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The Effect of Biochar Amendment, Microbiome Inoculation, Crop Mixture and Planting Density on Post-Mining Restoration

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Abstract: Ecological restoration with a multispecies and multifunctional approach can accelerate the re-establishment of numerous ecosystem services. The challenges with land that is degraded, damaged, or destroyed post-mining are the low productivity of soil and the high potential for contaminants. Herein, we evaluated the multispecies and multifunctional approach to restoration strategy through a mixture of woody and herbaceous species, microsymbiont and biochar amendments, and plant spacing. The experiments were conducted using greenhouse and field trials located in Quebec, Canada. We used a mixture of tree species (*Alnus viridis* (Chaix) DC. ssp. crispa (Aiton) Turrill, *Picea glauca* (Moench) Voss, *Populus tremuloides* Michx. and *Salix arbusculoides* Andersson) and herbaceous species (*Avena sativa* L., *Festuca rubra* L. and *Trifolium repens* L.) on two types of gold-mine waste materials (fine tailing and waste rock). The biochar amendment and microbial inoculation were applied on both greenhouse and field trials. We found both positive and negative effects of plant spacing, biochar amendment and inoculation depending on their interactions. The net positive effect was shown by combining high plantation density, biochar, and inoculation factors on *Alnus viridis* ssp. crispa. Overall, plantation density was shown to be the most important factor in generating the net positive effect. We suggest that the mechanism was correlated with the improvement in microclimate through soil plant water conservation and microbial activity enhancement over soil temperature modification. Hence, we propose to put emphasis on microclimate improvement for accelerating the restoration processes, along with other combined factors, including microbial inoculation and biochar amendment.

Keywords: restoration; facilitation; post-mining; plantation spacing; biochar; inoculation

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1. Introduction

Since the industrial era, land degradation, damage and destruction (3D) (hereafter, collectively referred to as degradation), have become some of the most significant environmental problems globally. These problems have diminished the biodiversity, functioning and resilience of ecosystems, which in turn negatively affects the resilience and sustainability of social–ecological systems [1]. The tradeoffs between economic benefits and the loss of ecosystem functions seem to be unsustainable in terms of natural resources. The ecological restoration paradigm, which focuses on enhancing ecosystem services and increasing resilience, is believed to be the best environmentally sustainable practice [2]. Accelerating land restoration with similar principles could be beneficial considering the expansion in demand for land and food [3].

Multi-species mixtures of trees and crops in agroforestry bring about a unique set of ecological interactions that can be positive, neutral or negative among different species [4–6].

If the agroforestry system formed by different multi-species mixtures of trees and crops makes it more resilient, eases recovery from disturbances and accelerates successional processes [2,7,8], then this system may in total accrue ecosystem goods and services greater than the output of those species if they were grown separately on an equal area of land [5]. The multi-species approach has been suggested in restoration practices [9–12]. However, the mechanism by which the species diversity and the ecosystem function is sometimes confusing [13], and most of the restoration experiments have used the trial-and-error approach [10], which makes it difficult to predict the optimum method in various cases. Therefore, understanding both the biophysical processes and the mechanisms involved in the allocation of resources is essential for the development of ecologically sound agroforestry systems that are sustainable, economically viable, and socially acceptable [4].

The diversity of the soil ecosystem has the same role as the diversity of aboveground species in providing ecosystem services. A healthy soil ecosystem is formed by various species of microorganism with specific roles and functions [14]. While some plants are hosts for other symbiotic microorganisms, their existence and diversity are highly correlated [15]. The introduction of inoculation with mixed microorganism species is expected to restore the soil ecosystem and ameliorate the soil conditions. At the same time, some microorganisms are also able to extract contaminants from soil, which are often used in phytobial remediation technologies [16,17]. Degraded post-mining soil is not an ideal material for plant growth. The physico-chemical and microbiological characteristics of the materials are too poor for optimal plant growth [18,19]. Fertilization may help the plant to grow better, but it can be costly and not sustainable in ecological restoration [20]. The introduction of soil amendments could be a sustainable option supporting plant growth, as it has been shown to improve soil properties and functions relevant to agronomic and environmental performance [21,22]. Hypothesized mechanisms for such a potential improvement are mainly enhanced water and nutrient retention (as well as improved soil structure and drainage). Furthermore, there is experimental evidence that soil microbial communities and their activity, which have key roles in sustaining soil health and functioning, are directly affected by the addition of biochar to soils [23–26].

Here, we evaluated the mixture of woody and herbaceous plant species with the introduction of microsymbionts through inoculation and the application of biochar amendments for accelerating the restoration processes. The spacing effect was also tested to find out the interaction mechanism between the plant species and their micro-environment. The aim of the research is to find the best method for the restoration of post-mining sites.

2. Materials and Methods

2.1. Biochar and Hydrogel Amendments

The biochar used in this experiment was the commercial Award-Maple-700 made through pyrolysis of maple bark at 700 °C for 20 min from the company Award Rubber (Windsor, QC, Canada). The physicochemical properties of the biochar are pH 8.39 ± 0.75, cation exchange capacity (CEC) 26.3 ± 2.2 $cmol^+ kg^{-1}$, C total 65.4%, ash content 14.2%, N 0.58 ± 0.01%, P 805 ± 22 mg kg^{-1}, $K_{exchangeable}$ 8.09 ± 0.4 $cmol^+ kg^{-1}$, $Na_{exchangeable}$ 2.03 ± 0.05 $cmol^+ kg^{-1}$, $Mg_{exchangeable}$ 2.23 ± 0.22 $cmol^+ kg^{-1}$, $Ca_{exchangeable}$ 13.9 ± 1.6 $cmol^+ kg^{-1}$, Electrical Conductivity (EC) 0.48 dS m^{-1}, total porosity 0.8 $m^3 m^{-3}$, and true specific density (ρs)1.76 ± 0.03 g cm^{-3}.

The commercial hydrogel from Solid Rain Corp. (San Diego, CA 92101, USA) was used in this experiment. This hydrogel is a soil amendment with the main component $(C_3H_3KO_2)_n$ (potassium polyacrylate). This polymeric material, with its hydrophilic structure, can hold a large amount of water in its three-dimensional networks. We used a dosage of 15 kg per hectare as recommended by the usage instructions of the product.

2.2. Plant-Microbial Organisms

The selected plant species were *A. viridis* subsp. *crispa*, *P. glauca*, *P. tremuloides* and *S. arbusculoides*. These four species are native to North America region and are commonly found growing in the Abitibi-Témiscamingue region. Each of those species can be dominant or codominant depending on the type of habitat [27]. The composition of the habitat may change over time with the dynamics of the ecosystem and successional processes. In this experiment, we combined all those four species at the initial stage and expected to obtain the potential benefit of different species' composition for accelerating restoration processes. *P. tremuloides* and *S. arbusculoides* are the fastest-growing species, followed by *A. viridis* subsp. *crispa*, which is also relatively fast-growing. The three species are light-demanding species and are considered pioneer species, while *P. glauca* is slow-growing species and is considered a mid- to late-successional species with shade-tolerant characteristics [28]. All the listed species are tolerant and adapted to poor soil and disturbed sites and are often used for restoration and rehabilitation projects [19,28–31], especially *A. viridis* subsp. *crispa*, which can grow well on poor soils because of its association with the nitrogen-fixing actinobacterium *Frankia* spp. and mycorrhizal fungi [32]. Herbaceous species associations were also included as one of the treatment factors. The herbaceous species included oat (*A. sativa*), red fescue (*F. rubra*), and white clover (*T. repens*). *A. sativa* is a grass species that is grown for its seeds for human consumption and as a livestock feed. The other grass species *F. rubra* is known for its tolerance of heavy metal contamination and is often used for phytoremediation in post-mining restoration [33]. *T. repens* is also quite tolerant of heavy metal contamination and also has the ability to fix nitrogen with Rhizobia [34]. As a legume species, *T. repens* can fix nitrogen up to 80 g N ha^{-1} h^{-1} in contaminated soil [34]. Apart from those benefits, herbaceous species have a faster turnover rate, which contributes to soil organic accumulation, which can be advantageous for associated woody species.

