was dried in a growth chamber at approximately 30°C for 3 weeks. Once dry, the FPK and silt were manually crushed using a pin roller to achieve a more consistent texture of particles size around 3.9-62.5 µm (Campbell and Bergeron unpublished data).

The five mixes of mineral soil were created: 1) 100% vermiculite, 2) 100% silt, and mixes of silt:CPK:FPK of at a ratio of 3) 20:60:20, 4) 20:80:0 and 5) 0:40:60, by volume. Each mineral soil was mixed with an additional 40% peat from Victor Mine, again by volume. To ensure the soil amendments were distributed evenly throughout, they were each mixed in a cement mixer for 30 minutes. Each soil mix was divided into twenty, 4" pots of each soil amendment, with approximately 500 mL in volume.

The pH was measured for each soil by placing the soil samples in a 100mL beaker containing 2 parts soil, 1 part distilled water. Using a calibrated waterproof hand held Fisher Scientific pH accumet probe, three replicates of each soil sample were taken to get the mean pH of each soil.

Two N-fixing species were used, white clover (*Trifolium repens*) and green alder (*Alnus crispa*). The white clover is non-native but is a common legume often used in reclamation, and the green alder is a native actinorhizal shrub common around the Victor Mine. The non-native white clover was used instead of a native species due to the lack of available seeds of native legumes during the time frame of this experiment. Native legume species to the Hudson Bay Lowland include from the Family Fabaceae; wild pea, *Lathynes palustris,* and American vetch, *Vicia Americana,* (Ritchie 1957) and should be tested to grow in the Victor diamond mine waste soil, if there was available seeds. White clover seeds were obtained from Southview Greenhouse Growers in September 2011. Green alder seeds were collected in 2009 from uplands in the Hudson Bay Lowlands near the Victor mine, and were air dried and stored at room temperature (C. Laurin, *pers. comm.*).

The white clover seeds were germinated directly on our soil mixes by sowing a pinch of seeds onto the mixes and covering them lightly. After germination, they were thinned to a single plant closest to the center of the pot. Green alder seeds were first germinated in trays with a 50:50 mixture of AllTreat Farms Premium Potting Mix® and vermiculite. Once the green alder established showing their true leaves they were transplanted carefully into the prepared pots of mixes. If individuals died the green alders were re-transplanted; no more than two additional transplant attempts were attempted for establishment of growth. Both species were germinated and grown in a BioChambers® growth chamber (model AC-60), where they received 16 hours of light per day under a mix of fluorescent and incandescent bulbs at 25°C and 8 hours of darkness at 15°C.

Once in the soil mixes, both species received an inoculation treatment to help with nodulation formation. The inoculation slurry consisted of multiple N-fixing plants to ensure optimum nodulation formation. Litter, humus, roots and root nodules from *Alnus rugosa*, *Alnus crispa* and *Myrica gale*, and also litter from N-fixing legumes *Lotus corniculatus, Trifolium repens,* and *Vicia cracca* were collected. As well, litter from *Sheperdia canadensis* was collected from Manitoulin Island in the fall of 2011. Nodules were carefully separated and crushed with a mortar and pestle and added to the litter mixture. The inoculation slurry was prepared almost directly after collection of material so water loss of the bacterial spores would not destroy the microorganism (Quoreshi 2008). Approximately 750 mL of leaf litter and humus material, 5-10 mL of fresh nodules, and 250 mL of roots from each plant were added to 26 L of distilled water. The inoculation material was left for 24 hours to infuse as a litter tea. The litter material was strained through a 2 mm sieve to extract the infused tea for inoculation. Green alder was inoculated with 60 mL once it was transplanted into the prepared soils, while white clover was inoculated with 60 mL once true leaves had formed.

Two phosphorus fertilizer treatments were created from 23.07g/100mL of KH_2PO_4 based on phosphorus treatments from Rorison solutions: a 1% (2.307g/L) and 0.2% (0.4614g/L) solution were tested. The pots were watered with 60 mL of 1% or 0.2% fertilizer 3 times a week. On days without fertilizer, each pot received 60 mL of distilled water to avoid drying out. This experiment was set up as a random block factorial design with the five soil mixes, two nitrogen-fixing plant species, and two levels of phosphorus fertilizer, set up in five blocks in the growth chamber.

After 6 weeks of growth for white clover and 8 weeks of growth for green alder, plants were removed from the growth chamber and carefully extracted from their soils so no biomass was lost. The amount of nodules per plant were counted and quantified as well as shoot length and longest root length. The roots and shoots were separated and placed in separate paper bags and dried in a drying oven at 80°C for 48 hours. The total dry plant biomass of the root and shoots was measured to an accuracy of 0.001g, and root/shoot ratios were calculated.

The data was analyzed using a 5×2×2 factorial ANOVA with a random block design, using SPSS software. Assumptions of the ANOVA were determined using graphical analyses of residuals, and data were transformed if necessary. Type I error level was set at $P<0.05$.

Results

Survival

The survival rate between white clover and green alder was very different. Just over a quarter (26%) of the alder survived the complete 8 weeks of growth in the fabricated soils whereas the clover had a 100% survival rate (Figure 1). The soil that fared the best for both plant species was the control vermiculite with 80% survival rate. Silt, 20-80, and 20-60-20 all had the same survival rate when supporting both plant species with 60% survival and the worst soil amendment was 60-40 with just over 50% of the survival (Figure 2). The phosphorus treatment showed that both species did much better overall when given the low 0.2% phosphorus solution, than the high 1% phosphorus solution (Figure 3).

In terms of the survival of both species together under different fertilization conditions and soil types, the vermiculite control had 100% success of survival for the low phosphorus, but the high phosphorus showed a 40% drop in survival, only surviving 60% of the time (Figure 4). Silt, 20-80, and 20-60-20 all had the same survival rate for both low phosphorus treatment, at 70% survival and showed a 20% drop between for high phosphorus treatment, to only a 50% survival rate. The worst soil again for supporting plants and different phosphorus levels was 60-40 mix of FPK to CPK.

When comparing the survival of the two species in each soil, all of the clover survived the growth chamber experiment in every soil amendment (Figure 5). The green alder had varying results. The alders in the vermiculite survived only 60% of the time, but in the silt, 20-80, and 20-60-20 amendments, only 20% of green alder survived, and in 60-40 it fared the worst, with only 10% survival.