Fine tailings and waste rocks have poor soil nutrients and organic matter, which may limit microbial activity in these challenging materials. Therefore, we included microorganism inoculation as part of the experimental factors. The microorganisms applied as inoculants were *Cadophora finlandia*, *Tricholoma scalptiratum*, *Azobacter chroococcum*, *Pseudomonas putida*, *Frankia alni*, and the commercial inoculum of arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* produced by the Company Premier Tech Biotechnologies (Rivière-du-Loup, QC, CA). The microorganisms were inoculated on tree seedlings based on their known symbiotic associations as follows: *C. finlandia*, *T. scalptiratum*, *A. chroococcum* and *P. putida* on *P. glauca* and *S. arbusculoides*; *C. finlandia*, *P. putida*, *F. alni* and *R. irregularis* on *A. virdis; T. scalptiratum*, *A. chroococcum*, *P. putida* and *R. irregularis* on *P. tremuloides*. For the herbaceous species, the inoculant was added to the hydroseeding mixture.

2.3. Greenhouse Mesocosm Experiment

The rectangular plastic containers of 34 cm × 54 cm × 18 cm were used as a mesocosm experiment unit. They were filled with two types of waste gold mine materials (fine tailing and waste rock). Biochar and Hydrogel amendments, a micro-symbiont inoculation, and combination of tree and herbaceous crop species (HerbMix) were used. The experimental design was a split–split plot with 3 blocks and 36 treatment combinations, resulting in a total number of 108 experimental units. A mixture of woody species was randomly planted in a Latin square arrangement, as shown in Figure 1, with 8 cm spacing. The purpose of this arrangement was to give a balanced interaction for all 4 plants randomly allocated amongst 16 plots, such that each species appears once in each of four column blocks and once in each of four row blocks. For each species, we had 4 individual plants, where 3 were planted on the border and another one inside the square.

The woody plants were first propagated for one week in small 10 × 20 mm pellets Jiffy-7 Forestry (Stuewe & Sons, Inc., Tangent, ON 97389, USA). Half of the seedlings were inoculated with specific microorganisms and planted in the designed containers. The crops were planted using the hydro-seeding medium in which the specific symbiotic microorganisms were mixed for the inoculation factor. Biochar was applied at a rate of 0.0075 m^3/m^2. The hydrogel was applied at 20 g/L of water mixed with soil.

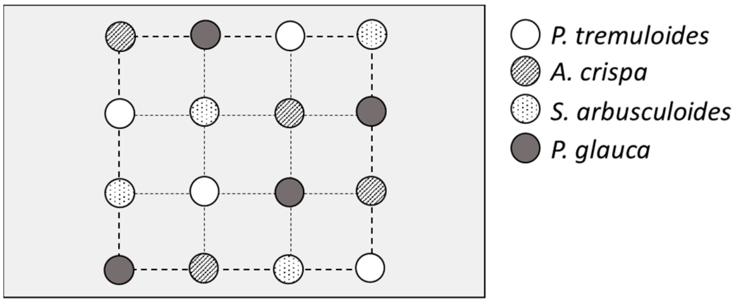


Figure 1. Tree seedling plantation arrangements. The seedling positions are shown using colored circles (gray shading), and the color legend shows different species.

Fertilization 20-8-20 (50 ppm) was applied once at the beginning of the experiment. The temperature of the greenhouse was maintained at 23 °C (daytime) and 16 °C (nighttime), with an average humidity of 50%. The experiment was set up in June 2016 and lasted for three months. After three months, soil respiration was measured using the LICOR LI-6400XT Portable Photosynthesis, Fluorescence, Respiration System (Lincoln, NE 68504, USA). The 6400-09 Soil CO_2 Flux Chamber was installed on the LI-6400XT system for measuring the CO_2 flux from the soils. The soil core at a 6 cm depth was used as the interface of the soil surface and the flux chamber. The plant shoot and root biomass were harvested at the end of experiment, and the dry weight was measured.

2.4. Field Trials

The field trials were established on two mining sites, Sigma-Lamaque (now called Eldorado Gold Lamaque, EGL) and Metanor Resources (now called BonTerra Resources, BTR) in Abitibi-Témiscamingue region, Quebec, Canada. The location of these two mining sites is shown in Figure 2. Two types of waste materials were selected: fine tailing and waste rock. The field coordinates are 48°06′38.4″ N and 077°44′44.74″4 W (fine tailing—EGL), 48°06′20.7″ N and 077°45′43.1″ W (waste rock—EGL), 49°29′40.1″ N and 076°08′49.9″ W (fine tailing—BTR), 48°59′03.8″ N and 075°46′18.4″ W (waste rock—BTR). The average daily temperature at BTR site is 1 °C, with the temperature range between −23° and 23 °C. The average precipitation is 702.3 mm and the snowfall 226.2 cm. The weather information is based on data obtained between 1981 and 2010 from the Canada Environment and Natural Resources website (http://climate.weather.gc.ca/climate_normals/index_e.html, accessed on 1 March 2020). Our weather station installed in June 2016 at EGL site showed the precipitation of 703.8 mm, with temperature range between −24.1°C and 25.4 °C and a mean annual temperature of 4.3 °C.

Gold mining operations produce waste materials such as soil, rock, and fine tailing during gold extraction. Waste rock is often stored in heaps or dumps on the mine site. Tailings are finely ground and can contain leftover processing chemicals such as arsenic (As). These tailings are usually deposited in the form of a water-based slurry in tailings ponds that are left to evaporate over time [35,36]. The tailings are often stored underwater to reduce contact with the atmosphere and prevents oxidation [36]. In a dry climate, evaporation from ponded tailings water and wet tailings can lead to a salinity concentration. The tailings in our field sites are mainly composed of biotite and Fe (Taner et al., 1986). The chemical analyses [37] are as follows: sulfur (0.48 to 0.51%), Al (5500 to 6100 mg kg^{-1}), Ca (21,000 to 23,000 mg kg^{-1}), Fe (14,000 to 16,000 mg kg^{-1}), Mg (4000 to 4500 mg kg^{-1}), P (0 to 560 mg kg^{-1}), and K (86 to 100 mg kg^{-1}). Zn, Mn, Cu, Mo, and Na were found in low concentrations and there was no N in the tailings. The pH of tailings was between 8.55 and 8.68. The arsenic (As) and cyanide concentrations were quite high (8 to 9 mg/kg and 3.7 to 6.3 mg/kg, respectively). Fine tailings have very low hydraulic conductivity in the range of 10^{-4} to 10^{-5} cm/sec, with a grain size <74 µm [35], while waste rock has very high hydraulic conductivity ranging from 10^{-1} to 10^{2} cm/sec, with particles' grain size ranging from sand (625 µm–2 mm) to gravel-free particles (<2 mm) [36]. Soil material with large particle size and high hydraulic conductivity such as waste rock is not suitable for plant growth. This material has a low water holding capacity and may lack water in dry periods. Silty clay soil such as fine tailing with very low hydraulic conductivity is also not good for plant growth. This silty clay can be sticky and plastic when wet and prone to drainage problems but hard when dry [38]. Both materials have very extreme physico-chemical properties that are not suitable for plant growth, and the ideal soil texture is between loam and silt, with a pH between 5.8–6.5 [38].