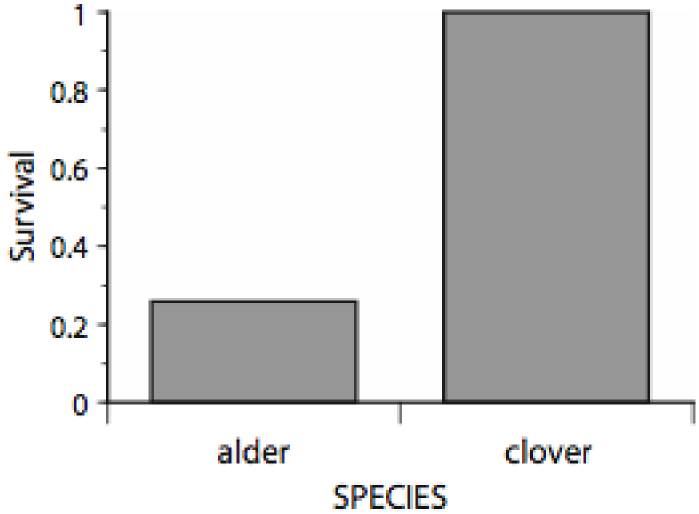

Figure 1: The proportion of survival rate in the amended soils from the De Beers Victor Diamond mine of green alder and white clover.

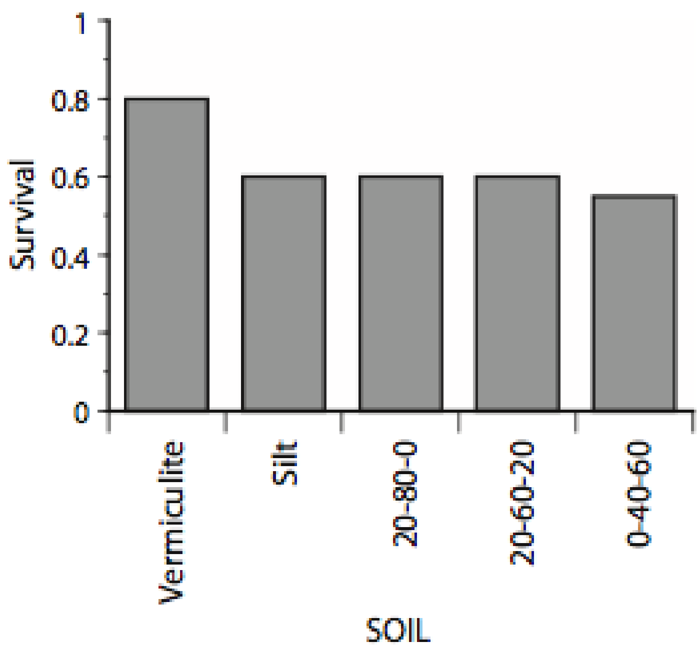

Figure 2: Survival rate of both Clover and Alder species in the different amended soils.

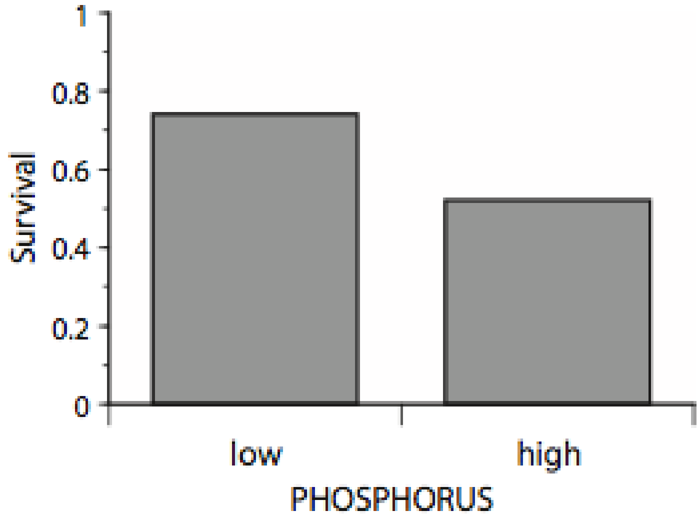

Figure 3: The survival rate proportions of both species with a low phosphorus 0.2% solution and a high 1% solution of KH_2PO_4 (23.07g/mL).

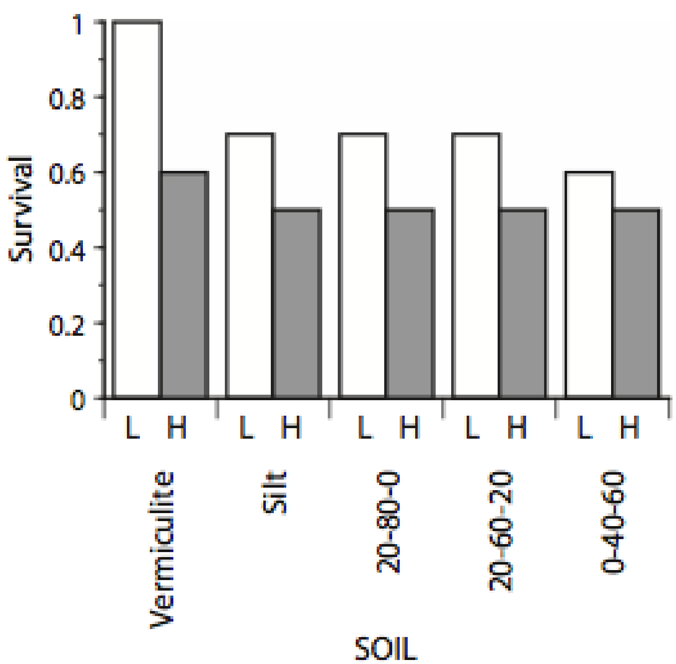

Figure 4: The proportion of survival rate when comparing the soil type and the phosphorus treatment (L, low versus H, high).

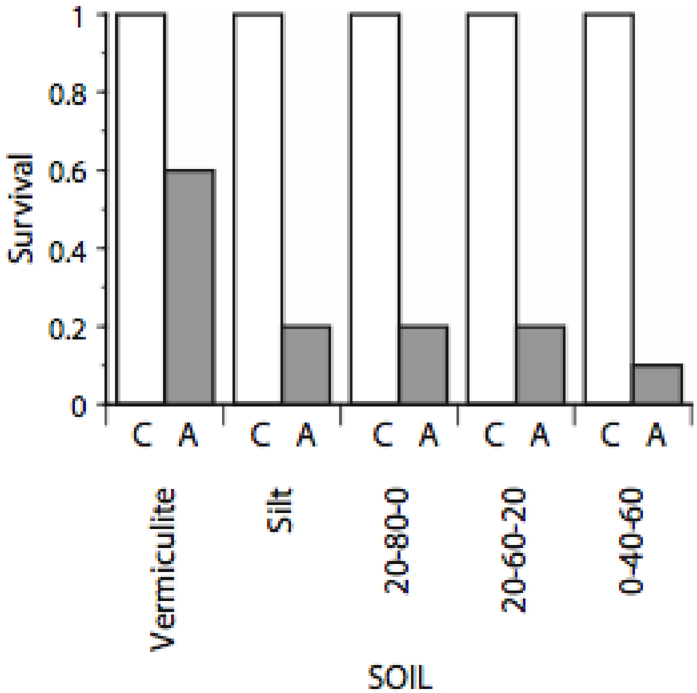

Figure 5: The survival rate proportions when comparing the soil types and of both species clover (C) and green alder (A).