Figure 2. The site location at the gold mine in Quebec, Canada.

Set up of Experimental Design Field Trials

The field trial was set up as a split–split plot design arrangement. The trial had 12 combinations of factors and 4 replication blocks in each waste rock and fine tailing sites on two mining sites, with a total of 192 plots. The treatment factors were biochar amendment, micro-symbiont inoculation, and a combination of tree and herbaceous crop species. The plot dimensions were 5 m × 8 m, and the tree plantation arrangement is shown in Figure 3. The plant seedling position was arranged in a patch with different inner spacings. Each patch contained 4 seedlings of different species: *P. tremuloides*, *A. viridis* subsp. *crispa*, *S. arbusculoides* and *P. glauca*. The inner spacings were 20 cm × 20 cm, 40 cm × 40 cm and 60 cm × 60 cm with 4 replicates in each plot. Each block consisted of 8 plots with tree seedlings and 4 plots with herbaceous crops. The block was arranged from the North (block 1) to the South (block 4) direction, and the distance between blocks was 4 m.

The seeds were germinated on 20 × 32 mm pellets Jiffy-7 Forestry in the greenhouse. The pellet was made from peat and coir. After 3 months, half of the seedlings were inoculated with specific microorganisms. The seedlings were then grown in the greenhouse for 4 months. The seedlings were moved outside the greenhouse for one week before being transported to the planting site. The planting took place in June 2016 and was monitored for two growing sessions in September each year.

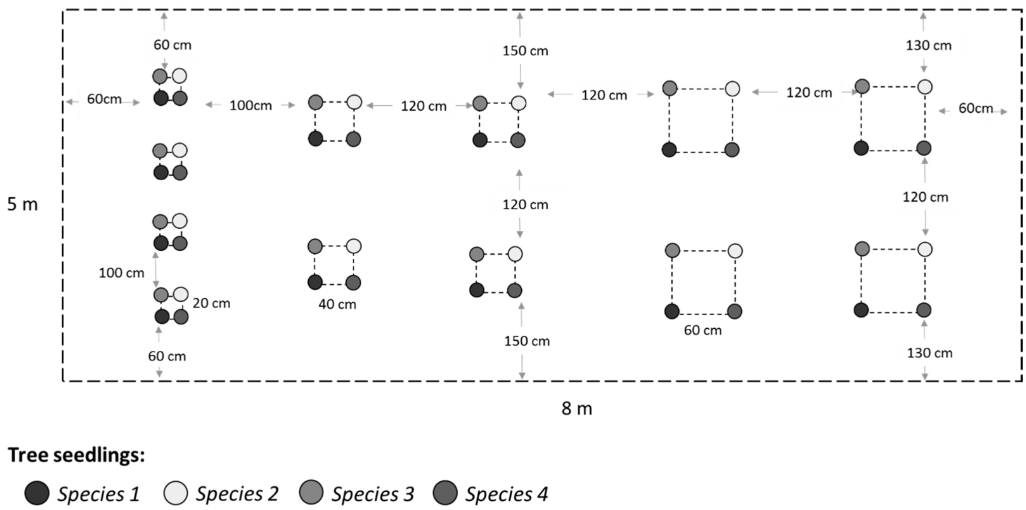


Figure 3. A plot showing tree seedling plantation arrangements. The seedling position is shown by the circle and the grayscale legend shows a distinct species.

The herbaceous crops were planted using hydro-seeding method with commercial mulch Beno-Vert made from recycled paper (Soprema-Quebec, Quebec, QC, Canada). The seedling rate ratio was based on the common seedling rate of oats (*A. sativa*) as a companion crop, which is 50 to 75 kg/ha [39]. However, here, we increased the seedling rate ratio for 10% (84 kg/ha) to compensate for the higher mortality rate on degraded soil. For the other herbaceous species, the weight ratio was adjusted for the same number of seeds as oats, 8 kg/ha for red fescue (*F. rubra*) and 4 kg/ha for white clover (*T. repens*). The application used Beno-Vert and additional Beno-Tack, a vegetal adhesive for the complement binder. The Beno-Vert application was 1500 kg/ha and Beno-Tack 60 kg/ha mixed with 250 g/ha of 15-30-15 fertilizer in water (40,000 L/ha). Therefore, the crop seed composition was 8 kg/ha of red fescue seed, 4 kg/ha of white clover, and 84 kg/ha of oat seeds, mixed with Beno-Vert solution. Biochar application was 75 m^3/ha or about 20 ton/ha, mixed with the soil on the surface down to 5 cm depth in fine tailing. The tilling machine was used for mixing the biochar. On the waste rock site, the biochar was spread on the surface without mixing.

Soil moisture sensors were installed in two blocks of fine-tailing material at the EGL site. The probes were installed at 15 cm and 30 cm depths on each experiment plot within the two blocks. Lysimeters were also installed on the same blocks at a 15 and 30 cm depth, and water was sampled weekly. A weather station was also installed on this site to measure daily precipitation rate, air temperature, relative humidity, and soil moisture (at 15 cm depth).

2.5. Statistical and Data Analyses

The data collected in the field trials were stem diameter and height increments for tree species. The herbaceous plants were not measured because they only grew during the first growing season. The plant diameter was measured at the ground surface level. The measurement was performed at the beginning of the experiment (June 2016), in September 2016 for the first growing season, and in September 2017 for the second growing season. Since data were collected in a shorter time range (4 months) during the first growing season and could have been biased due to the adaptation factors, we decided to use only growth data from the second growing season (one-year growth). Apart from that, we also excluded the height increment data because we found bias on some plants that were broken and/or re-sprouting, resulting in negative increments and high variabilities.

For all statistical analyses, we used the general linear mixed-effects regression model for a split–split plot design [40,41]. The fitting uses the restricted maximum likelihood (REML) method from the lme4 package [42] in R Statistic software (version 4.2.1, Vienna, Austria) [43] for both greenhouse experiments and field trials with unbalanced data. A general statistical term with the assumption of fixed-effect (α, β) and split–split plot factors ($\varnothing$, ρ, δ) is as follows [40]:

$$\begin{gathered} y_{dhijqrt} = \mu + \theta_h + \alpha_i + (\varnothing 2)_{q_2} + (\rho 2)_{r_2} + \epsilon^W_{d(h)} + \beta_j + \alpha\beta_{ij} + (\varnothing 3)_{q_3} + (\varnothing_2\varnothing_3)_{q_2 q_3} \\ +(\rho 3)_{r_3} + (\rho_2\rho_3)_{r_2 r_3} + \epsilon^{SS}_{ijq_3r_3(dhq_2r_2)} + \delta_t + (\beta\delta)_{jt} + \epsilon^{SS}_{t(dhijqr)}, \\ \theta_h \sim N(0,\ \sigma^2_\theta),\ \epsilon^W_{d(h)} \sim N(0,\ \sigma^2_W),\ \epsilon^S_{ijq_3r_3(dhq_2r_2)} \sim N(0,\ \sigma^2_S),\ \epsilon^{SS}_{t(dhijqr)} \sim N(0,\ \sigma^2_{SS}) \end{gathered} \tag{1}$$

θ_h's, $\epsilon^W_{d(h)}$'s, $\epsilon^{SS}_{ijq_3r_3(dhq_2r_2)}$'s, $\epsilon^{SS}_{t(dhijqr)}$'s are mutually independent.
d, h, i, j, q, r, t are the observation levels for each corresponding factor.