Growth and nodulation

The productivity of growth was quantitated by the mass of dry roots, dry shoots dry root/shoots and by the mean number of nodules produced for nitrogen fixing comparing it to each species and each soil type. Due to the large mortality of green alder under the higher phosphorus treatment, we could not test for 3-way interactions between soil type, species and phosphorus, however main effects and 2-way interactions could be evaluated.

The dry root biomass varied significantly depending on species ($P < 0.001$), as well as soil amendment ($P < 0.001$; Table 1). However phosphorus fertilization did not show an effect, nor were there any interactions. White clover had a much higher average dry root mass, nearly 3 times greater than the average dry root mass of green alder

(Figure 6). The amended soil that established the greatest amount of root biomass was the vermiculite control, which produced almost 3.5 times as much dry root biomass than the second best soil, which was 100% silt (Figure 7). Both species developed the smallest root system in the soil with 20-60-20 ratio of silt:CPK:FPK (Figure 7).

Table 1: ANOVA of rank transformed total dry root biomass of *T. repens* and *A. crispa* in the growth chamber experiment. Significant effects with $P < 0.05$ are shown in bold.

Source	df	SS	MS	F	P
BLOCK	4	284.8	71.2	0.8	0.524
SOILS	4	6200.3	1550.2	17.7	**<0.001**
SPECIES	1	1281.5	1281.5	14.6	**<0.001**
PHOSPHORUS	1	6.2	6.2	0.1	0.792
SOILS * SPECIES	4	535.7	133.9	1.5	0.211
SOILS * PHOSPHORUS	4	317.7	79.4	0.9	0.469
SPECIES * PHOSPHORUS	1	15.8	15.8	0.2	0.673
Error	43	3768.9	87.7[b]		

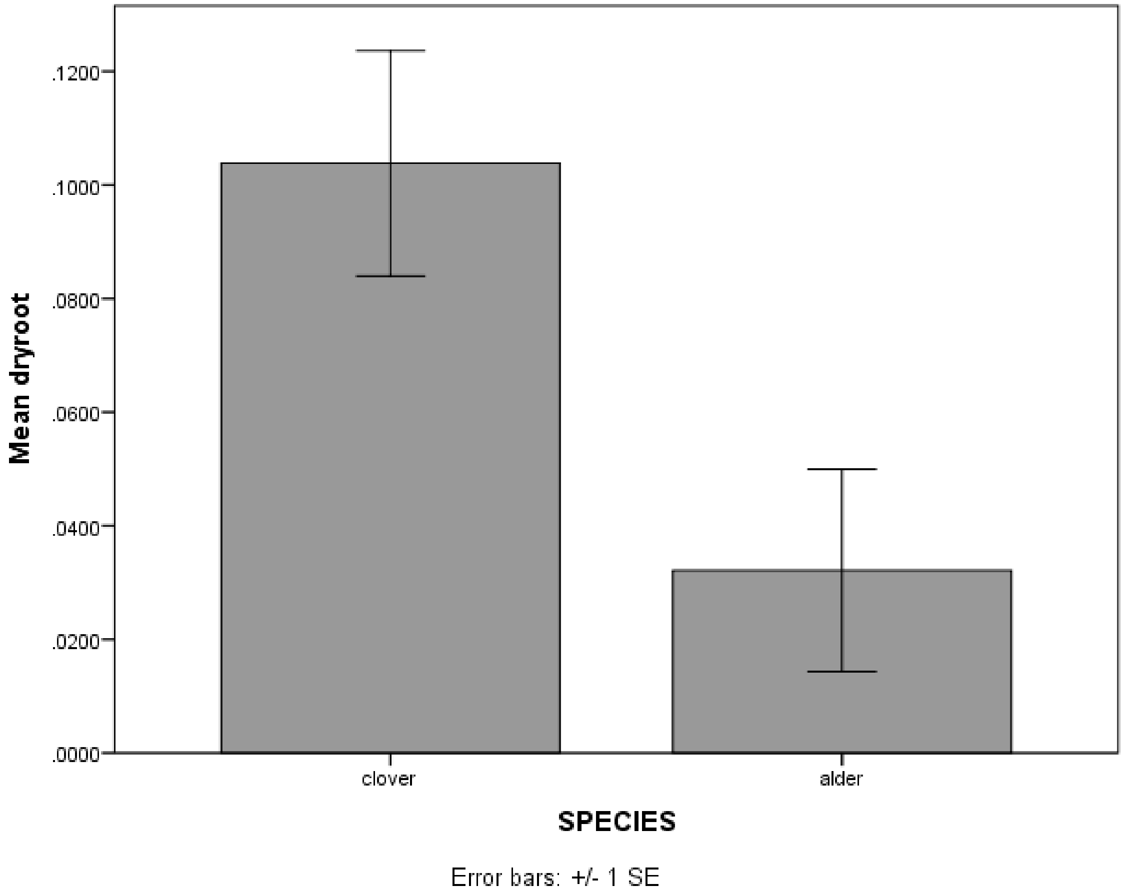

Figure 6: The dry root mass of *T. repens* and *A. crispa* across all soils (mean ± 1SE, N_{clover} = 50, N_{alder} = 13).