The dependent variable for greenhouse experiments are dry plant biomass and root:shoot ratio. The fixed effects are tailings, soil supplement, inoculation, and herbaceous mixture (HerbMix). The random effects on a split–split plot design are inoculation nested under supplements, under tailings, and under blocks. The statistical term for lmer method on R software is as follows [40,41]:

$$Y = Tailings * Supplement * Inoculation * HerbMix + (1 \mid Block/Tailings/Supplement/Inoculation) + \epsilon \tag{2}$$

where Y is a dependent variable for plant biomass and the shoot:root ratio.

The field trial data analyses excluded the spacing factor on the waste rock site because the planted seedlings had a mortality of up to 80%, which made the spacing arrangement no longer consistent. The analysis was then split into two regression models, with the spacing factor (fine tailing only) and without the spacing factor (fine tailing and waste rock).

The dependent variable for field trials was the plant diameter growth. The fixed effects are species, tailings, initial diameter (InitDiameter), biochar, inoculation, and herbaceous mixture (HerbMix). The random effects on the split–split plot design were herbaceous mixture nested under inoculation under biochar, under blocks, under tailings, and under site location. The statistical term for the lmer method on R software is as follows:

$$Growth = Species * Tailings * InitDiameter * Biochar * Inoculation * HerbMix + (1 \mid Location/Tailings/Block/Biochar/Inoculation/HerbMix) + \epsilon \tag{3}$$

The statistical term for field trials with the inclusion of spacing effect on fine tailing only (removing the tailings factor) is as follows:

$$Growth = Species * InitDiameter * Biochar * Inoculation * HerbMix * Spacing + (1 \mid Location/Block/Biochar/Inoculation/HerbMix) + \epsilon \tag{4}$$

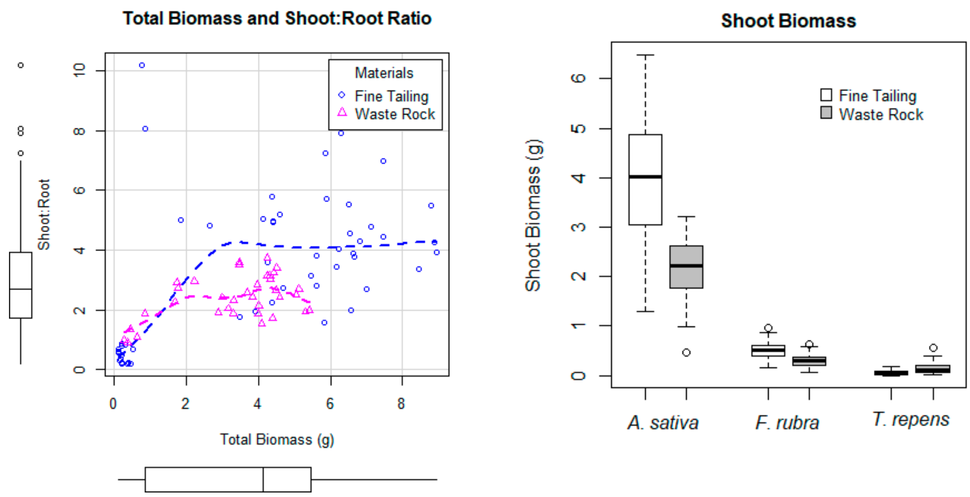
The plot of marginal effects interaction terms is displayed with error bars showing the 95% confidence intervals, unless mentioned otherwise in the captions.

3. Results

3.1. Greenhouse Experiment

The woody species were not grown in the greenhouse experiment, and the average survival rate was only about 10% in the mesocosms. Thus, species-specific aboveground biomass data analysis was only applied to herbaceous crop species, but the total above- and below-ground biomass was for all species, including the woody species.

The total biomass was dominated by *A. sativa* and seemed to be higher in fine tailing than in waste rock material, as shown in Figure 4. Waste rock material has low water retention and high hydraulic conductivity, which may lead to nutrient leaching with daily watering. On the other hand, fine tailing has high water retention, which allows he conservation of nutrients, but the retention was too high to permit penetration of the water deeper in the soil. This fact seems only beneficial for herbaceous crop species with shallow fibrous root characteristics.

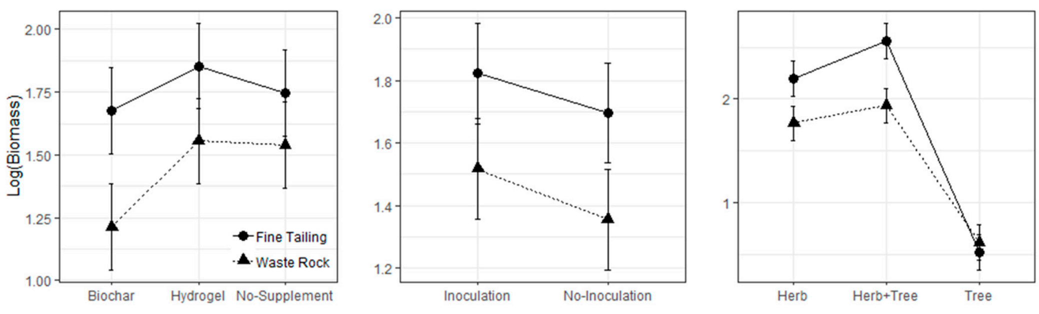


(**a**) (**b**)

Figure 4. (**a**) Dry weight biomass and shoot:root ratio of herbaceous and woody species, compared between fine tailing and waste rock growth materials after 3 months of growth. The loess regression curve shows a tendency of constant shoot:root ratio on bigger plants. (**b**) The biomass yield of herbaceous species on fine tailing material tends to be higher compared to that on waste rock material.

The statistical analysis is shown for fixed effects in Table S1 and for random effects in Table S2. The total biomass has a significant interaction between herbaceous mixing with tailings as well as herbaceous mixing and amendments. The shoot:root ratio was shown to be lower on waste rock material (Figure 4), which could be an indicator of higher limitations of nutrients compared to fine tailing material. However, statistically, we found no correlation between the shoot:root ratio with all treatment factors in our experiment (Table S1), although in Figure 3, we can see some differences between tailing materials. The stunted seedlings of the tree treatment (perennial species) with a low survival rate, caused a high variability in the shoot:root ratio, and thus the statistical results for the tree treatment should be interpreted cautiously.

Figure 5 shows that some interaction effects of the main factors on the total biomass were significant, but we could not find other slightly significant effects on the interactions between some main effects at $p < 0.05$ (see Table S1). Herein, the biochar amendment had a negative effect on total biomass, while hydrogel showed a slightly positive effect but as not significant at $p < 0.05$.



(**a**) (**b**) (**c**)

Figure 5. (**a**) The interaction effect of substrates materials and soil amendment on total biomass. (**b**) The interaction effect of substrates materials and microbial inoculation on total biomass. (**c**) The interaction effect of substrates materials and plantation method on total biomass. The error bars represent the 95% confidence interval (CI) of means.