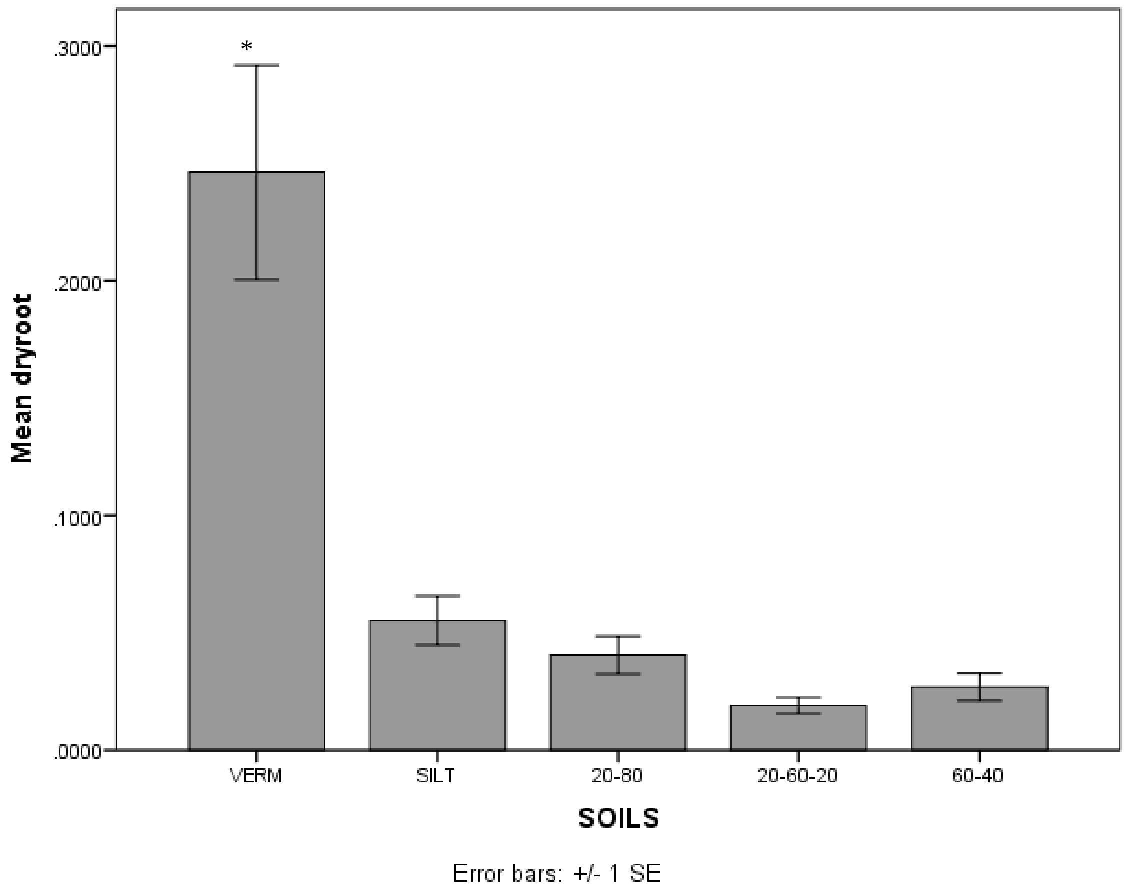

Figure 7: The dry root mass in each amended soil across both species (mean ± 1SE). Tukey's HSD (P<0.010)

The dry shoot biomass showed a similar pattern as the root biomass. It varied significantly with species (P = 0.017) and with soil amendment (P < 0.001; Table 2). There was no effect of phosphorus, nor were there any interactions. The white clover produced a greater mean shoot biomass than the green alder, with only a slight overlap of the lower standard end of clover mean shoot biomass and at higher standard mean biomass of alder (Figure 8). The manufactured soil that produced the largest dry shoot biomass on average was the vermiculite control soil; it produced almost 4 times more dry shoot biomass than the second best soil which was 100% silt (Figure 9). The soil that developed the least amount of shoot biomass was the 40-60, CPK:FPK (Figure 9).

Table 2: ANOVA of rank transformed total dry shoot biomass of *T. repens* and *A. crispa* in the growth chamber experiment. Significant effects with $P < 0.05$ are shown in bold.

Source	df	SS	MS	F	P
BLOCK	4	184.9	46.2	0.7	0.617
SOILS	4	7427.2	1856.8	26.9	**<0.001**
SPECIES	1	425.0	425.0	6.1	**0.017**
PHOSPHORUS	1	150.8	150.8	2.1	0.147
SOILS * SPECIES	4	317.1	79.3	1.1	0.347
SOILS * PHOSPHORUS	4	469.5	117.4	1.7	0.168
SPECIES * PHOSPHORUS	1	0.1	0.1	0.001	0.973
Error	43	2971.9	69.1[b]		

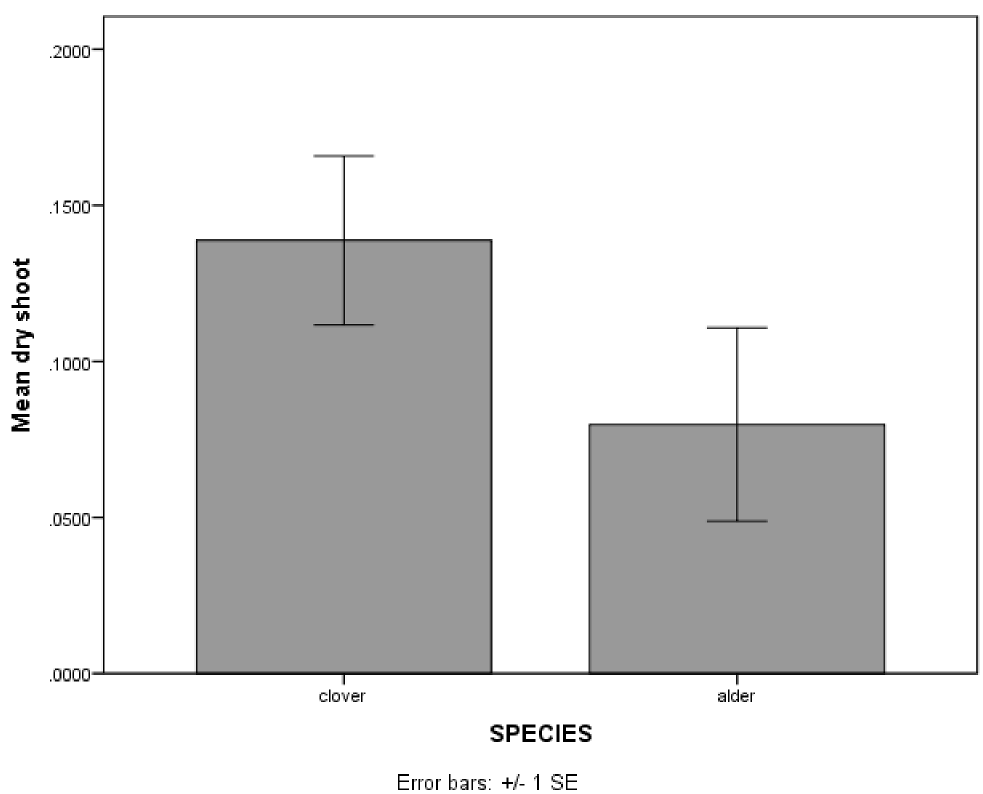

Figure 8: The dry shoot mass of *T. repens* and *A. crispa* across all soils (mean ± 1SE; N_{clover} = 50, N_{alder} = 13).

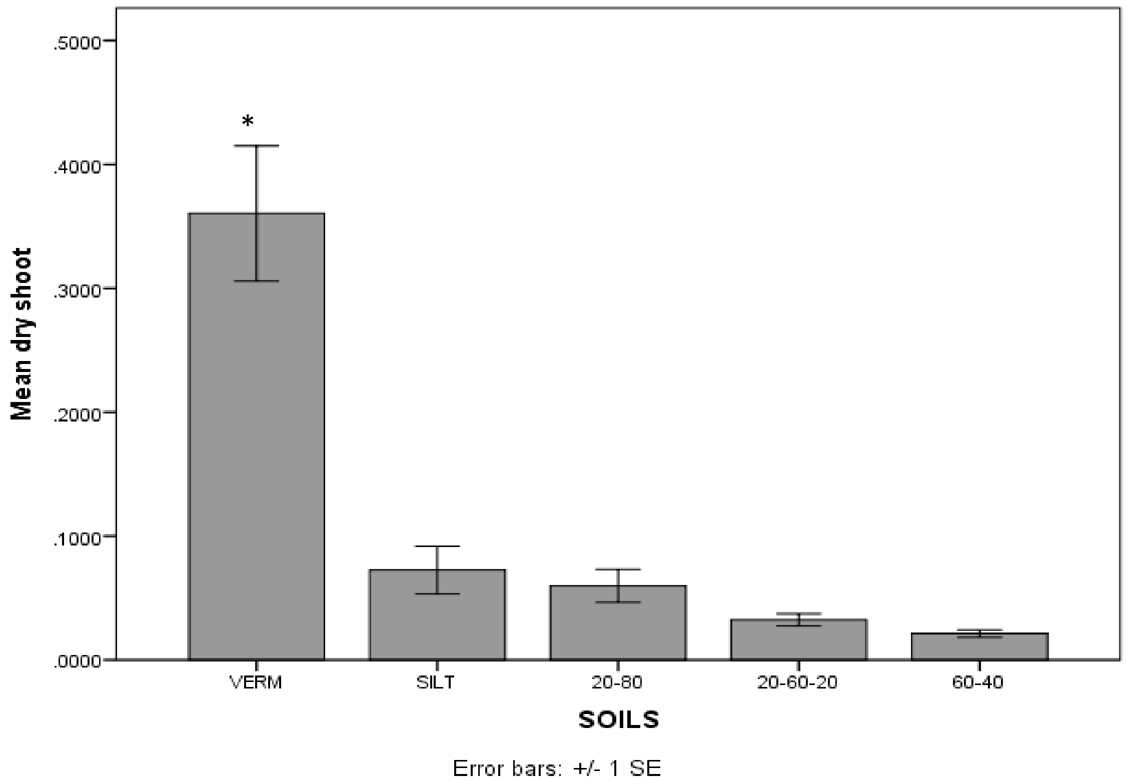

Figure 9: The dry shoot mass in each amended soil across both species (mean ± 1SE). Tukey's HSD (P<0.003)

The dry root/shoot ratio varied significantly between species ($P < 0.001$) but there was no effect associated with soil amendment, phosphorus fertilizer or any interaction (Table 3). The white clover had over twice the mean root:shoot ratio as green alder (Figure 10). The placement within the block formation in the growth chamber produced a significance difference ($P < 0.001$) between dry root:shoot ratios (Figure 11). Block 4 placement produced a significant amount less root to shoot ratio with in the growth chamber.

Table 3: ANOVA of rank transformed dry root/shoot ratio of *T. repens* and *A. crispa* in the growth chamber experiment. Significant effects with $P < 0.05$ are shown in bold.

Source	df	SS	MS	F	P
BLOCK	4	3058.3	764.6	5.8	**0.001**
SOILS	4	255.0	63.8	0.5	0.750
SPECIES	1	2000.4	2000.8	15.1	**0.001**
PHOSPHORUS	1	340.3	340.3	2.6	0.117
SOILS * SPECIES	4	250.1	62.6	0.5	0.757
SOILS * PHOSPHORUS	4	776.3	194.1	1.5	0.230
SPECIES * PHOSPHORUS	1	.4	0.4	0.003	0.958
Error	43	5707.7	132.7		

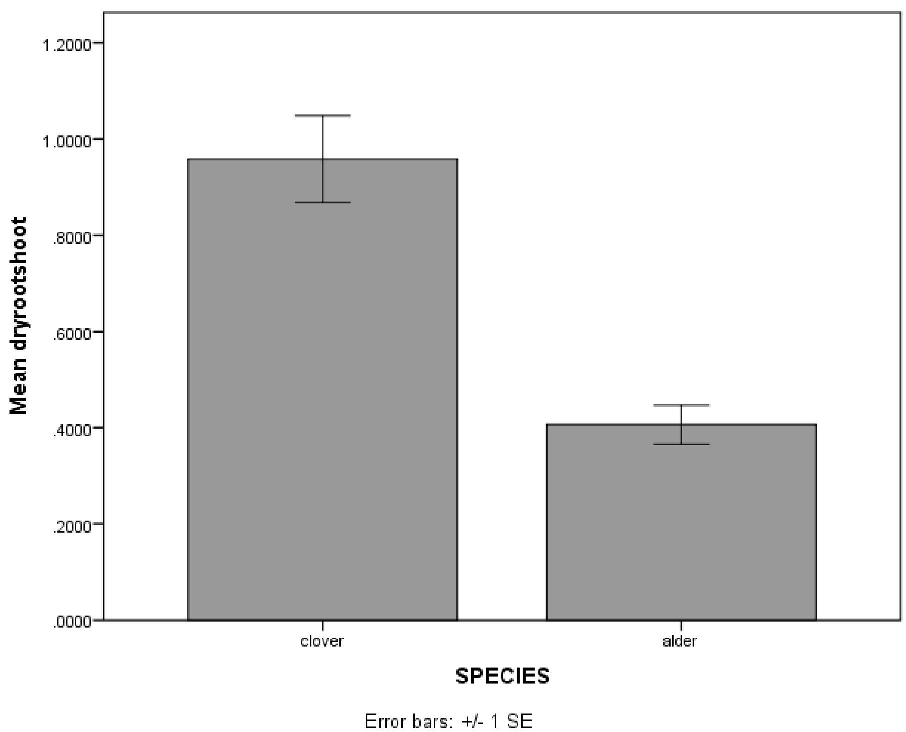

Figure 10: The dry root/shoot ratio of *T. repens* and *A. crispa* across all soils (mean ± 1SE; N_{clover} = 50, N_{alder} = 13).