The measured soil temperature using the LICOR temperature sensor showed that the biochar treatment has a slightly higher temperature. Our assumption is that biochar might increase the soil surface temperature (by lowering the soil albedo), which may accelerate the evaporation and limit the available water for the plant.

The inoculation showed a positive effect on biomass yield ($p < 0.01$). The consistent effect between fine tailing and waste rock materials showed that microbial inoculation helped accelerate plant growth in both tailing materials. The positive effect of tree species inclusion on total biomass could be more due to the additional biomass from tree species. However, the analysis of the aboveground biomass of herbaceous species also showed the same positive effect on the inclusion of tree species, suggesting that there could be an indication of facilitative effects from tree species. In a separate analysis, we found that the benefits of the addition of tree species are higher with biochar treatment, thus suggesting that the inclusion of tree species might help in reducing the negative effect of biochar on soil surface temperature. Since we did not observe this specific effect of biochar on this aspect, this hypothesis might need to be verified with another experiment.

3.2. The Field Trials

The plant mortality was high at the waste rock site (60% at Sigma and 80% at Metanor), while fine tiling showed a better survival rate (2% and 10% mortality on the Sigma and Metanor sites, respectively) during the first month after initial planting. Surprisingly, the rest of the plants on the waste rock were able to survive until the second growing session with a mortality of less than 10%. Thus, we assumed that the cause of high mortality (up to 80%) was only during the initial adaptation to the transplant shock, which could be due to the harsh climate and worse soil conditions on the waste rock material. The spacing analysis was then excluded for the waste rock materials as it no longer had the proper spacing arrangement.

We analyzed plant diameter increment as dependent variable, and the factors of species, initial diameter, time of measurement, soil material, biochar, inoculation, and species mixing. The analysis of the variance table is shown in Tables S3 and S5, while the standard deviation for random effect is shown in Tables S4 and S6. Table S3 is the analysis of variance for all data on waste rock and fine tailing but without the spacing factor, while Table S5 is the analysis of variance for fine tailing only with the inclusion of the spacing factor.

We found interactions between all factors ($p < 0.01$) and that it is difficult to interpret all the interactions at once. Thus, we used the marginal interaction effect from the model analysis. The interaction was mostly consistent between fine tailing and waste rock tailings. Figure 6 shows the marginal interaction effect for each plant species and tailings with biochar treatment on plant diameter increments.

The marginal interaction effect in Figure 6 shows that *A. viridis* subsp. *crispa* has the biggest diameter increment compared to the other tree species (*P. glauca, P. tremuloides* and *S. arbusculoides*). The inoculation treatment had a positive effect on *A. viridis* subsp. *crispa* but showed no significant effect on *P. glauca* or *S. arbusculoides*. The inoculation effect was the opposite between fine tailing and waste rock on *P. tremuloides*.

Biochar showed positive interactions with inoculation treatment on fine tailing for *A. viridis* subsp. *crispa* and *P. tremuloides* (Figure 6). However, the effect was the opposite on waste rock for *A. viridis* subsp. *crispa*. The interaction of biochar and inoculation seems to have no significant effect on the rest of the species.

The effect of biochar was found to be negative in a greenhouse experiment on herbaceous biomass in both fine tailing and waste rock tailings (Figure 5). Thus, the biochar effect varies depending on the plant species and environment. Figure 6 showed that the biochar effect also differed for various plant spacings. The various effect also applies to inoculation treatment on different plant species, the environment, and plant spacings. Hence, the interaction cannot be interpreted easily because of the ecophysiological complexity of plant responses to the different amendments and plant spacings.

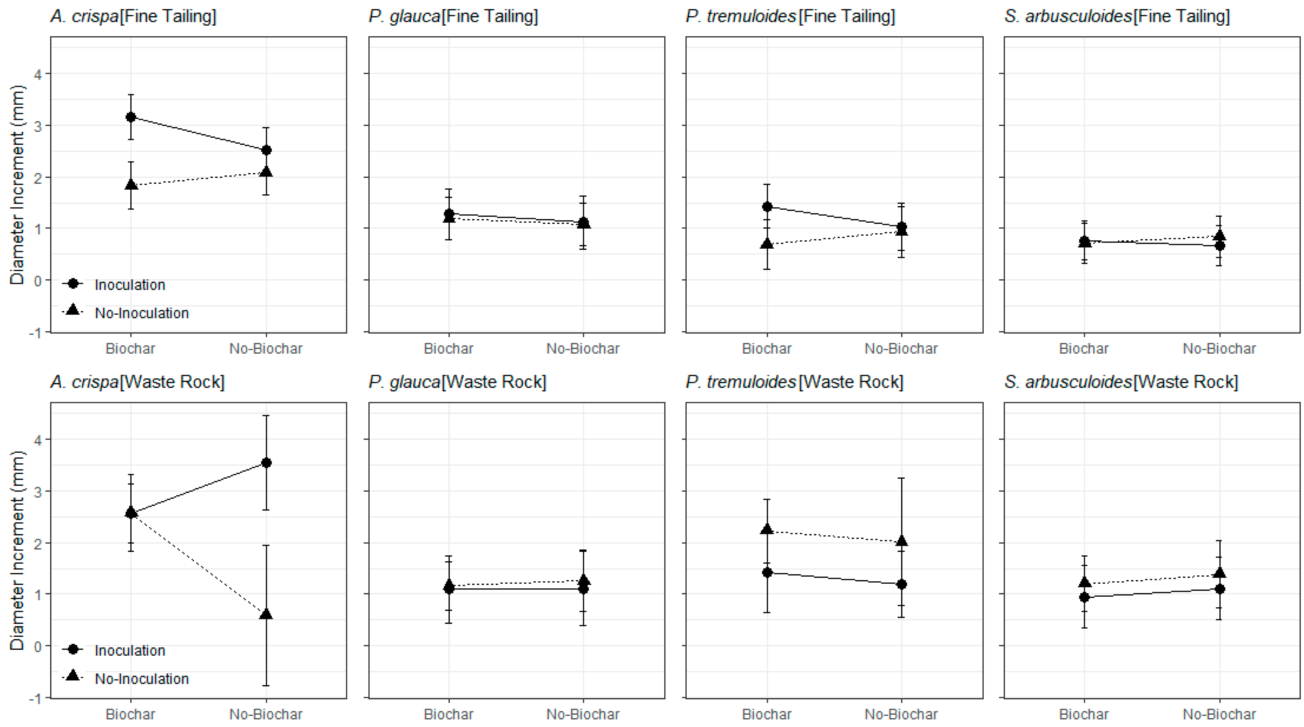


Figure 6. The marginal interaction effect of plant species, tailing, biochar, and inoculation treatment on plant diameter increments. The error bars represent the 95% confidence interval (CI) of means.

The spacing treatment showed unexpected results, as shown in Figure 7. The plant mixing with the highest density or the smallest inner spacing did not necessarily show the lowest growth rate because of higher competition. Only *S. arbusculoides* without biochar and *P. tremuloides* without biochar and inoculation showed the positive linear trend with the spacing. For most other interactions, the growth rate was shown to be decreasing from the largest spacing (60 × 60 cm) to the middle spacing (40 × 40 cm) and increasing again with the smallest spacing (20 × 20 cm), except for *S. arbusculoides*.

Different responses were also shown by *A. viridis* subsp. *crispa* and *P. tremuloides* with biochar and inoculation treatments, with an increasing growth rate from the largest spacing to the intermediate spacing and a decreasing effect for the smallest spacing. The greatest soil water loss was observed with the smallest spacing, where competition dominated, while facilitation was observed at the intermediate spacing and little to no interactions for the largest plant spacing.

The positive balance between competitive and facilitative effects was also amplified by the addition of biochar and inoculation treatments (Figure 7). The positive response of *A. viridis* subsp. *crispa* and *P. tremuloides* with the biochar and inoculation treatments at 40 × 40 cm spacing showed the impact of density or spacing configuration on the interaction of the treatments (Figure 7). The 40 × 40 cm spacing was the optimum spacing for *A. viridis* subsp. *crispa* and *P. tremuloides,* where a balance is achieved between competition and facilitation.

The addition of herbaceous crop plants did not show a significant effect on woody plant growth (Figure 8), except one noted interaction shown on herbaceous crops and inoculation treatment on *A. viridis* subsp. *crispa*. Without inoculation, the herbaceous plants tend to improve the growth rate of *A. viridis* subsp. *crispa*. The herbaceous plants may perform as a cover crop in this case, which maintains the evaporation and soil surface temperature around the woody plants. However, the plant response changed when the inoculation was added, thereby improving the herbaceous growth and the competitive effect on the perennial woody plant.

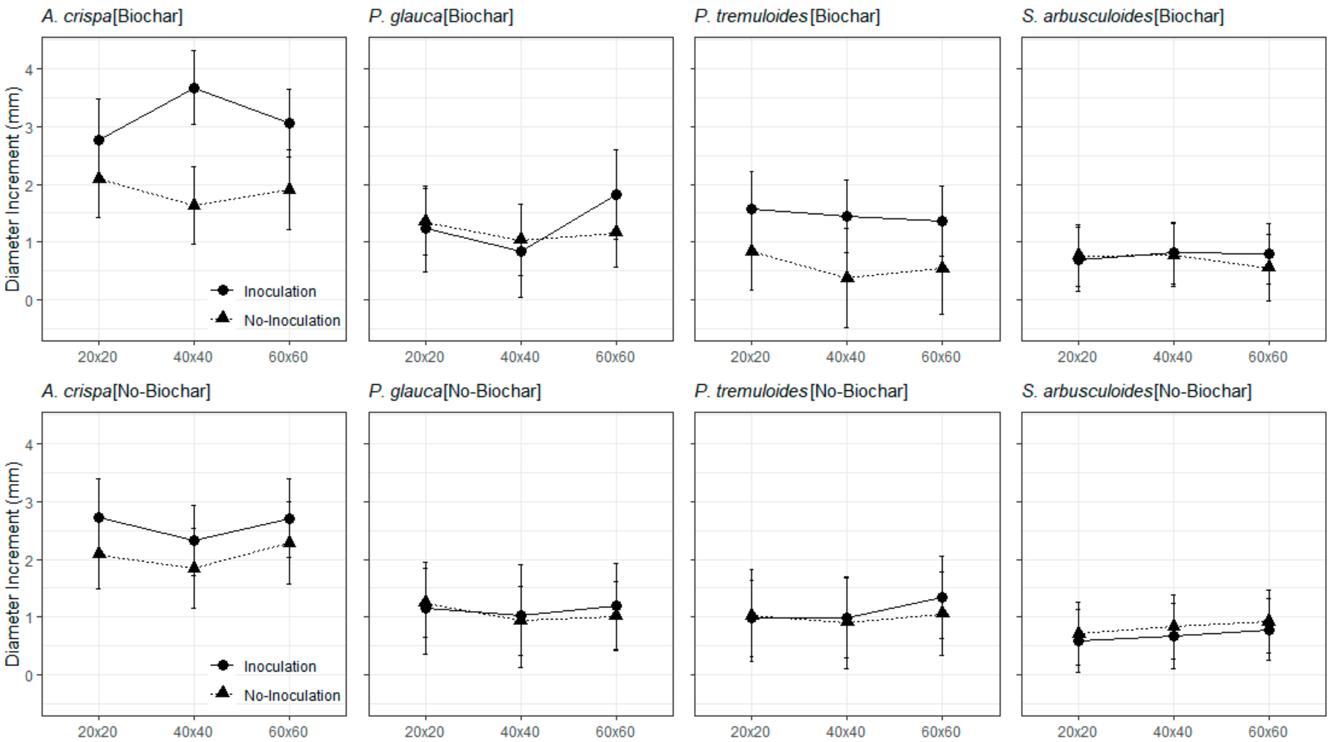


Figure 7. The interaction effect of biochar, inoculation, and spacing treatments in the fine tailing site. The error bars represent the 95% confidence interval (CI) of means.

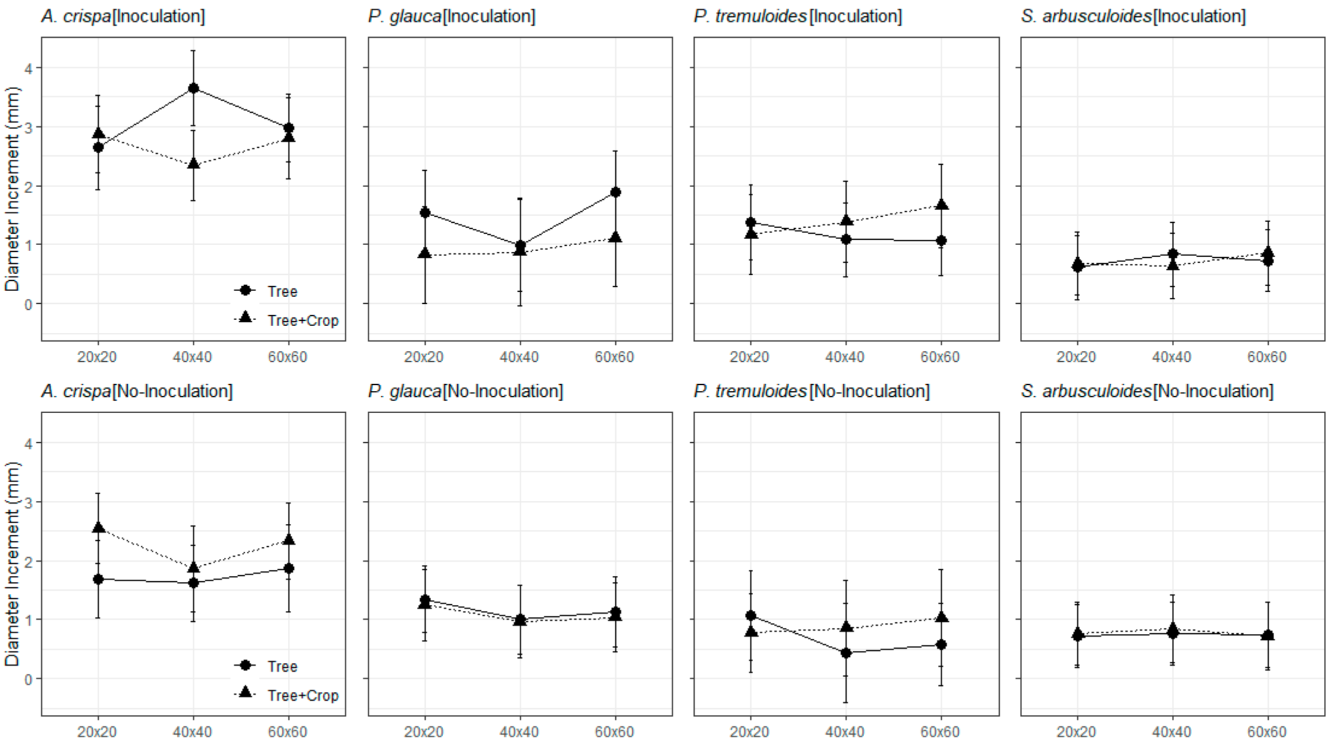


Figure 8. The interaction effect of inoculation, tree-crop, and spacing treatments in the fine tailing site. The error bars represent the 95% confidence interval (CI) of means.