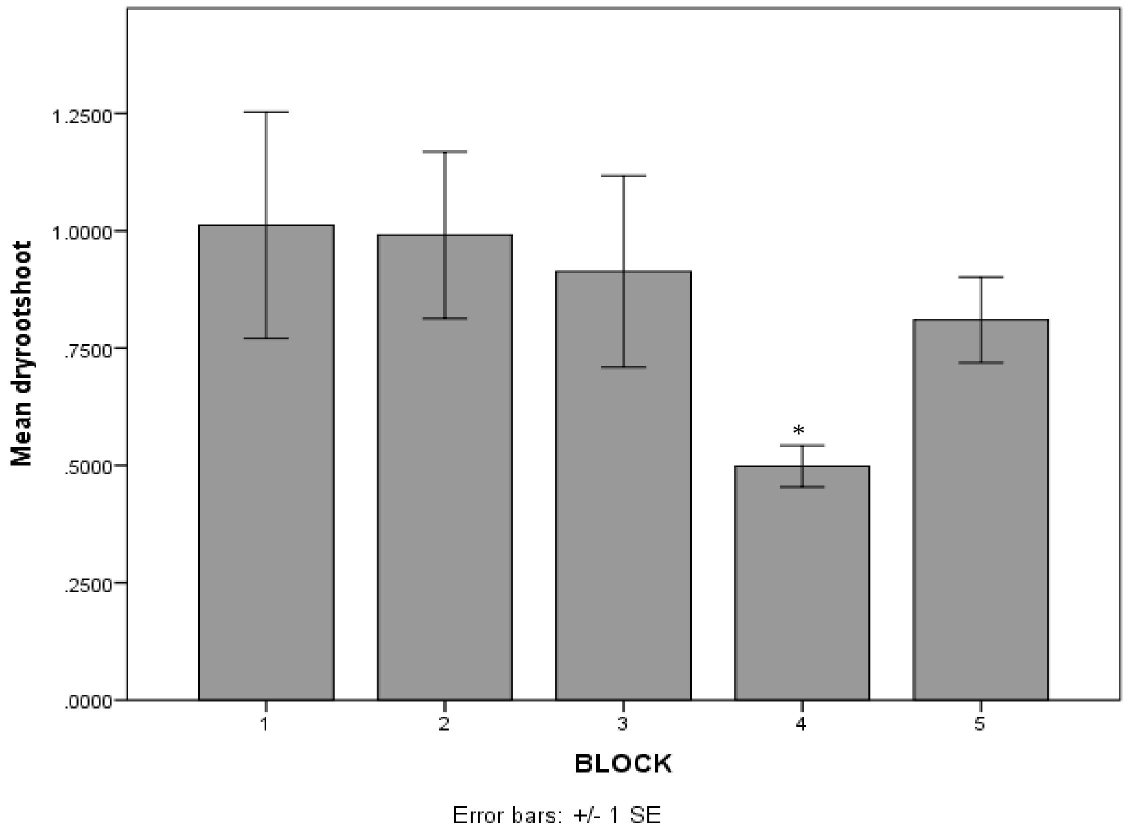

Figure 11: The dry root/shoot ratio in each block across both species (mean ± 1SE). Tukey's HSD (P=0.032)

The number of nitrogen-fixing nodules present on each plant varied significantly between species (P = 0.006) as well as among soil amendments (P = 0.045; Table 4). Nodule numbers did not vary with phosphorus fertilization, nor were there any interactions. The white clover developed a greater number of nitrogen fixing nodules, more than 4 times the average of the alder (Figure 12). The manufactured soil that produced the highest average amount of nitrogen fixing nodules was 100% silt, followed closely in second by the vermiculite control (Figure 13). Nodulation decreased in soils based on kimberlite. The 40-60, CPK:FPK soil was the least successful at developing nodules (Figure 13).

Table 4: ANOVA of square-root transformed total number of nodules of *T. repens* and *A. crispa* in the growth chamber experiment. Significant effects with $P < 0.05$ are shown in bold.

Source	df	SS	MS	F	P
BLOCK	4	1.73	0.43	0.1	0.978
SOILS	4	41.15	10.29	2.7	**0.045**
SPECIES	1	32.98	32.98	8.5	**0.006**
PHOSPHORUS	1	4.37	4.37	1.1	0.293
SOILS * SPECIES	4	13.52	3.38	0.9	0.486
SOILS * PHOSPHORUS	4	6.05	1.51	0.4	0.813
SPECIES * PHOSPHORUS	1	0.16	0.16	0.04	0.839
Error	43	165.98	3.86		

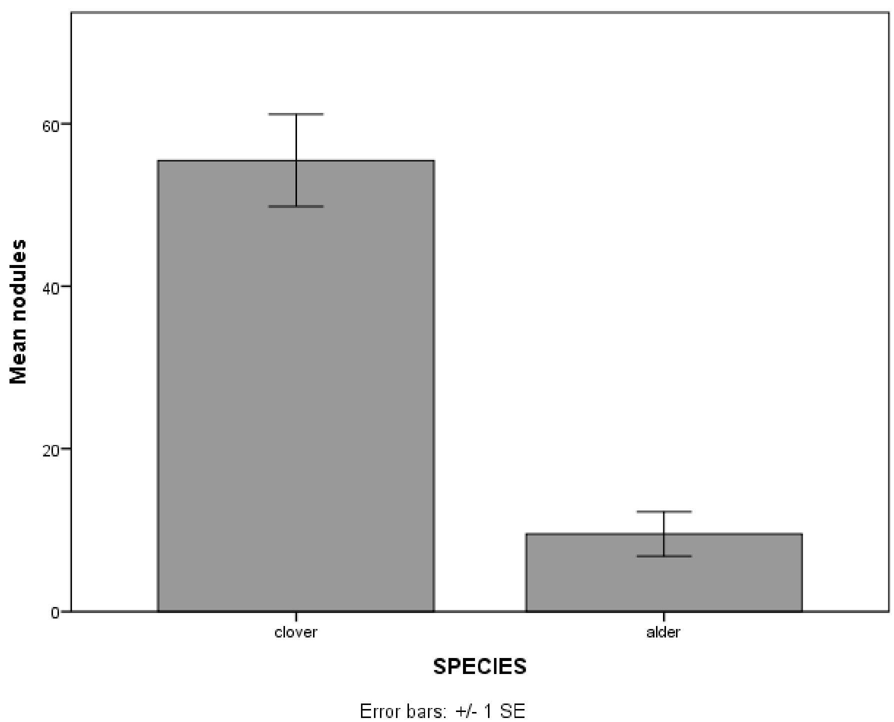

Figure 12: The number of nitrogen fixing nodules on the root system of *T. repens* and *A. crispa* across all soils (mean ± 1SE; $N_{clover} = 50$, $N_{alder} = 13$).