4. Discussion

The physical characteristics of the fine tailing with very high moisture retention [44] and very low hydraulic conductivity [35] were not ideal for plant growth. The particle size was between that of clay and silt, which has a high water content at permanent wilting point (±27%) [45]. The soil moisture content at 15 cm below the surface was always below 30% for the whole year based on our measurements. Although the site had a mean rainfall of 700 mm, the soil moisture never reached the field capacity level (±40%), suggesting that the water was unable to infiltrate the soil.

Fine tailing and waste rock soil tailing had inadequate characteristics for plant growth in both greenhouse and field trials. The tree species exhibited better survival than herbaceous species in the field trial after two growing sessions, and better survival in the field trial than in the greenhouse trial. This can be explained by the fact that the woody perennial species used in the field trials are native and ecologically well adapted to the boreal Forest of Abitibi-Témiscamingue region, while the herbaceous species were species that were allochthonous to the region. These results corroborate those of [19,46], who showed the importance of using ecologically well-adapted native mycorrhizal fungi with their host plants for a successful long-term revegetation program. On the other hand, the herbaceous species showed better growth in the greenhouse experiment. Indeed, because of the overgrowth of the herbaceous species, the perennial woody plant species were suppressed in our greenhouse mesocosm experiment since the first month of plantation, although these annual herbaceous crop species started dying after 3 months of experiment time. Plant seedling size was shown to be important for the survivability and adaptability of tree species. While the biochar and inoculation treatments did not influence plant survivability, they did have an effect on the biomass yield productivity.

4.1. Biochar Amendment

Biochar is a highly stable and rich source of carbon and is a potential carbon sink in relation to climate change mitigation [47]. Biochar is also known as a soil amendment for improving the soil's properties and functions in agronomic applications [47–49]. It has the capacity to enhance the water and nutrient retention and improves soil structure and drainage [47]. There is evidence of an effect of biochar on microbial activity and plant symbiosis, which improve crop productivity [47,49]. The possible mechanisms involved include the immobilization of plant-available N, and the mineralization of labile, high-C-to-N fractions of biochar into microbial biomass [48]. Other possible mechanisms are the alteration of soil physico-chemical properties, mycorrhiza helper bacteria, plant–fungus signaling interference and detoxification of allelochemicals on biochar, and provision of refugia from fungal grazers [26,50].

In opposite to those reports above, our greenhouse experiment with biochar amendments showed a negative effect on total biomass yield (Figure 4). In fact, the plants did not benefit from the improvement in soil water retention physical characteristics by the biochar, while the control plants grew better with only daily watering treatment. The field trials also showed a negative effect of biochar without the inoculation treatment. However, when combined with inoculation in the field trial, the biochar showed a positive effect, especially on *A. viridis* subsp. *Crispa* and *P. tremuloides* (Figure 6). However, surprisingly, the effect became negative with bigger spacing (60 × 60 cm), when the plant competition was lower. This could mean that the biochar and inoculation effect was less strong than the effect of density. At the same time, we also noted the positive effect of density in our trials, which could be due to an improvement in the microclimate condition due to higher density [51–53].

There was another effect of biochar amendment on soil, which was the reduction in soil albedo [54]. The lower albedo of biochar can make the soil warmer, which can result in more soil water evaporation compared to the higher albedo, resulting in a negative effect on plant growth. Some interesting results on the effect of biochar on soil temperature in the temperate zone have shown that it can increase the average soil temperature, but it has a lower temperature on the hottest day of the year [55]. This means that biochar was also able to stabilize the soil temperature in an extreme zone. The stable soil temperature was favorable for the plant and also for the soil microbial activity [56]. The improvement in microbial activity in warmer soil could be another interaction mechanism between biochar and microbial inoculation in our field trial experiments.

4.2. Microbial Inoculation

A healthy soil ecosystem is formed by various microbial species with specific roles and functions [14]. We believe that soil ecosystem biodiversity is as important as aboveground biodiversity. We also believe that above- and belowground biodiversity is highly correlated, as some microorganisms may require specific host plants [15]. Thus, providing the mixture of root inoculants for the plants is expected to introduce and increase below-ground biodiversity. The mixture of microbial processes is expected to regulate the nutrient mineralization, biological nitrogen fixation, and other functions that can improve soil physico-chemical and microbiological properties [57–59].

The inoculation treatment showed a positive effect on plant growth in the greenhouse experiment. It had a slightly positive interaction with biochar, although it was not significant. The inoculation was beneficial for plant growth in both fine tailings and waste rock tailings. Since the mining waste tailings are mostly deprived of beneficial microorganisms [18], the inoculation with beneficial microbes was shown to be a good way of introducing beneficial symbiotic microorganisms into these challenging reclamation tailings, as reported in other studies [19].

The field trials showed a similar positive effect of microbial inoculation on *A. viridis* subsp. *crispa* and *P. tremuloides*, but not for *P. glauca* and *S. arbusculoides*. The different responses could be due to the specificity and efficiency of different microsymbionts [19]. The symbiotic relationship can range from parasitism to true mutualism depending on the microsymbiont, the plant host, and the soil fertility [59–61]. *Alnus viridis* subsp. *crispa* as an ectomycorrhizal plant benefits symbiotic mutualism due to both nitrogen-fixing *Frankia* actinomycete and mycorrhizal fungi [32]. *P. tremuloides* is not a nitrogen-fixing plant but can form both ectomycorrhizal and arbuscular mycorrhizal [62] and benefit from the co-occurrence of the mycorrhizal network with *A. viridis* subsp. *crispa* [63–65]. This hypothesis can be explored by another specific experiment involving *P. tremuloides*, *A. viridis* subsp. *crispa* and their interaction with mycorrhiza.

The benefit of inoculation on *A. viridis* subsp. *crispa* and *P. tremuloides* was shown to be enhanced by the addition of biochar. Biochar is known as a soil amendment that provides a good environment for mycorrhiza colonization [49,66,67]. However, the direct mechanism of how it affects mycorrhizal dynamics is still unclear [49]. The known mechanism in correlation with mycorrhiza is through soil chemical and physical alteration [48,49]. Another possible mechanism is through the soil temperature stabilization by the reduction in soil albedo, as discussed above. In fact, the microorganisms are known to be sensitive to soil temperature and microclimate conditions [56,68–71].

4.3. Mixed System Interactions

The field trials showed a positive effect of the herbaceous crop on tree species without inoculation treatment. One of the reasons for this finding is the well-known role of cover crops in reducing the evapotranspiration [72]. The herbaceous crops became disadvantageous and competitive to the woody perennial species when the inoculation treatment was applied (Figure 8). The literature on the use of bioinoculants in agroforestry systems is very scanty [73]. We conducted the first test on the use of bioinoculants in boreal agroforestry in the context of ecological restoration of post-mining areas with the aim of improving nutrient availability for plants while reducing the use of inorganic or organic fertilizers, pesticides, and water. More research is needed in that area to develop a broad conceptual framework and methodology that is supported by robust scientific data for the large-scale use of bioinoculants in the ecological restoration industry.