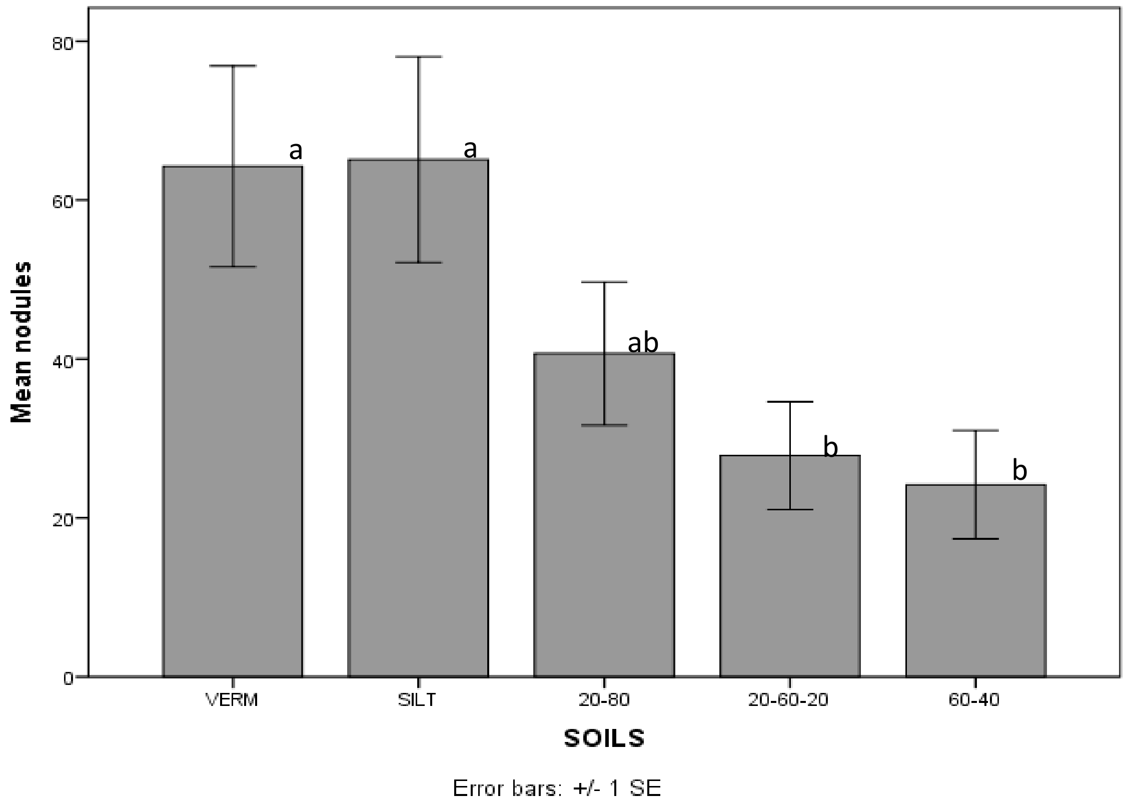

Figure 13: The number of nitrogen fixing nodules on the root system in each amended soil (mean ± 1SE). Tukey's HSD ($P<0.018$)

The average pH of each soil varies. The lowest average pH observed was the control soil of the vermiculite mixture (pH= 6.03). The silt has the lowest average pH in the amended soils from the Victor mine (pH=7.87). Any soils that contained kimberlites resulted in an average pH over 8.08. The highest average alkaline pH was observed in the 60:40, FPK:CPK (pH=8.44).

Table 5: The average pH of each amended soil tested

Soils	Test 1	Test 2	Test 3	AVG pH
Vermiculite	5.97	5.84	6.53	6.03
Silt	7.6	8.03	8.25	7.87
20 80	7.9	8.24	8.18	8.08
20 60 20	8.03	8.39	8.37	8.23
60 40	8.34	8.45	8.54	8.44

Discussion

Ecological succession is a transition over an ecological time of species composition. Primary succession occurs in an area with no topsoil, and is very slow to develop due to many physical and chemical stresses (Keeton and Gould 1986). Secondary succession occurs on fertile soil for root development, and can take over much more rapidly due to the soil being well developed, and allowing a new community to develop (Keeton and Gould 1986). The goal is to recover the De Beer Victor diamond mine to establish primary succession to recover the soil so that secondary succession can occur. This will result in new species that will grow and sustain the land little to no maintenance.

Chemistry of soil mixes appears to be a key factor responsible for differences in plant growth. The experiment showed that with an increased composition of processed kimberlites in the soil, there was a reduction in root and shoot development and nodulation. Higher amounts of FPK in the mix also reduce plant growth, compared to the coarser kimberlites (CPK), which was also shown by Rouble (2011). Kimberlite and serpentine soils are known for low exchangeable Ca:Mg ratios (<1), but they also have a high transition element concentration with heavy metals (Cr, Mn, Fe, Co and Ni) that can cause minor toxicity problems from heavy metals to turn into serious problems (Alexander 2009: Moore & Zimmerman 1977). Slightly elevated Cr content in the processed kimberlite at Victor mine, especially in the FPK (K. Bergeron, unpublished data), may be related to the lower growth in kimberlite soils. The pH of the FPK and CPK is also high, with a pH range of 8-8.6, higher than the upland soils of the Hudson Bay (pH 7-8) (K. Garrah and K Bergeron, unpublished data). The inclusion of peat (pH ~6) could reduce the alkalinity of the soil, but Rouble (2011) did not reduce the pH even after seven weeks mixed with 40% peat.

Physical properties of the soil, such as texture and particle size also appear to be of primary importance for plant development. The fine grained soil mixtures in this experiment, (100 % silt, and 60:40 FPK:CPK) underwent drying periods in the growth chamber in between watering times that could have affected plant growth. These drying periods lead to hardening and mud cracks that fine grained soil are susceptible to as well as shrinking and swelling periods. Once the hardening of the soil occurred it was difficult for roots to grow and absorb water and nutrients. The hardening and excess water loss to the plants may be due to the dark green/grey colouration of the kimberlite soils (Reid & Naeth 2005). The dark colour promotes a high summer surface temperature to be retained in the soil and increases the water loss to evaporation from the surface layer (Reid & Naeth 2005).

Coarse sized grained soil mixtures used in this experiment (20:80 silt:CPK) typically have lower water retention capabilities, and lower water holding capacities than the fine grained mixtures (Rouble 2011). These soils did stay loose in between watering times, allowing roots to grow and access water and nutrients with in the soils allowing roots to easily penetrate in between particles. With the addition of organic peat, it helped increase the watering holding capacity in the soil matrix and soils did not become too hard or show signs of cracking**.**

Silt soil that produced the most roots and shoot biomass besides the vermiculite control and was the most productive soil in producing nodule formation. The medium texture of silt allows for optimal pore spaces provide good water movement and is considered for optimal water-holding capacity and nutrient availability (Dexter 2002). This silt is essentially ground calcium carbonate, so its amendment also acts to increase the Ca:Mg ratio in these soils.

Peat performed an important role for these amended soils by increasing the moisture content in the soils, and possibly increasing the Ca:Mg ratio. The addition of peat was advantageous to the amended soil mixtures because it held more water and nutrients for the roots. It also allowed the soil to stay loose, making it less difficult for the roots to penetrate further into the soil (Reid & Naeth 2005). The addition of peats may also explain why the 100% silt mixture was able to produce the largest biomass in the amended soil. The texture and particle size was small but with the addition of peat, it helped loosen up the soil allowing for optimal growth and available nutrients for the nitrogen fixing plants.

Inoculation of a litter tea mixture to help nodulation was observed to be successful on every plant in every soil. Hutton et al. (1997) found that inoculating soils greatly increased the mycorrhizal potential for growth of plants of Epacridaceae in a highly disturbed mined area in Australia. Inoculation seems to be of the utmost importance. A study by Chaia et al. (2010) of crushing nodule and releasing *Frankia* bacteria into the soil allows the nodules to trap and harness the power to fix nitrogen in nitrogen limiting conditions.

The use of these fast-growing nitrogen-fixing legumes would reduce the costs of importing excess nitrogen fertilizers to the isolated area of Victor Diamond mine in the Hudson Bay Lowland and would be a catalyst in sustaining nitrogen supply naturally. The supply of nitrogen within the soil for other plants for secondary succession to begin would be very low, because most fixed nitrogen goes directly into the plant producing it (Lindemann & Glover 2003). But when the nitrogen-fixing legumes die and start decomposition the nitrogen supply would be recycled and returned to the soil (Lindemann & Glover 2003). White clover was used in this experiment as a control because of its fast growth and early colonization and tolerance to disturbed site, and its capability to fix nitrogen (van Eekeren et al. 2009). Legumes species including *T. repens* can fix nitrogen even under limited nutrient conditions. The non-native white clover was used instead of a native species due to the lack of available seeds of native legumes during the time frame of this experiment. The use of native legume species that are found in the Hudson Bay Lowland for remediation are important variable to pursue. Common native species to the Hudson Bay Lowland from the Fabaceae Family including alpine milk-vetch (*Astragalus alpinus*), wild pea, (*Lathyrus palustris*), and American vetch, (*Vicia americana*), should be tested if there was an available seed bank of these, to grow these species in the Victor diamond mine waste soil. Wild pea, *Lathyrus palustris*, was studied by Vince & Snow (1984) and showed it can grow successful on riverbank levees in a silty delta in Alaska.

Actinorhizal shrubs are another group of plants used for reclamation projects that fix nitrogen and are among the best plants to establish in a nitrogen poor habitat (Bargali 2011; Roy et al 2007). Alder sp. has been tested many times to examine the success of reclamation and rate of growth on landscapes affected by mining using the existing mining waste materials as soil (Robb 2001; Roy *et al* 2007). Many separate studies show varying pH levels that produce optimal growth for the *Alnus* sp.. A study by Berry & Torrey (1986) found that optimal nodulation and shoot growth was at a pH 5.5 compared to Wheeler *et al* (1986) discovered that *Alnus* sp. could live and sustain growth at a pH of 4.5 to 6.5. Schalin (1968) tested specifically for *A. crispa* and found that it preferred growth at a pH closer to 4. All of these experiments found that *Alnus sp*. have optimal growth in low pH, acidic soils. The high alkaline of the kimberlite soils from the Victor Diamond mine (pH 8-9) could have reduced the growth capacity and resulted in so many deaths and lack of survival. CaCO3 tolerance of *Alnus,* is extreme low as well as having a pH tolerance between 4.8-7.0 (Natural Resources Conservation Services 2012). The high survivability of the green alder in the vermiculite soils could be do to the low average pH (pH= 6.03), which is with in the optimal range set by Natural Resources Conservation Services (2012) for *Alnus* sp. The amended silt soil sample had the lowest pH from the Victor Diamond mine (pH=7.87), which is just outside of the optimal range, but the high alkaline pH ranges where the soil samples that contained kimberlites, pH 8.08-8.54 did not allow for the green alders to establish and survive as well. Sader et al. (2003) found that Kimberlite pipe samples had a very average high alkaline pH around 9.54 in Kirkland Lake in Northern Ontario. Nutrient-imbalance in the soils can highly affect the re-establishment of plant growth. Disturbed mining waste soils can greatly decrease the nitrogen-fixing bacteria of the actinorhizal shrubs, and also affect the mycorrhizae (Hutton et al. 1997).

Limiting available nutrients in the Victor diamond mine affect the growth, productivity and the ability to nodulate in nitrogen fixing plants. There is no need to add nitrogen fertilizers when using nitrogen-fixing plants because it reduces their ability to fix nitrogen, and has also been observed to damage *Frankia* vesicles in *Alnus sp*. (Burgess & Peterson 1987; Koo et al.1996). Conversely, phosphorus fertilizers enhance the formation of nodules in infertile soils, but reduce the formation of mycorrhiza (Koo et al.1996; Seilers & McCormick 1982). Koo et al. (1996) did an experiment that phosphorus fertilizers actually enhanced the amounts of nitrogen that was fixed by the red alder (Koo et al.1996). As nitrogen-fixing plants start to mature rapidly in the early stages increasing the amount of nitrogen biomass, requiring more phosphorus, there is an increased demand of phosphorus to the plant.

Almost all phosphorus is not immediately available to the plants for uptake. It usually is bound to soil constituents before the plants uptake through their roots (Grant et al. 2005). A study by Brown and Courtin (2003) testing the growth productivity of red alder, *Alnus rubra*, in acidic soils with limiting nutrients show that fertilization with phosphorus increased the seedling growth and overall growth of the whole plant mass. The effects of phosphorus additions showed greater biomass production in lower pH soils tested (Brown and Courtin 2003). Increased concentrations of phosphorus fertilizers levels, similar to the concentrations used in this study, showed an increase in exchangeable Ca concentrations (Brown and Courtin 2003). This increased exchange of Ca, could help balance the Ca:Mg ratio that is less than one, which is seen in the Victor mine waste soils. The increase concentrations of phosphorus fertilizers also showed that the whole-plant and root nodules proportions increased (Brown and Courtin 2003). Increased phosphorus had a greater effect on *Alnus* sp. growth in more acidic soils than the higher pH soils (Brown and Courtin 2003). The N/P ratio of a plant is also very particular. As the nitrogen fixing plant species matures, the plant increases the amount of nitrogen in its biomass, requiring more phosphorus. However, higher phosphorus treatments aid in overall plants biomass development but have reduces mycorrhizal development in actinorhrizal shrubs (Grant et al. 2005). The presence of mycorrhiza can help the plants grow by directly increasing plants growth through the uptake of other nutrients such as phosphorus as well as indirectly improving soil conditions, such as adding aggregates and improving stability to the soil (Tisdall & Oades 1982; Abbott et al. 1992). Phosphorus amendments must therefore be sufficient to encourage N-fixation, but cannot be excessive, which would prevent mycorrhizal development (Grant et al. 2005).

Conclusions

Nitrogen-fixing plants were able to grow in kimberlite based on available mining waste material; however success depended on the species and on the phosphorus amendments. The legume species white clover (*Trifolium repens*) was able to survive in all high alkaline soils but the actinorhizal shrub species, green alder (*A. crispa*) could not tolerate the transplantation and/or the amended soils from the Victor Diamond mine, especially at higher phosphorus levels. The locally available 100% silt amendment performed the best for nitrogen-fixing plants tested in this study, contrary to previous studies with non-N-fixing grasses, which showed only moderate growth in silt as compared to soils with higher CPK content. Using nitrogen-fixing plants helps the amended soils to be less dependent on inorganic fertilizers and able sustain growth naturally. Increasing FPK and lower CPK also reduced the growth and nodulation of species. Inoculation proved to be successful for promotion of nitrogen fixation in both species. Phosphorus fertilization did not promote plant development, and the high phosphorus treatment killed almost all of the green alder. Since each plant species has certain characteristic that are optimal for growth, further studies need to be conducted to test the nitrogen fixation of more native nitrogen fixing plants found with in the Hudson Bay Lowland area. This study does however demonstrate clearly that N-fixing species can be a long-term solution to nitrogen amendments and remediation on mining wastes at the De Beers Canada Victor Mine. Using nitrogen-fixing plants helps the amended soil to be less dependent on inorganic fertilizers and able sustain growth naturally.

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