In general, we also found the positive effect of density which is supported by the principles of "Allee" effect in ecological theory [52,74,75]. The positive effect on higher density could be explained by the below-ground facilitative mechanism or aboveground microclimate improvement. The improved microclimate could be an important factor on spacing configuration, which correlates with the sensitivity of microorganisms on soil temperature and the effect of biochar on soil albedo [55,56].

We found that the interactions between components in mixed systems were not straightforward and required a comprehensive scenario for the intervention of the system. Figure 9 shows the ecophysiological complexity of plant responses to the different interactions between the factors that we studied in our experiment. The addition of biochar, inoculation treatment, and herbaceous crops could improve plant growth, and at the same time, it can have negative effect. The planting density treatment not only affects the plant competition, but also alters the microclimate around the plants. These conditions may change as the plants grow and yield different outcomes in the long term. The modeling effort can help estimate the growth dynamics in this restoration processes at the later stages. Some aspects that need to be considered in the long-term stages are the nutrient cycle and phytoremediation processes. The species mixing and planting configuration are other elements that need to be considered in the modeling scenario.

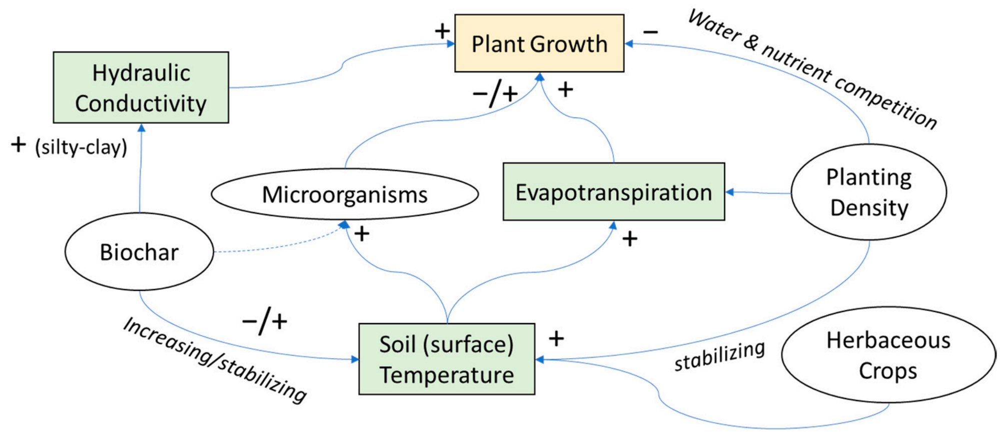


Figure 9. Hypothetical interactions between component factors in a restoration trial experiment. The interactions are complex, and all the factors can contribute to plant growth dynamics.

Soil temperature, soil cover, evaporation, and evapotranspiration affect soil water availability. Therefore, the comparison of volumetric water content between biochar-amended and control soils in field experiments may be confounded by indirect effects, that is, on plant growth and soil thermal properties. In addition to the chemical stabilization of nutrients, the modification of the physical structure of the bulk soil may result in biochar not simply increasing the capacity of soil to retain water, but also nutrients in the soil solution. The multispecies approach has shown some advantages in our restoration experiment. Mixing species at the early stage of planting proved to have no significant effect on spacing competition, although the outcome can differ in the later stages, when the plant is growing and both aboveground and below-ground competition occurs. At the early stage, we found a positive balance between plant competition and microclimate improvement on high-density plantation. Another facilitative effect was shown on the inoculation treatment between *A. viridis* subsp. *crispa* and *P. tremuloides*. *Alnus viridis* subsp. *crispa* may have served as a nurse species for *P. tremuloides* through mycorrhizal network associations. The addition of herbaceous crop species showed a positive effect on the perennial plant growth rate through their function as cover crops. In the long term, we expect to find more interactions between the species through their functions on nutrient cycling and successional dynamics.

The positive effect of plant density confirms the "Allee" effect, which showed the benefit of living in groups for inducing the facilitation within individuals [52]. The practical implementation of high-density planting can be very costly. The proposed "Nucleation" method introduced by [51] could be a low-cost alternative. The seedlings are planted in patches or "islands" to facilitate forest recovery that is less expensive than planting large areas. However, a study in tropical forest restoration in Costa Rica highlighted the importance of broad spatial replicated studies to account for high variability and make generalizable restoration recommendations [76]. The improvement in microclimate on high-density cluster planting is known to increase the survivability of the seedlings [51,77,78]. The combination with other enabling biotechniques such as microbial inoculation and biochar amendment may improve the whole successional process. Reinstalling the biological life through microbial inoculation of seedlings planted in reconstructed anthroposols after mining operations has shown successful plant growth and health and improved soil quality [19,32].

Biochar has the capacity to increase the hydraulic conductivity on the soil or tailings with very fine grain size, such fine tailing waste tailings, and at the same time, it is also able to reduce the hydraulic conductivity on the tailings with large particle size such as waste rock [48,49,79]. Biochar also reduces the soil albedo, which may increase the average soil surface temperature [54,55]. Warmer soil temperature can have negative effects on some ecosystems, but it seems to be advantageous for colder climate zones, as warmer soil temperature may increase the soil microbial activity and accelerate the plant recovery processes [56].

To improve the microclimate, it is suggested focus be placed on the initial planting, along with planting configuration and multispecies approach. In the long term, the nutrient cycle, phytoremediation processes and successional dynamics are some other aspects that are also important. The combination of annual and perennial plants in agroforestry systems can be applied to accelerate the nutrient cycle and successional processes for forest recovery of severely disturbed ecosystems [3]. The herbaceous crops serve as cover crops for supporting other woody plants [72,80]. We believe that this multispecies and multifunctional approach can be advantageous for ecological restoration projects of mining sites.

5. Conclusions

There were both positive and negative effects on plant spacing, biochar amendment and inoculation, depending on their interactions. The net positive effect was shown by combining high plantation density, biochar and inoculation factors on *Alnus viridis* ssp. crispa. Overall, planting density was shown to be the most important factor in generating a net positive effect. We suggest that the mechanism was correlated with microclimate improvement through soil plant water conservation and microbial activity enhancement over soil temperature modification. Hence, we propose emphasizing microclimate improvement for accelerating the restoration processes, along with other combined factors, including microbial inoculation and biochar amendment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14040856/s1, Table S1: The ANOVA P-value for total biomass, shoot biomass of *A. sativa*, *F. rubra*, *T. repens*, and shoot:root ratio; Table S2: The standard deviation of random effect factors on split-split plot design for each dependent variable data group of total biomass, shoot biomass of *A. sativa*, *F. rubra*, *T. repens*, and shoot:root ratio; Table S3: Type III Analysis of Variance Table with Satterthwaite's method on diameter growth of tree species on waste rock and fine tailing without spacing factor; Table S4: The standard deviation of random effect factors on split-split plot design for regression model on data group of fine tailing and waste rock without spacing effect. The total observation data is 2795; Table S5: Type III Analysis of Variance Table with Satterthwaite's method on diameter growth of tree species on fine tailing only with the inclusion of spacing factor; Table S6: The standard deviation of random effect factors on split-split plot design for regression model on data group of fine tailing with spacing effect. The total observation data is 2132.

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